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The number above an abstract corresponds to its designation in the program of the 1988 APS Annual Meeting in San Diego, CA, November 13–17. If a presentation was not given at the meeting, the abstract is not printed among the following pages.

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1

INTERACTION BETWEEN ACID RAIN AND DROUGHT STRESS ON FIELD CORN. R. Knittel, E. J. Pell, and D. P. Knievel, The Pennsylvania State University, University Park, PA 16802.

Field and greenhouse experiments were conducted in 1986 and 1987 to test the hypothesis that acid rain could predispose Zea mays (cv B73 X Mo17) to drought stress. Plants were treated with 3.0 cm/wk of simulated acid rain (SAR) of pH 3.0 or 5.0, and ambient rain was excluded. Two droughts were imposed, one following silking and a second following pollination. Effects were determined by physiological and histological parameters and by yield determination. Drought but no pH significantly reduced yield both years. In 1986 there was a drought X pH interaction in which plants treated with rain pH of 3.0 and drought had more kernels/ear and lower stomatal and cuticular conductance. In 1987 there were no interactions and conductance was lower for plants treated with rain of pH 5.0. Scanning electron microscopy shows no correction between presence of plate-like epicuticular wax formations and rain pH on foliage of greenhouse grown plants treated with SAR of pH 3.0.

2

INFLUENCE OF O_3 AND NITROGEN ON GROWTH AND PARTITIONING OF ASSIMILATE IN RADISH PLANTS. E. J. Pell and C. Vinten-Johansen. The Pennsylvania State University, University Park, PA 16802.

Raphanus sativus L. 'Cherry Belle' were grown with suboptimal, optimal and supraoptimal soil nitrogen (N). Plants were treated with charcoal filtered air or O_3 levels averaging 38 or 66 ppb delivered from 1000 to 1930 h for 32 d. Dry weight and total nonstructural carbohydrate (TNC) were measured as determinants of growth and assimilate. 66 ppb O_3 induced significant reduction in weight of hypocotyls and roots, and root/shoot ratio while elevated N resulted in increased weight of all plant parts. Effects of O_3 were more apparent at the optimal and supraoptimal N treatments. TNC levels were reduced at higher levels of N but lower in hypocotyls and roots harvested from plants treated with 66 ppb O_3. Elevated O_3 levels increased TNC content of foliage in the supraoptimal N treatment.

3

GAS EXCHANGE RESPONSES OF SOYBEAN CULTIVARS TO SHORT TERM EXPOSURE OF SULFUR DIOXIDE AND OZONE. Wen S. Sheng and Boris Chevone. Dept. of Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

Soybean cultivars 'Dare', 'Williams 82' and 'Essex' were exposed to 0.7 ppm sulfur dioxide (SO_2), 0.2 ppm ozone (O_3) or filtered air for 4 hr in environmentally controlled fumigation chambers. Gas exchange measurements were taken at intervals of 30 min during the fumigation. Both O_3 and SO_2 resulted in a reduction in net photosynthesis (Pn) and stomatal conductance (Cs). All the cultivars developed typical SO_2 or O_3 symptoms during or after fumigation. Suppression of Cs and Pn by SO_2 occurred primarily within the first 60 min of exposure. However, dramatic effects of O_3 on Cs and Pn did not occur until 90 min after the fumigation was initiated. Control plants exposed to filtered air maintained consistent Pn and Cs throughout the 4 hr period.

4

RESPONSE OF FIELD-GROWN LOBLOLLY PINE TO OZONE OVER THREE GROWING SEASONS. S. R. Shafer and A. S. Heagle, USDA/ARS, N. C. State Univ., Dept. of Plant Pathology, Raleigh, NC 27695-7616.

Seedlings (4-mo-old) of Pinus taeda were planted in a field and exposed daily during 3 growing seasons (May 27-Oct. 24, 1985; Apr. 8-Oct. 16, 1986; Apr. 8-Oct. 1, 1987) to charcoal-filtered (CF) air, nonfiltered (NF) air, or NF air supplemented (12 hr/da) with O_3 to produce O_3 concentrations in proportions of 1.25, 1.50, 1.75, or 2.00 x NF in open-top chambers (128 seedlings/chamber). Plants were harvested at the end of each growing season (4 families in 1985 and 1986; one of the 4 in 1987). Significant dose-response models (seasonal 12-hr/da mean O_3 concentrations vs. above-ground biomass components) developed for plants harvested in Oct. 1985 were linear. After remaining plants were exposed in subsequent years, data for some family-variable combinations were fitted by Weibull models that indicated a level of O_3 near 1.25 x NF was required for noticeable plant responses. However, data for one family continued to indicate linear dose-response relationships. Depending on the year-family-variable combination, models predicted yields in NF air (3-season average of the 12 hr/da seasonal mean O_3 concentrations=0.048 µl/l) that were suppressed as much as 21% from those predicted for CF air (3-season average O_3=0.025 µl/l).

5

OZONE EFFECTS ON LEAF CARBOHYDRATE CONTENT OF DIFFERENTIALLY SENSITIVE RADISH POPULATIONS. C.T. Gillespie and L. D. Moore. Dept. of Plant Pathology, Physiology, and Weed Science, VPI & SU, Blacksburg, VA 24061.

Three populations of radish, Raphanus sativus L. cv 'Cherry Belle', previously selected for differential sensitivity to ozone were exposed to either filtered air or 0.10 ppm ozone for 4 hr/day, 3 day/week, for 3 weeks. At intervals of 5 days, one leaf from each of the first two leaf pairs was harvested and analyzed for sugar and starch levels. Exposure to ozone increased the free sugar level in leaves in all populations as compared to filtered air treated plants, but to a lesser degree in the ozone resistant population than in the ozone sensitive or non-selected populations. Leaves from these two populations exposed to ozone also had elevated starch levels. Increases in the leaf carbohydrate pool may be a product of decreased carbon translocation to sinks such as the roots and hypocotyl and could account for the greater sensitivity of below ground parts to ozone.

6

RELATIONSHIP BETWEEN COMMON ROOT ROT AND WINTER WHEAT FORAGE PRODUCTION. J. T. Mathieson, C. M. Rush, and K. B. Porter. Texas A&M University, Texas Agricultural Experiment Station, Bushland, Texas 79012.

Both Imazalil treated and untreated seed of seven winter

wheat varieties were planted in a field naturally infested with Bipolaris sorokiniana. Plants were sampled for disease and forage production at the seedling and jointing stages of growth. At both sampling dates, the plants from the treated seed had a significantly lower incidence of disease and disease index than plants from the untreated seed; however, no difference was seen with regard to forage production. Significant differences in disease incidence and disease index also occurred among varieties at each sampling date. The varieties Scout 66 and Siouxland consistently had significantly less disease and a lower disease index. In final forage production, however, no difference was seen among the varieties.

7

VERTICILLIUM WILT OF ALFALFA IN UTAH. S. V. Thomson and R. D. Buhler, Dept. of Biology, Utah State University, Logan, 84322-5305.

Verticillium wilt of alfalfa was first observed in northern Utah in 1985. Isolations confirmed the presence of Verticillium albo-atrum Reinke and Berth. with characteristic dark mycelial growth. Verticillium wilt was found in 25% of the 323 fields examined in a random statewide survey conducted in August and September, 1987. The widespread distribution suggests that the disease had been present in the state for several years before it was first observed. Verticillium wilt was found in Millard and Sevier counties and most counties north of these but was not observed in southern counties. The alfalfa variety Deseret appears to be unusually susceptible to Verticillium wilt based on the high frequency of infection in the state and significantly higher incidence of infection in a variety trial.

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VERTICILLIUM WILT OF ALFALFA: DISSEMINATION VIA SCLEROTIA OF SCLEROTINIA. R. G. Gilbert, USDA-Agricultural Research Service, P. O. Box 30, Prosser, WA 99350.

Commercial seedlots from alfalfa fields, infected with Verticillium wilt, were cleaned and sized. Sclerotia of *Sclerotinia sclerotiorum*, because of prevalence and similar size, were detected in each alfalfa seedlot. Approximately 45-50 sclerotia were isolated from each 450 g of alfalfa seed. When sclerotia were incubated on sterile water agar and/or acidified PDA, *Verticillium* sp. grew out from ca. 10% of these sclerotia. Pathogenicity tests of resultant colonies demonstrated that all isolates were the virulent, pathogenic strain of *Verticillium albo-atrum*, the cause of Verticillium wilt in alfalfa. The presence of sclerotia of *Sclerotinia sclerotiorum* in alfalfa seedlots, colonized by *Verticillium albo-atrum*, contributes to the dual dissemination of both diseases, Verticillium wilt and Sclerotinia crown and stem rot.

9

EFFECT OF MELOIDOGYNE HAPLA ON SEEDLING DAMPING-OFF AND ROOT ROT CAUSED BY PHYTOPHTHORA MEGASPERMA F. SP. MEDICAGINIS IN ALFALFA. F. A. Gray, G. D. Griffin, D. A. Johnson and J. W. Eckert. Wyo. Agric. Exp. Sta., Laramie, WY 82071, USDA-ARS and Utah Agric. Exp. Sta., Logan, UT 84322-6300.

The interaction between Phytophthora megasperma f. sp. medicaginis (Pmm) and Meloidogyne hapla (Mh) was studied during the seedling growth phase in three alfalfa cultivars (Nevada Synthetic XX, resistant to Pmm and highly resistant to Mh; Apollo II, resistant to Pmm and susceptible to Mh; Deseret, susceptible to Pmm and Mh). Post-emergence damping-off, attributed to Pmm, was suppressed when both pathogens were applied in combination at planting as compared to the application of Pmm alone. Percent loss (percent of control) at 2 wk was 33 and 26, 45 and 21, and 87 and 35, in the Pmm alone and Pmm + Mh treatments for Apollo II, Nev Syn XX and Deseret, respectively. After 7 weeks when the experiment was terminated, Phytophthora root rot was increased in the surviving plants in the combination treatment as compared to the Pmm alone treatment.

10

EFFECT OF ENVIRONMENTAL FACTORS ON THE PATHOLOGICAL RELATIONSHIP OF MELOIDOGYNE HAPLA AND PHYTOPHTHORA MEGASPERMA F. SP. MEDICAGINIS ON ALFALFA. G. D. Griffin*, F. A. Gray**, D. A. Johnson*, and D. L Crebs*. *USDA-ARS, Forage and Range Research, Utah State Univ. Logan, UT 84322-6300, and **Plant Science Dept., Univ. Wyoming, Laramie, WY 82071.

There was a significant interaction ($P < 0.05$) between Meloidogyne hapla (Mh) and Phytophthora megasperma f. sp. medicaginis (Pmm) on Deseret alfalfa, susceptible to both Mh and Pmm, and Apollo II alfalfa, susceptible to Mh and resistant to Pmm. Plant mortality and plant growth suppression were increased when inoculation with Mh preceded that of Pmm. Nematode reproduction was significantly affected by simulataneous or preinoculation with the nematode or fungus. There was a greater percentage of plant mortality in clay soil than in sandy-clay or sandy-loam soil. The greatest mortality rate in plant growth suppression occurred at 28 C. A combination of Mh-Pmm had minimal effect on the growth of Nevada Synthetic XX alfalfa, resistant to both pathogens.

11 Withdrawn

12

POPULATION DYNAMICS OF OVERWINTERING NEMATODES ASSOCIATED WITH PEACH IN WEST VIRGINIA. J. B. Kotcon, Div. Plant & Soil Sci., West Virginia Univ., P. O. Box 6057, Morgantown, WV 26506.

Six plots of four trees each were permanently marked in each of 15 orchard blocks infested with Xiphinema spp. and representing a variety of soil types, rootstocks, and scion cultivars. Soil was sampled on 11 dates from October 1986 to March 1988. Overwintering survival (ratio of spring to fall population densities) was less than 20% in 3 of 15 plots in 1987 and in 6 of 15 plots in 1988. Population declines were not significant in the remaining plots and actually increased 10-fold each year in one plot. Similar variability in overwintering survival was observed with Meloidogyne hapla, Pratylenchus spp., Criconemella spp., and Paratylenchus hamatus. Population densities of Mononchids increased significantly over winter in 2 of 15 blocks in each year, but did not change significantly in the remaining blocks. Results suggest that predictions of spring population densities based on fall sampling of soil are not reliable.

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SUSCEPTIBILITY OF FINNISH PINES TO NORTH AMERICAN PINEWOOD NEMATODES. T. S. Panesar and J. R. Sutherland, Pacific Forestry Centre, Canadian Forestry Service, 506 West Burnside Road, Victoria, BC, Canada V8Z 1M5

In 1984 Finnish authorities found the pinewood nematode (PWN) in imported Canadian wood chips and we were asked to determine pathogenicity of Canadian PWN isolates (and for comparison, a pathogenic MO isolate) to Finnish Scots and lodgepole pines. In March 1987, and every 2 months thereafter, 7 Scots and 20 lodgepole pine seedlings were inoculated separately with 2000 PWN of each of the following isolates (geograhic origin in parenthesis): MO(Missouri), ALB(Alberta), BC(British Columbia), QUE 1(Quebec, isolate Q14-26) and QUE 2(Quebec, isolate Q52A). Seedlings were kept in a greenhouse (18h day/28°C; 6h night/23°C) and mortality recorded for 4 months. Percent mortality and mean numbers of PWN recovered/g of lodgepole pine stems over all inoculation dates were: 61% and 3540(MO), 54% and 3570(ALB), 44% and 2740(BC), 29% and 2300(QUE 1) and 28% and 2340(QUE 2). For Scots pine, mortality and PWN numbers were: 39% and 2130(MO), 71% and 1930(ALB), 71% and 3370(BC), 46% and 2970(QUE 1), and 36% and 1320(QUE 2). These results show the susceptibility of Finnish Scots and lodgepole pines to North American PWNs, but inoculations of field-grown trees are needed to confirm our results.

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SOYBEAN CYST NEMATODE ASSOCIATIONS WITH SUDDEN DEATH SYNDROME OF SOYBEANS. G. W. Lawrence, K. W. Roy, and K. S. McLean. Dept. of Plant Pathology and Weed Science, Mississippi State University, Miss. State, MS 39762.

Symptom development of sudden death syndrome (SDS) was examined in the greenhouse on soybeans (Glycine max (L.) Merrill) inoculated with a race 3 population of soybean cyst nematode (Heterodera glycines Ichinohe) (SCN) and the strain of Fusarium solani (Mart.) Sacc. (FS-A) implicated as the causal agent of SDS. Although SCN was not required for infection by FS-A, symptoms developed 3 to 5 days earlier and were significantly more severe in the SCN + FS-A combination. The result was the same when Coker 156 soybeans were planted in FS-A and FS-A + SCN infested soil stored at 5C for 90 days to simulate overwintering. Pure colonies of FS-A were isolated from 38 and 35 percent of the mature SCN cysts from FS-A + SCN combinations at harvest and after overwintering for 90 days, respectively. FS-A isolates from SCN cysts, when used as inoculum on Coker 156 soybeans, produced all symptoms of SDS.

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PHYLOGENETIC AND FUNCTIONAL IMPLICATIONS OF THE FINE STRUCTURE OF PHASMID DEVELOPMENT IN JUVENILES AND MALES OF *MELOIDODERA FLORIDENSIS, M. CHARIS, HETERODERA SCHACHTII* AND *VERUTUS VOLVINGENTIS (NEMATODA: HETERODERIDAE)*. L. K. Carta and J. G. Baldwin, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

The fine structure of the phasmid sensory organ is observed throughout development in juveniles and males in four members of the Heteroderidae. The phasmid is best developed at the end of molts in *M. floridensis* and *M. charis*. The phasmid becomes reduced in size with succeeding stages. During the molt the dendrite elongates and sheath cell lamellar infoldings transiently disappear. The phasmid degenerates after hatch in *H. schachtii* and *V. volvingentis* and cannot be found in males dissected from sugar beet and buttonweed roots, respectively. The functional and phylogenetic significance of phasmid morphology and phasmid loss is discussed.

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TRANSIMISSION AND CONTROL OF SEED-BORNE INOCULUM (XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS) IN CAULIFLOWER. Alvarez, A. M., R. L. Gabrielson*, and J. E. Yuen. University of Hawaii, Honolulu, Hawaii 96822 and Washington State University*, Puyallup, Washington 98371.

Serological methods were used to monitor low levels of the black rot pathogen, Xanthomonas campestris pv. campestris in cauliflower seed, seedlings, and transplants. The relationship between seed-borne inoculum, latent infections in symptomless seedlings, and infection rates in mature plants was evaluated in field and greenhouse experiments. Chlorine gas was the most effective seed treatment assessed in seedbeds and field plots in land not previously planted to crucifers. A 1.5% infection rate in untreated naturally infected seed resulted in 100% infection of mature plants under tropical conditions. The implications for evaluation of seed treatments are discussed.

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STUDIES ON ERADICATION OF BLIGHT BACTERIA FROM BEAN SEED. A.W. Saettler, C. Heyuan, and M. Adimihardja, ARS/U.S. Department of Agriculture, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Infected seed is a major inoculum source for bean common (Xanthomonas campestris pv. phaseoli, Xcp) and halo blight (Pseudomonas syringae pv. phaseolicola, Psp) diseases. Organic solvent infusion was used to eradicate Xcp and Psp from infected bean seed. Eradication of Xcp occurred when infected Navy bean seed was immersed for 45 min at 45°C in methanol containing 800 ppm tetracycline; seed germination was reduced approximately 50%. Partial eradication of Psp resulted when infected Kidney bean seed was immersed in the same solution for 60 min at 45°C; seed germination was unaffected. Xcp was eradicated from Navy bean seed by conditioning the seed osmotically for 5 days in 65% glycerol containing 800 ppm chlorotetracycline, with no effects on germination.

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MONOCLONAL ANTIBODIES USED IN AN ELISA AND AN IMMUNOFLUORESCENCE PROCEDURE FOR THE DETECTION OF SEED BORNE ERWINIA STEWARTII IN MAIZE. G. L. Lamka, D. C. McGee, J. H. Hill, and E. J. Braun, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

Monoclonal antibodies (Mab) to Erwinia stewartii were derived from BALB-c mice by injecting them intraperitoneally with washed whole cells. A specific Mab was used as the detection antibody and polyclonal antibodies produced in rabbits as the capture antibody in a ds-ELISA. Positive ELISA results were obtained from seed lots that originated from A632 parent plants that were leaf inoculated in the field with Erwinia stewartii, while seed from uninoculated control plants reacted negatively in the assay. Seed sample preparations from the same two lots were fixed on glass slides. The specific Mab was then added. After rinsing, a fluorescein-labeled antimouse antibody was added. Fluorescing bacteria-like cells were detected microscopically in the seed lot from inoculated parent plants but none were found in the control lot.

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GROWTH CHAMBER EVALUATIONS OF SEEDBORNE FUNGI AS SEED AND SEEDLING PATHOGENS OF SUGARBEET. S. K. Kober and J. J. Gallian, Dept. of Plant, Soil and Ent. Sciences, Div. of Plant Pathology, University of Idaho, 1330 Filer Avenue East, Twin Falls, ID 83301.

Thirty isolates in three fungal genera were isolated from sugarbeet (Beta vulgaris L.) seeds produced near Salem, OR, and tested for pathogenicity in growth chambers. Diurnal temperatures fluctuating through the 30 day test period began at 10/2 C (day/night) and gradually increased to 20/6 C. Seedling emergence from previously disinfested seed which was individually inoculated by vacuum infiltration with one Alternaria and two Fusarium isolates was 30 and 17-20% lower, respectively, than the water inoculated controls. Seed inoculated with 11 Phoma isolates resulted in emergence reductions of 25-92% compared to the controls. These results suggest that cool temperatures at planting contribute to the ability of seedborne microorganisms to be pathogenic to sugarbeet seedlings, resulting in reduced plant stands.

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DEVELOPMENT OF PATHOGEN EXTRACTION METHODS FOR NONDESTRUCTIVE SEED HEALTH TESTS. P. M. Higley, D. C. McGee, and J. S. Burris, Seed Science Center, Iowa State Univ., Ames, IA 50011.

Germplasm collections can be a major source of potentially harmful seedborne pathogens. Standard procedures to test for these pathogens destroy the seeds and, therefore, are inappropriate for germplasm collections that contain small numbers of high value seed. Methods are being developed to extract pathogens from seed while preserving the capacity to restore the seed to a safe physiological condition for storage. Various extraction methods have been evaluated. Phaseolus bean seed was soaked to leach out bacteria or incubated for a short time on semiselective media to assay seedborne bacteria. Tissue from dry or slightly imbibed corn and soybean seed was surgically removed with a metal drill or with a sharpened syringe needle. The intact cores were assayed for pathogens. Following each extraction method, seed was returned to storable moisture and germination of seed was tested. Advantages and limitations of the extraction methods have been identified.

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COMPARATIVE KERNEL INFECTION FROM EAR INOCULATION OF MAIZE WITH ASPERGILLUS FLAVUS AND ASPERGILLUS PARASITICUS IN THE FIELD AT STARKVILLE, MISSISSIPPI. N. Zummo and G. E. Scott

Assays of kernels from ears in replicated plots of 23 maize hybrids inoculated with Aspergillus flavus Lk. ex Fr. or Aspergillus parasiticus Speare showed similar levels of infection. Inoculation of four maize hybrids with the needle inoculation method and the pinbar method in 1986 using either fungus also resulted in similar levels of kernel infection. Kernels from ears inoculated with A. flavus, when assayed, yielded only A. flavus in culture, while isolations from kernels from ears inoculated with A. parasiticus yielded only A. para-siticus. Kernels from noninoculated ears in replicated control plots yielded only A. flavus. When corn cobs were evaluated over a 2-year period as a carryover source for A. flavus at Starkville, Mississippi, all Aspergillus isolates recovered were A. flavus. These data indicate that although A. parasiticus did infect maize kernels, it was not as prevalent on maize in these tests as was A. flavus.

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RISK, RIDOMIL, AND CONTROL OF TOBACCO BLUE MOLD IN VIRGINIA. C. S. Johnson, Virginia Polytechnic Institute and State University, Southern Piedmont Agricultural Experiment Station, P.O. Box 448, Blackstone, VA 23824.

Prior probabilities of crop losses caused by tobacco blue mold were estimated from Tobacco Disease Loss Evaluation Committee reports and the results of annual county agent surveys conducted from 1979 through 1987. Expected crop losses due to blue mold were then compared with the cost of metalaxyl application. The average prior probability of blue mold occurring in Virginia, based upon reports over the last 9 years, was 22% and 78% for flue-cured and burley tobacco, respectively. Crop losses in Virginia due to blue mold averaged 3.05% and 3.67% for flue-cured and burley tobacco, respectively. Broadcast application of 2.3 L/ha Ridomil was economically justified when blue mold-induced crop losses greater than 1% of the gross value of the crop were certain to occur, or when there was at least a 30% probability of crop losses of at least 3%.

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THE SELECTIVE CUTTING OF TREES INFECTED BY FUSIFORM RUST (CRONARTIUM QUERCUUM (BERK.) MIYABE EX SHIRAI F. SP. FUSIFORME) IMPACTS THE GROWTH AND MORTALITY OF SLASH PINE (PINUS ELLIOTTI ENGELM. VAR. ELLIOTTI)' AND LOBLOLLY PINE (PINUS TAEDA L.) PLANTATIONS. R. P. Belanger, T. Miller, and J. F. Godbee. USDA Forest Service, Southeastern Forest Experiment Station, Carlton Street, Athens, GA 30602, and Union Camp Corporation, P.O. Box 216, Rincon, GA 31326.

Trees severely infected by fusiform rust were removed from 21 merchantable slash and loblolly pine plantations as a means of salvaging potential mortality and thereby increasing the growth and final yield of diseased stands. Volume growth was determined for treated and untreated portions of the study plantations 5 years after the salvage cutting was completed. Net gains in volume growth were greatest in the treated portions of slash pine plantations. Conversely, growth in the loblolly pine plantations was greatest in the untreated areas. Trends in mortality account for these differences.

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NATURALLY OCCURRING TOLERANCE TO METALAXYL IN PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA (PMG). K. M. Howard and A. F. Schmitthenner, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Pmg isolates obtained from soil treated 6 years with metalaxyl or nontreated were evaluated in vitro for naturally occurring resistance or tolerance to metalaxyl. No resistance has been detected, however 12% of single zoospore isolates selected for growth on metalaxyl supplemented agar were inhibited less than 50% and were considered tolerant. Only 10% of these selected isolates were inhibited more than 90% at 100 ppb. In contrast, 50% of the isolates selected for sensitivity to the fungicide were similarly inhibited. None of these sensitive isolates were inhibited less than 50% at 100 ppb. No growth of any isolate was observed at concentrations exceeding 250 ppb. It was concluded that sublethal concentrations of metalaxyl under field use may select for tolerant isolates.

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EFFECT OF COVER CROP, POTASSIUM, AND VARIETY ON VERTICILLIUM WILT OF COTTON. J.C. Broome, J.J. Marois, K.C. Cassman, Dept. of Plant Pathology, University of California, Davis CA. 95616

The experiment was a split-split plot design with a barley cover crop as the mainplot and potassium (K) amendments as the subplot, and two cotton genotypes as the sub-subplot. The vertical distribution of propagules was on average 8.01 propagules per gram of soil (ppg) at 0-20 cm; 13.1 at 20-40 cm; and 0.89 at 40-80 cm. Pathogen populations were significantly effected by the barley cover crop only at 40-80 cm, with a significant increase in propagules from 0.5 to 1.26 ppg. The high K application (480 kg/ha over 3 years) increased the propagule numbers at 0-20 cm from 3.8 ppg to 12.2 ppg. Disease incidence as measured by foliar necrosis was significantly lower in the high K plots, 48.7% vs 40.5%. However, vascular discoloration was not reduced by the K treatments. In soil planted for 3 years to GC510 there were significantly lower propagule numbers (7.6 ppg) than with SJ2 (10 ppg).

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DEVELOPMENT OF A RAPID, FIELD USABLE IMMUNOASSAY FORMAT, FOR DETECTION AND QUANTITATION OF PYTHIUM, RHIZOCTONIA, AND SCLEROTINIA SPP. IN PLANT TISSUE. J. H. Rittenburg, F. P. Petersen, G. D. Grothaus, and S. A. Miller. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077.

Double antibody enzyme-linked immunosorbent assays (ELISA) have been developed for detection and quantitation of Pythium, Rhizoctonia, and Sclerotinia spp. in plant tissue samples. The methodology allows rapid aqueous extraction of plant tissue samples into an aqueous form suitable for assay. The assays are performed on absorbent plastic devices, and are highly sensitive and specific yielding color end-points with wide dynamic range. Each assay device contains an internal positive control zone, a calibration zone and a sample test zone. The analysis, including sample preparation, can be completed within 60 minutes, and the actual immunoassay in less than 15 minutes.

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RAPID DETECTION AND MONITORING OF PYTHIUM BLIGHT OF TURFGRASS BY MEANS OF A FIELD USABLE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA). S. A. Miller, K. A. Plumley, J. H. Rittenburg, F. P. Petersen, and G. D. Grothaus, Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077.

Pythium Blight was detected and quantified in golf course turfgrass by means of a rapid immunoassay designed for field use. The immunoassay utilizes a monoclonal antibody directed against Pythium aphanidermatum, which also reacts with other Pythium spp. associated with Pythium Blight in warm- and cool-season grasses (Phytopathology 76:1057). Leaves sampled from pre-symptomatic turfgrass or from the margins of visible patches were pulverized on an abrasive pad, and a standardized amount of ground tissue was extracted in buffer. Immunoassays were carried out on absorbent plastic devices and completed in less than 15 minutes. The relative amount of Pythium spp. present in the sample was indicated by the intensity of the color of the final reaction product, which was quantified using a reflectometer. The assays clearly differentiated Pythium Blight from other turfgrass diseases and detected Pythium spp. at an early stage of disease development.

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RESPONSE OF IN VITRO PROPAGATED PEACHES IN CULTURE, GREENHOUSE, AND MICROPLOTS TO MELOIDOGYNE INCOGNITA. R. N. Huettel and F. A. Hammerschlag. USDA/ARS, Beltsville, MD.

Five in vitro propagated (IVP) peach (Prunus persica L. Batsch) scion cultivars (Suncrest, Rio Oso Gem, Compact Redhaven, Redhaven, Jerseyqueen) and 2 rootstocks (Nemaguard and Lovell) were screened for their susceptibility to the root-knot nematode, Meloidogyne incognita. Screening was conducted in tissue culture, in the greenhouse and in microplots. IVP cultivars were evaluated in tissue culture for galling at 5 wk. IVP cultivars transferred to the greenhouse were evaluated after 6 mo for galling. IVP cultivars transferred to microplots were evaluated for 3 yr for nematode populations, growth of trees, and yield. Results indicated that galling observed at 5 wk on IVP cultivars in tissue culture was indicative of the galling observed in the greenhouse and microplots. The size and number of galls observed in vitro appeared to be related to resistance of peaches to root-knot nematodes in the field.

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IMMUNODETECTION AND QUANTIFICATION OF ASPERGILLUS NIGER AND BOTRYTIS CINEREA ON HARVESTED WINE GRAPES. R. W. Ricker and R. M. Bostock, Department of Plant Pathology, University of California, Davis, CA 95616.

Polyclonal rabbit antibodies were used to detect the fungal pathogens Aspergillus niger and Botrytis cinerea on harvested wine grapes. Antibody cross-reactivities with extracts from grapes and grape fungal pathogens were measured by enzyme and fluorescence immunoassays. Anti-fungal antibodies did not cross-react with grape juice. Binding of anti-Botrytis antibodies to Aspergillus extracts was low, whereas binding of anti-Aspergillus antibodies to Botrytis extracts was extremely high. Antiserum specificity was increased by selective cross-absorption, and absorbed antisera were used in competitive inhibition assays. Magnetic microbeads provided rapid separation of microbead-bound Antigen-Antibody complexes to determine mold levels in grape juice samples from harvested wine grapes.

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APPLICATION OF POLYCLONAL AND MONOCLONAL ANTIBODIES IN THE DETECTION AND IDENTIFICATION OF PHYTOPHTHORA SPECIES AND RACES FROM PLANT TISSUE. H.S. Pepin and C.M. Prager. Agriculture Canada Research Station, Vancouver, B. C., Canada V6T 1X2.

Polyclonal antiserum specific to Phytophthora spp. was used in an indirect sandwich ELISA and in indirect immunofluorescence staining for the detection of Phytophthora infection in plant tissue. Monoclonal antibodies developed from Balb/C mice spleen cells sensitized to Phytophthora fragariae and fused to Fox NY myeloma cells were specific to races or groups of races of P. fragariae and were used to identify these races in pure culture by indirect sandwich ELISA. Although most monoclonals reacted with more than one race of P. fragariae, it was possible to differentiate each race using a combination of monoclonal antibodies.

31

GENOME ORGANIZATION OF MAIZE CHLOROTIC MOTTLE VIRUS BY NUCLEOTIDE SEQUENCE AND IN VITRO TRANSLATION. R. C. Nutter, K. M. Scheets, N. Siu* and S. A. Lommel*. Dept. of Plant Path., Okla. State Univ., Stillwater, OK 74078; *Dept. of Plant Path., Kansas State Univ., Manhattan, KS 66506.

The entire nucleotide sequence of cDNA clones of the maize chlorotic mottle virus (MCMV) has been determined. The virus is at least 4435 nucleotides in length. Analysis of the DNA sequence reveals open reading frames of 32, 55, 8.8 and 25 kd. However, suppression of the terminators of the 55 and 8.8 kd polypeptides would result in readthrough proteins of 110 and 32 kd, respectively. The predicted amino acid sequence of regions of MCMV shows striking homology to regions of carnation mottle virus and turnip crinkle virus. A 4.435 kb cDNA clone has been constructed by joining three subclones of the virus. The genome organization of MCMV is being analyzed by producing in vitro transcripts from the full length clone and from a series of ExoIII deletions and translating them in a reticulocyte system.

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EXPRESSION OF TOBACCO VEIN MOTTLING VIRUS HELPER COMPONENT PROTEIN IN TRANSGENIC TOBACCO
P. H. Berger, A. G. Hunt, G. M. Hellmann, and T. P. Pirone.
University of Kentucky, Lexington, KY 40506-0091.

The helper component (HC) of the potyvirus tobacco vein mottling virus (TVMV) is a 48-kDa virus-encoded protein which is required for aphid transmission. A cDNA clone was constructed which contained the first three cistrons (34K-HC-42K) of the TVMV RNA genome, the first six nucleotides of the adjacent CI protein cistron, and a synthetic translation termination codon. Previous experiments had indicated that a proteolytic activity responsible for cleavages which produced mature HC was encoded by this cDNA segment. Translation of *in vitro*-synthesized RNA transcripts and expression in *E. coli* indicated, based on the detection of HC by Western blot or immunoprecipitation analysis, that the protease was active. This cDNA segment was next cloned in a Ti-plasmid-based vector for *Agrobacterium*-mediated transformation of tobacco. Western blot analysis revealed low levels of an HC-specific protein in transgenic plants which comigrated with authentic HC from TVMV-infected tobacco. Biologically active HC was obtained when sap extracts from transgenic plants were concentrated before bioassay.

33

HYBRIDIZATION ANALYSIS OF MAIZE STRIPE VIRUS VIRION RNAS. B. W. Falk, V. Klaassen, and J. H. Tsai. Dept. of Plant Pathology, University of California, Davis, CA 95616; and University of Florida, Fort Lauderdale, FL 33314.

DNAs complementary (cDNAs) to the maize stripe virus (MStV) virion RNAs were cloned and hybridized with the MStV virion RNAs. Clones were obtained to all 5 virion RNAs. Each clone hybridized with a single-specific denatured virion RNA. When northern blots of nondenatured MStV virion RNAs were compared, the clones again hybridized with a specific ssRNA, and with a slower migrating dsRNA. ^{32}P-labeled ssRNA riboprobes transcribed from recombinant plasmids containing RNA promoters also hybridized with denatured MStV virion RNAs, although one polarity always reacted more intensely than the other. These data demonstrate that the MStV virion RNAs are encapsidated as both polarities.

34

THE CLONING AND IN VITRO TRANSCRIPTION OF CMV-WL SATELLITE. C. M. Kearney and D. Gonsalves, Dept. of Plant Pathology, NYS Agric. Experiment Station, Cornell Univ., Geneva, NY 14456.

The three genomic RNAs of cucumber mosaic virus strain WL plus its satellite (sat-WL) cause white-leaf in tomato. Without sat-WL, the genomics cause a mild leaf curl. We have produced a cDNA clone of sat-WL which differs from the published RNA sequence by four bases. The in vitro transcript of this cDNA elicited white-leaf symptoms in tomatoes when inoculated with native CMV-WL genomic RNA, indicating that the sequence is active. The cDNA was produced from LiCl-purified sat-WL dsRNA. Plus and minus RNA strands were eluted from a strand separation gel, hybridized to an oligomer specific to the 3' end, and reverse transcribed. The RNA templates were destroyed by NaOH and heat, and the plus and minus cDNAs were hybridized together and filled in with Klenow. Since the primers also had a Pst I site, the ds cDNA was cut and ligated into this site in pUC18. It was later subcloned into pT7T318 for dideoxy sequencing and T7 RNA polymerase transcription.

35

DOUBLE STRANDED RNA-DEPENDENT PROTEIN KINASE ACTIVITY INDUCED BY TMV INFECTION. C.E. Crum, J. Hu, H.J. Hiddinga and D.A. Roth. Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071.

A Mr 68,000 host encoded protein (p68) is phosphorylated in extracts of mock inoculated tobacco tissue. The basal level of phosphorylation increases 3-4 fold by TMV infection or addition of dsRNA. Nucleotide photoaffinity labeling indicates that the protein has a specific ATP binding site, characteristic of protein kinases. The phosphorylated protein can be immunoprecipitated by antiserum produced against Mr 68,000 dsRNA dependent protein kinase from interferon-treated, virus infected human cells. In addition, p68-containing immunocomplexes can catalyze the incorporation of ^{32}P from (γ^{32}P) ATP into endogenous p68. This protein has immunological and biochemical similarities to dsRNA dependent protein kinases implicated in the regulation of protein synthesis and poliovirus replication.

36

INHIBITION OF TOBACCO MOSAIC VIRUS REPLICASE ACTIVITY BY PHOTOAFFINITY LABELING. H.J. Hiddinga, D.A. Roth, University of Wyoming, Laramie, WY 82071

Nucleotide photoaffinity labeling was used to investigate template independent TMV replicase activity. Replicase activity was inhibited in a concentration dependent fashion by 8-N_3ATP or 5-N_3UTP. Inhibition in the presence of azidonucleotides was dependent upon UV photolysis Azidonucleotide-mediated inhibition of replicase activity was diminished by the presence of excess ATP, CTP, GTP, and UTP but not by deoxribonucleotide triphosphates, adenosine, or AMP. Specificity of inhibition was demonstrated by slot-blot hybridization of in vitro replicase products to a collection of TMV cDNA sequences. A TMV induced Mr 120,000 protein was labeled using (γ^{32}P) 8-N_3ATP with kinetics similar to those exhibited in inhibition studies. These data demonstrate that covalent modification of the TMV replicase nucleotide binding site results in decreased activity and suggest that the Mr 120,000 protein is involved in the replication complex.

37

EFFECTS OF GENES I AND VI OF TWO CAULIMOVIRUSES IN TRANSGENIC PLANTS. K.-B. Goldberg, J. M. Kiernan, S. Gowda, J. E. Schoelz, and R. J. Shepherd. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Genes I and VI of figwort mosaic virus (FMV) and gene VI of cauliflower mosaic virus (CaMV) were transferred to the genomes of Datura innoxia and Nicotiana edwardsonii using either pGA472 or pKYLX-7 Ti-plasmid gene vectors. N. edwardsonii is a systemic host of both viruses, but D. innoxia is a systemic host for only FMV. Gene VI of FMV and its homologous promoter produced no disease effects in either host. However, expression of gene VI of CaMV (with its homologous 19S promoter) produces a chlorotic mottling disease of transgenic D. innoxia, an effect similar to that in transgenic Nicotiana tabacum. No disease effects were associated with transformation of plants with gene I of FMV.

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COMPARATIVE ANALYSIS OF CAULIMOVIRUS PROMOTERS IN PROTOPLASTS. F. C. Wu, S. Gowda, H. B. Scholthof, J. M. Kiernan, and R. J. Shepherd. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Promoter sequences of figwort mosaic virus (FMV) ligated to a reporter gene of chloramphenicol acetyltransferase (CAT) or β-glucuronidase (GUS) were electroporated into protoplasts of Nicotiana edwardsonii cell cultures and mesophyll tissue. The cauliflower mosaic virus (CaMV) 35S promoter with CAT gene was used for comparison. Enzymatic assays for reporter gene were carried out 24 h after electroporation. DNA sequences in the large intergenic region of the FMV genome that gave the maximum gene expression were defined. The upstream elements of the FMV 35S promoter extend into the 3'-end of gene VI. FMV 35S promoter stimulated gene expression at comparable levels to the CaMV 35S promoter while the FMV 19S promoter produced a lower level of expression. Significantly stronger expression of CAT gene with CaMV 35S or FMV 19S promoter was observed in transfected protoplasts from plants infected with FMV.

39

COMPLEMENTARY DNA CLONING AND SEQUENCING OF THE BEAN YELLOW MOSAIC VIRUS COAT PROTEIN GENE AND COMPARISON WITH OTHER POTYVIRAL CAPSID PROTEINS. John Hammond and Rosemarie Hammond. USDA-ARS, PSI, Beltsville, Md. 20705.

A complementary DNA (cDNA) clone useful as a probe for virus detection encodes the complete bean yellow mosaic virus (BYMV) coat protein gene (identified immunologically and by hybridization to a cDNA probe specific for the viral RNA 3' end). The nucleotide and amino acid (AA) sequences were compared to those of other potyviral capsid genes. The AA sequences were found to be more highly conserved than nucleotide sequences. The major differences were at the amino terminus (exposed on the virion surface), to which most virus-specific antibodies are produced. The conservation of internal AA sequences is probably necessary for capsid structure. This also explains why antigen-coated forms of ELISA (which lead to partial disruption of the virion and thus expose internal sequences), with polyclonal sera, reveal relationships between viruses not obvious in double antibody sandwich ELISA with the same sera.

40

PRODUCTION OF FULL-LENGTH IN VITRO TRANSCRIPTS OF TOBACCO VEIN MOTTLING VIRUS. Kathleen Franklin, Leslie Domier, John Shaw, and Robert E. Rhoads. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

A full-length cDNA clone of the single-stranded RNA genome of the potyvirus, tobacco vein mottling virus (TVMV), has been constructed and inserted into SP6 and T3 transcription vectors. Oligonucleotide site-directed mutagenesis was used to remove non-viral sequences between the transcription promoter and the 5' terminus of the TVMV cDNA. Transcripts produced from the SP6 promoter have one 5' non-viral G residue and those from the T3 promoter have two non-viral G residues; a non-viral C residue remains at the 3' end of transcript RNAs. In vitro transcripts from both constructions were translated in rabbit reticulocyte and wheat germ in vitro translation systems, and the resulting protein products processed properly when compared to in vitro translations programmed with viral RNA. Infectivity of the transcripts in tobacco plants and protoplasts is being investigated.

41

ISOLATION OF THE MAIZE STRIPE VIRUS GENE ENCODING THE NONCAPSID PROTEIN. R. E. Gingery, and M. D. McMullen, USDA-ARS, Departments of Plant Pathology and Agronomy, respectively, The Ohio State University, OARDC, Wooster 44691

Maize plants infected with maize stripe virus (MStV) synthesize large amounts of a 16-kd noncapsid protein (NCP). The isolation of cDNA clones of the MStV-NCP gene would provide probes for investigating NCP expression. cDNA was prepared to MStV RNA by random priming and reverse transcriptase synthesis. The NCP gene was isolated by screening a λgtll MStV cDNA library for clones expressing fusion proteins that reacted with antibodies raised against purified NCP. Antibodies immunoselected by the λgtll fusion protein reacted with purified NCP and a protein from MStV-infected tissue that co-migrated with NCP on SDS-PAGE confirming that the λ inserts encoded NCP. There was no reaction to proteins from healthy tissue. The gene was subcloned into pUC119 for sequencing and preparation of single- and double-stranded hybridization probes. The NCP-cDNA probe hybridized specifically to RNA from MStV-infected maize.

42

HETEROGENEITY IN THE COAT PROTEIN SEQUENCE OF A MILD MUTANT OF PAPAYA RINGSPOT VIRUS. B. L'Hostis and D. Gonsalves. Dept. of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456.

Clones to the RNA of a mild mutant of papaya ringspot virus (PRV HA 5-1) were obtained via 3 different methods: a) dAdT tailing (Phytopathology 77:119, 1987), b) cloning of HindIII restriction fragments and c) size fractionation on sucrose gradient followed by methylation and linker ligation. Hybridizations were conducted with ^{32}P end-labeled PRV HA 5-1 RNA. Up to 4kbp-long inserts were obtained by the last method. Recombinants were screened with 1) a ^{32}P-labeled oligonucleotide representing a conserved sequence in the coat protein of potyviruses and 2) a ^{32}P-labeled plasmid carrying the sequences coding for the coat protein, part of the nuclear inclusion protein and the 3' non-translated region of a watermelon strain of PRV (PRV-w). Restriction map data show that clones corresponding to the coat protein region obtained by either method present some sequence heterogeneity. These clones are being sequenced. The possibility of sequence heterogeneity involving other regions is being investigated.

43

ORGANIZATION AND IN VITRO TRANSLATION OF BEAN COMMON MOSAIC VIRUS (BCMV) RNA. A W Millar and P R Mills, Department of Mycology and Plant Pathology, The Queen's University of Belfast, Northern Ireland, BT9 5PX.

BCMV-RNA was isolated from purified Netherland's strains NL3 and NL4. The RNA's of both strains were shown to have a poly (A) tract by oligo$_6$ (dT) cellulose column chromatography and a Mr of 3.0± 0.15 x 10^6 under denaturing conditions. RNA was translated in vitro in message dependant rabbit reticulocyte lysate to give approximately 20 ^{35}S methionine labelled polypeptides ranging in Mr from 28000 (28 K) to 250 K. Translation was stimulated by the cap analogue pm^{7}G. Seven major polypeptides, produced for both strains, had estimated Mr's of 90 K, 78 K, 63 K, 55 K, 51 K, 31 K and 28 K. The NL3 31 K product co-migrated with authentic coat protein and reacted with antisera to NL3 and the closely related pathotype NL5. In time course experiments the 31 K peptide appeared as early as 15 minutes. Preliminary experiments have revealed no evidence for the processing of a high molecular weight polypeptide containing the capsid protein sequence.

44

Red Clover Necrotic Mosaic Virus Genome Organization. Z. Xiong and S. A. Lommel, Dept. of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506.

Red clover necrotic mosaic virus (RCNMV) RNA-1 directed the synthesis of 90 kDa (p90), p50, p39, p32 and RNA-2 directed the synthesis of p35, in vitro. Only p39 was immunoprecipitated by capsid protein antiserum. A full length RNA-2 cDNA clone was synthesized and a clone representing all of RNA-1, was constructed from smaller overlapping clones. Cistrons were mapped by translation of transcripts derived from a series of 5' deletion constructs from RNA-1 and -2 cDNA clones. The organization of the RCNMV genome is 5'-p32-p50-p39-3' for RNA-1 and p35 for RNA-2. The RNA-1 specific p90 is presumed to be a readthrough product of p32 into the p50 cistron. The high messenger activity for p39 from a deletion transcript representing the 3' 1.5 kb of RNA-1 indicated that RCNMV capsid protein was probably translated from a subgenomic RNA species in vivo. The RNA-2 sequence has been determined and the sequencing of RNA-1 is in progress. Sequence analysis confirms the organization described above.

45 Withdrawn

46

DIFFERENTIATING LEUCOSTOMA PERSOONII FROM L. CINCTA BY ISOZYME POLYMORPHISMS. Rupa Surve and Gerard Adams, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824- 1312

Ascocarps of the pathogens Leucostoma cincta and Leucostoma persoonii are rarely found, hence cultural characteristics of the asexual stage are used to differentiate them. However, the characteristics of Leucostoma cincta and Leucostoma persoonii are highly variable and identifications are questionable. We used cellulose acetate and starch gel electrophoresis to assay the mycelium derived from several isolates of each pathogen from Michigan and elsewhere for the activity of selected enzymes. Polymorphisms were found for a number of loci (phosphoglucoisomerase, aconitase, phosphoglucodehydrogenase, malate dehydrogenase, hexokinase, isocitrate dehydrogenase and general esterases). Electromorphs of Leucostoma persoonii differed from those of Leucostoma cincta. Within Leucostoma cincta different patterns were revealed, some of which resembled those of Leucostoma persoonii.

47

VEGETATIVELY COMPATIBLE GROUPS OF FUSARIUM MONILIFORME COLONIZING ASPARAGUS TISSUES. J. A. LaMondia and W. H. Elmer, The CT Agric. Expt. Sta., Box 1106, New Haven, CT 06504.

The population composition of Fusarium moniliforme colonizing asparagus tissue was studied by determining the vegetative compatibility groups (VCG) on three 5-yr-old field-grown asparagus plants (cv Mary Wash.). Five isolations were made on Komada's medium from each of the following symptomatic and asymptomatic tissues: feeder roots, storage roots, crown tissue and basal stem segments. Heterokaryosis of complementary nitrate-nonutilizing mutants placed 99 isolates into 13 vegetative compatibility groups (VCG). All isolates were virulent on asparagus in seedling tests. When placed into their respective VCGs, significant differences were detected among

the mean disease ratings for each VCG. The VCGs that contained the greater number of isolates were among the more virulent groups. No correlation existed between VCG composition and the sampled plants, plant part, symptomatic or asymptomatic tissue.

48

PHYTOPHTHORA SPECIES FROM REMOTE FORESTS OF WESTERN NORTH AMERICA. E.M. Hansen and P.B. Hamm, Dept of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331.

Phytophthora was recovered by baiting in the fall of the year from streams and rivers in forested areas of Alaska and Oregon. Waterways were sampled during a period of extreme drought. Several species were isolated but one resembling P. drechsleri was most common. The P. drechsleri-like fungus was the only species found in Alaska and in headwaters of streams in undisturbed watersheds of the Cascade Mountains in Oregon. Diversity of Phytophthora species increased in downstream areas adjacent to human settlement. Isolates of the P. drechsleri-like fungus were morphologically and electrophoretically similar to isolates from forest tree nurseries in Oregon, but differed from P. drechsleri isolates from other crops in California. This fungus appears to be a widespread inhabitant of remote, undisturbed, mountainous forest areas in western North America. There is no unusual mortality in native vegetation adjacent to infested headwater streams. The fungus may be endemic in these areas.

49 Withdrawn

50

ISOENZYME STUDIES OF THE GENUS *PHYTOPHTHORA*. Peter Oudemans and Michael Coffey, Dept. of Plant Pathology, Univ. of California Riverside, CA 92521.

The current systems available for classification of species of *Phytophthora* are inadequate due to the lack of "good" morphological characteristics. Isoenzyme analysis is emerging as one method which can be used to increase the number of characters useful for developing a more natural system of classification. Our objectives were to examine well described *Phytophthora* species and approach the species definition from a numerical viewpoint based on data derived from isoenzyme studies. To determine the extent of interspecific variability, 327 isolates from 14 species were examined using 15 to 20 isoenzyme loci. Comparisons based on Nei Genetic Identity calculations showed identities between 0.75 and 1.0 for most species. A minority of species, such as *P. capsici* showed lower intraspecific identity (0.5 - 1.0). Values for interspecific comparisons ranged from 0.0 to 0.2. Arguments in favor of this approach in taxonomic studies will be presented.

51

ECOLOGY OF *PHYTOPHTHORA CITROPHTHORA* ROOT ROT OF CITRUS. J. D. Sjoerdsma, J. A. Menge and E. V. L. Johnson, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Phytophthora citrophthora (*Pc*) is active as a citrus root rot pathogen from November through May in southern California. Populations of *Pc* in the rhizosphere increase with the onset of rain and cool temperatures and peak during January and February. As temperatures increase and rains cease, populations decline, and *Pc* is barely detectable from July through September. Chlamydospores, detected in soil during summer months and in dead roots, are believed to be the major survival structure. *Pc* populations increase in summer soil incubated at 6° or 16°C for 3-10 days. Rhizosphere populations of *Pc*, root length, and yield all were affected significantly by metalaxyl after two years. Treatment with metalaxyl during winter months only was as effective as a year-round treatment program.

52

UNUSUAL ISOLATES OF FUSARIUM MONILIFORME FROM SORGHUM IN KANSAS. C.J.R. Klittich and J.F. Leslie, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Fusarium moniliforme strains with an unusual appearance have been isolated from sorghum plants in Kansas. On semi-synthetic minimal or complete medium, the strains produce a yellow-brown pigmentation. Nitrate-nonutilizing mutants of these strains are also brown and often have a speckled, metallic appearance. On carnation leaf agar, most microconidia are produced in false heads, but a few short chains are present. No polyphialides or chlamydospores are produced. The brown strains do not cross with fertile testers from Fusarium section Liseola. Brown strains are isolated almost exclusively from sorghum. Over 20% of the 250 Fusaria isolated from sorghum stalks and seeds were brown strains, but only one of 230 isolates from maize was a brown strain. The brown strains belong to several different vegetative compatibility groups, and therefore are genetically distinct rather than clones of a single strain.

53

NUCLEAR NUMBER AND PATHOGENIC VARIABILITY IN ALTERNARIA CRASSA PROTOPLASTS. N. L. Brooker, D. O. TeBeest, and F. W. Spiegel, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701.

Although research has emphasized production of protoplasts from fungal mycelium, few studies have considered nuclear number within protoplasts or pathological variability of regenerated protoplast isolates. Studies were conducted with Alternaria crassa protoplasts produced after 2, 6, and 20 h incubation in Novozyme 234. Fixation in 5% glutaraldehyde and staining with DAPI showed that protoplasts produced after 2 h incubation had the highest nuclear number, 5.3 nuclei/protoplast, while 6 and 20 h cultures had 2.3 and 2.6 nuclei/protoplast, respectively. An evaluation of 88 isolates from regenerated A. crassa protoplasts on Datura stramonium showed that 85.2% of the isolates were less virulent, while 6.8% of the isolates were more virulent than the parent culture. These data suggest that changes in nuclear number and variability in disease ratings resulted from the protoplast production and regeneration process.

54

ULTRASTRUCTURAL STUDY OF SHOT HOLE OF ALMOND AND THE CAUSAL ORGANISM *STIGMINA CARPOPHILA*. J.E. Adaskaveg and J.M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

Bright-field and electron microscopy were used to examine *Stigmina carpophila* and shot hole disease of almond. The multi-celled spore of the fungus consisted of an outer wall (560 nm), an inner wall (450 nm), and a double wall (380 nm) separating each cell. Spores germinated by rupturing the outer wall. Cells of the spore did not germinate simultaneously and were capable of germinating upon separation. Hyphae penetrated leaves directly from appressoria and indirectly through stomata, and ramified within intercellular spaces of infected leaves. Diseased leaf cells were collapsed, devacuolated, and had disrupted chloroplasts. Healthy host tissue adjacent to an infection produced an abscission layer of mostly globose, thick-walled, vacuolated cells. Hyphae aggregated under the cuticle of the leaf epidermis forming pulvinate sporodochia with densely packed conidiophores that ruptured the cuticle. Conidia formed as clavate, aseptate extensions of the conidiophore and developed into mature, multi-celled spores.

55

USE OF NITRATE NON-UTILIZING MUTANTS OF VERTICILLIUM DAHLIAE IN VEGETATIVE COMPATIBILITY ANALYSIS TO DETECT PATHOTYPES FROM POTATO. T. R. Joaquim and R. C. Rowe, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

One-hundred and forty-two isolates of Verticillium dahliae collected from potato plants or soil in Ohio were assigned to vegetative compatibility groups (VCG) based on pairings of mutants unable to use nitrate (nit) (Phytopathology 77:1750). The distribution of isolates in each VCG were as follows: one isolate belonged to VCG#1, five to VCG#2, 23 to VCG#3, 32 to VCG#4, and 79 to VCG#5. Two isolates were capable of complementing testers of both VCG#4 and #5. Root dip inoculation of 10- to 15-cm-tall rooted potato sprouts with each isolate (10^5 conidia/ml) revealed that potato and soil isolates in VCG#4 were more virulent ($P<0.01$) than most isolates in VCG#2, #3, or #5, and could be differentiated as a distinct potato pathotype. Several potato isolates from other states were also assigned to VCG#4 and were similar in virulence to VCG#4 isolates from Ohio.

56

A SEROLOGICAL PROBE FOR DIFFERENTIATING TYPHULA SNOW MOLD FUNGI. A. J. Olson, D. K. Arora and N. W. Schaad, Pl. Path. Div., Univ. of Idaho, Moscow, Idaho 83843

Serology was investigated as a rapid method to differentiate

Typhula idahoensis, T. ishikariensis, and T. incarnata, the causal agents of snow mold of wheat in Idaho. Polyclonal antisera to a partially purified cell wall fraction of two isolates of T. idahoensis were prepared and tested by Ouchterlony double diffusion against four isolates each of T. idahoensis and T. ishikariensis, one isolate of T. incarnata, two isolates of low-temperature basidiomycetes, and four saprophytic soil fungi. Antigen from T. idahoensis and T. ishikariensis isolates resulted in a precipitation band of identity when tested against either antiserum. In contrast, T. incarnata resulted in a band of non-interaction and the other fungi failed to react. These results suggest that serology is a useful tool for differentiation of snow mold fungi.

57

COMPARISON OF PYTHIUM SPP. BY MYCELIAL PROTEIN ELECTROPHORESIS. W. Chen, R. W. Schneider, J. W. Hoy and M. C. Rush. Dept. of Plant Path. and Crop Physiol., La. Agric. Exp. Sta., LSU Agric. Center, Baton Rouge, LA 70803

Isoelectric focusing (IEF) and SDS-PAGE were employed to separate total mycelial proteins of Pythium spp. isolated from sugarcane and rice roots. These species include P. arrhenomanes, P. irregulare, P. catenulatum, P. dissotocum, P. myriotylum, and P. spinosum. Buffer soluble proteins were extracted from Pythium mycelia grown in a protein-free, chemically defined cultural medium. Some isolates within species showed minor differences in protein banding patterns or band intensities; however, distinctive patterns in both IEF and SDS-PAGE were recognized for each Pythium species. Isolates from different geographic locations with species showed identical or similar banding patterns. Phylogenetic analyses to deterime genetic diversity within and among species are being conducted, and the potential for development of species-specific serological assays is being investigated.

59 Withdrawn

60

DEVELOPMENT OF A POLYCLONAL ANTIBODY-BASED SERODIAGNOSTIC ASSAY FOR DETECTION OF XANTHOMONAS CAMPESTRIS PV. PELARGONII IN GERNAIUM PLANTS. M. J. Anderson and S. T. Nameth. The Ohio State University, Columbus, OH 43210.

Polyclonal antisera were produced by immunizing New Zealand white rabbits with a phosphate-buffered saline suspension of Xanthomonas campestris pv. pelargonii (Xcp). Titer of the raw antisera was determined using an indirect enzyme-linked immunosorbent assay (ELISA) in a multi-well format. A dilution of 1/8000 of the raw antisera was used to develop an immuno-gold silver staining dot blot for the detection of Xcp in geranium plants. This assay was used to determine the presence of Xcp in symptomatic and asymptomatic plants. Cross-reactivity was examined using a direct ELISA. Isolates of Xcp, various pathovars of X. campestris, unrelated bacteria, and fungal pathogens of geranium were tested. A high degree of specificity to only Xcp isolates was observed. Results indicate a rapid, more reliable technique for the detection of Xcp in geranium plants.

61

BOTRYTIS CINEREA CONIDIAL CONCENTRATION WITHIN A GERANIUM PROPAGATION GREENHOUSE. M. K. Hausbeck and S. P. Pennypacker, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Botrytis cinerea conidial concentration in a commercial greenhouse was monitored using a Burkard recording spore trap within a geranium (Pelargonium x hortorum) propagation system. In cropping cycles observed during 1986 and 1987, conidial peaks (>75 conidia/m^3 air/hour) were associated with grower activity including planting unrooted cuttings, watering, pesticide application, and shipping finished cuttings. In a 1 April to 21 April, 1986, cropping cycle, all conidial peaks were associated with grower activity. A minimum of 75% of conidial peaks were associated with grower activity when 2 benches were each monitored during a 16 December, 1986, to 28 January, 1987, cropping cycle. Results also showed that during the 7-12 day period between planting and fungicide application, newly-planted cuttings were exposed to peak conidial concentrations associated with grower activity involving nearby established cuttings.

62

DEVELOPMENT OF A DETERMINISTIC MODEL FOR GREENHOUSE ROSE POWDERY MILDEW USING MODIFIED LEAF WETNESS SENSORS. R. E. De Long and C. C. Powell, Department of Plant Pathology, Ohio State University, Columbus, OH 43210.

Greenhouse environments were continuously monitored using leaf wetness sensors (LWS) modified by coating them with acrylic latex paint. Consistently high LWS readings were commonly observed during a 9-12 hour period at night during powdery mildew (PM) epidemics. This finding led to research on the use of LWS as monitoring tools to predict PM epidemics. Laboratory environments resulting in different LWS readings were developed by placing sponges saturated with varying salt solutions within plastic boxes. Under low and high LWS conditions, conidial germination on glass slides was depressed compared to higher germination rates for midrange LWS conditions. Conidial germination and haustorial establishment on detached leaves in the lab boxes is now being studied.

64

PITCH CANKER IN CALIFORNIA. A. H. McCain, J. C. Correll, and T. R. Gordon, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Pitch canker incited by Fusarium subglutinans is causing mortality and loss in aesthetic value of Pinus radiata in landscape plantings in the Santa Cruz area of California. The California Department of Transportation has already spent $250,000 removing dead and dying trees. Many of the removed trees had bole cankers and extensive branch infections. The disease is continuing to spread and intensify in landscape plantings and eventually may threaten native stands. There are a number of reasons for believing that the disease is a recent introduction to California: the seedborne nature of the disease, limited geographic distribution, unique symptomology, and limited vegetative compatibility of California isolates of the fungus.

65

NITROGEN FORM AFFECTS ALTERNARIA LEAF SPOT OF SCHEFFLERA. J.H. Blake, Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

The effect of nitrogen form on Alternaria leaf spot of Brassaia actinophylla Endl. (schefflera) caused by Alternaria panax Whetzel was investigated. Ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 nitrate to ammonium were applied for 8 wk at

the recommended rate (1X = 0.063 g N/12.5-cm pot/wk) in test one. In the second test, ratios of 100:0, 50:50, and 0:100 nitrate to ammonium were applied at 0.5X, 1X, and 2X. Soluble salts were monitored bimonthly using a leachate method. Rates of fertilizer which resulted in leachate electrical conductivity levels between 400-3000 μmhos/cm produced good plant growth (fresh top weight and plant height). Rates above or below these reduced fresh top weight, plant height, leaflet area, leaflet count, and plant quality. Although in general nitrogen form did not affect plant growth parameters, in the second test 100% nitrate significantly reduced plant quality, leaflet area, and leaflet count. The number of lesions was affected quadratically with highest counts at the 1X rate. Plants receiving higher proportions of nitrate nitrogen had significantly more lesions than plants receiving higher proportions of ammonium nitrogen. Good quality schefflerias can be produced with higher ratios of ammonium nitrogen while reducing severity of Alternaria leaf spot.

66

TAXONOMY AND PATHOGENICITY OF A NEW GAEUMANNOMYCES SP. FROM TURFGRASS ROOTS. P.J. Landschoot, N. Jackson, and B.B.Clarke, Dept. of Plant Sciences, Univ. of Rhode Island, Kingston, RI 02881 and Rutgers Univ., New Brunswick, NJ 08903

An undescribed species of Gaeumannomyces was isolated from turfgrass roots during an investigation into the etiology of summer patch disease. The teleomorph was produced by crossing opposing mating types on sterile wheat stems in Sach's agar. Taxonomic features of this fungus conformed to Walker's emended description of the Gaeumannomyces genus. The identity of this fungus was confirmed by Mr. John Walker of the Biological and Chemical Research Institute in Rydalmere, Australia. Isolates have been obtained from Poa pratensis L., Poa annua L., Festuca sp., Cynodon sp., and Zoysia sp. in several Midwestern and Eastern States. The fungus was mildly pathogenic on P. pratensis and P. annua at 28°C. The pathogenicity of this species on other turfgrasses and cereals will be discussed.

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STRESS-RELATED PITH NECROSIS AND ROOT ROT OF *PINUS PINEA* CAUSED BY *FUSARIUM* SP. C. M. Sandlin and D. M. Ferrin, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

A species of *Fusarium* was isolated from the pith of wilted 4-month-old container-grown Italian stone pine (*Pinus pinea*) from San Diego County. Root symptoms included discoloration of the stele and sloughing of the cortical tissue. Wilt and death of seedlings occurred from June through September and coincided with periods of hot dry weather. Pathogenicity was tested by planting seeds in noninfested soil layered over soil infested with *Fusarium*. No differences in growth were evident between inoculated and control seedlings after 3 mo of growth in the greenhouse. The seedlings were then placed in a growth chamber set at 32°C for 6 h per day. Seedlings grown in infested soil wilted and died under these conditions; seedlings in noninfested soil remained healthy. Similarly, seedlings wound-inoculated at the hypocotyl wilted only when subjected to low-water stress. The pathogen most resembles *Fusarium oxysporum*; however, chlamydospores have not been observed.

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MONOCLONAL ANTIBODIES FOR DIAGNOSIS OF NECROTIC RING SPOT OF TURFGRASS. W. W. Shane and S. T. Nameth, Department of Plant Pathology, Ohio State University, Columbus, OH 43210.

Rapid diagnosis of necrotic ring spot disease of Kentucky bluegrass is hindered by the lack of definitive symptoms and culture characteristics. Verification currently requires the production of the sexual stage--a process that takes > 1 month in culture. Monoclonal antibodies were produced against mycelial homogenates of Leptosphaeria korrae (LK), strain ATCC 56289, for development of a diagnostic test. Spleen cells, from immunized Balb/c mice, were fused with NS-1 myeloma cells and supernatants from the resultant hybridoma were screened by indirect ELISA. Eleven lines testing positive against LK56289 were subcloned and tested twice. A subclone was selected exhibiting positive reactions against 11 LK strains from 5 states. There was no reaction with non-LK fungi such as Magnaporthe poae, Rhizoctonia spp., Fusarium spp., and Gaeumannomyces spp., or with healthy plant sap.

69

EFFECT OF WATER STRESS ON THE GROWTH OF MAGNAPORTHE SP. AND THE DEVELOPMENT OF SUMMER PATCH. K.E. Kackley, A.P. Grybauskas, and P.H. Dernoeden, Depts. of Botany and Agronomy, University of Maryland, College Park, MD 20742.

Summer patch of Kentucky bluegrass (Poa pratensis L.) occurs during hot, dry weather. The role of water and temperature stress on the growth of the pathogen and disease development were studied. Two isolates of Magnaporthe sp. were grown at 20, 25, 30 and 35 C on a minimal salts medium adjusted with KCl to achieve a range of osmotic potentials (-0.1 to -2.6 MPa). Growth of the isolates decreased with a decrease in osmotic potential at 20-30 C. Growth at 35 C was optimal between -1.0 and -1.5 MPa, but the growth rate was reduced compared to 20-30C. Kentucky bluegrass cultivars (S-21 and Aspen) and Poa annua L. were inoculated, and exposed to one of three water stress treatments (-0.05, -0.2, or -0.4 MPa) in growth chambers at either 20. 25, 30 or 35 C. Preliminary results revealed no relationship between disease and the soil water potentials assessed.

70

THE EFFECT OF SALINITY STRESS ON THE DEVELOPMENT OF PYTHIUM BLIGHT OF AGROSTIS PALUSTRIS. S. L. Rasmussen and M. E. Stanghellini, Dept. of Plant Pathology, University of Arizona, Tucson 85721.

Salinity stress predisposed Penncross creeping bentgrass to cottony blight caused by P. aphanidermatum at two temperature regimes. At the 25-32 C regime, complete necrosis of all inoculated plants occurred at Ec levels from 4.3 to 7.1 ds/m in 2 days, while at Ec levels of 0.5 to 2.8 ds/m death occurred within 3 days. At the 25-27 C regime, complete necrosis of all inoculated plants occurred at Ec levels from 4.3 to 7.1 ds/m within a period of 5 days. No death was observed in control or inoculated plants at an Ec level of 0.5 ds/m. Increased salinity levels apparently affected the bentgrass rather than P. aphanidermatum. Mycelial growth rate of the fungus was increased by salinity levels up to 7.1 ds/m.

71

SPATIAL AND TEMPORAL DISTRIBUTION OF PLANT PARASITIC NEMATODES IN PUTTING GREENS IN THE NEW ENGLAND REGION.
R. L. Wick, and P. M. Vittum, University of Massachusetts, 240 Beaver St., Waltham, MA 02154 and S. R. Swier, Nesmith Hall, University of New Hampshire, Durham, N.H. 03824.

During 1985, composite soil samples were taken every 2 weeks, May through August on 3 putting greens each from 11 different golf courses. Nematode population fluctuations between golf courses and between greens on the same golf courses were not consistant. During 1986 and 1987, samples were taken from new sites at 5 cm depth increments to 30 cm, every two weeks. Stratification of different species was found: Tylenchorhynchus and Criconemella were more concentrated in the upper 5 cm, Hoplolaimus was generally more distributed to 10 cm. Most of the Longidorus population occurred below 10 cm. The data indicate that no single time of the year can be recommended for "predictive" sampling of putting greens in New England and that considerable variation in the number of nematodes recovered occurs depending on sample depth.

72

CONTROL OF PHYMATOTRICHUM ROOT ROT OF WINE GRAPES IN NON-CALCAREOUS SOILS WITH AMMONIUM-THIOSULFATE APPLIED IN DRIP IRRIGATION SYSTEMS. M.W. Olsen, R.B. Hine, and G.R. Dutt. Dept. Plant Pathology, Dept. Soil Sciences, University of Arizona, Tucson, AZ 85721.

Phymatotrichum root rot, caused by Phymatotrichum omnivorum, is the most serious disease of wine grapes in Arizona and New Mexico. In southern Arizona the disease occurs at elevations as high as 1600 m in non-calcareous soils. Starting in 1980, 3 yr-old diseased vines were treated annually with ammonium-thiosulfate at the rate of 0.9 kg/sulfur/vine/yr applied through drip irrigation lines. Surveys made in 1987 indicated disease control in 2 acid soil series, Bernadino and Hathaway (pH 4-5 after treatment), but not in the calcareous series, White House (pH 7 after treatment). In in vitro studies with acid-treated Bernadino and Hathaway soils, Trichoderma viride was detected at an average of 30 cfu/g of soil, and actively parasitized P. omnivorum. T. viride was not detected in treated White House soil nor in any of the untreated soils.

73

BIOLOGICAL CONTROL OF SEEDLING DAMPING-OFF CAUSED BY Pythium ultimum BY Pseudomonas putida GR12-2 WHICH ALSO STIMULATES SEEDLING EMERGENCE DIRECTLY. R. Lifshitz, R. Zablotowicz, E.M. Tipping, S. Young, and J.W. Kloepper. Allelix

Agriculture, 6850 Goreway Drive, Mississauga, Ontario, L4V 1P1

The root-colonizing bacterial strain, Pseudomonas putida GR12-2 stimulated root elongation of canola (Brassica campestris cv Tobin) and tomato (Lycoperisicon esculentum Mill.) grown in sterile growth pouches. The bacterial seed treatment also enhanced the emergence rate of these crops in raw field soil, and improved the seedlings' emergence rate, final stand and dry weight in Pythium infested soil (50 propagules/g). However, this strain did not show any antagonistic activities against P. ultimum or other pathogenic fungi in vitro. It is speculated that a direct plant growth-promoting effect, induced by P. putida GR12-2, may provide a protection to Pythium-induced damping-off.

74

RELATIVE IMPORTANCE OF FLUORESCENT SIDEROPHORE AND PHENAZINE ANTIBIOTIC BY PSEUDOMONAS FLUORESCENS 2-79 IN SUPPRESSION OF TAKE-ALL. H. HAMDAN, L.S. Thomashow and D.M. Weller, Dept. of Microbiology, WSU, and USDA-ARS, Pullman, WA 99164

Pseudomonas fluorescens 2-79 is suppressive to Gaeumannomyces graminis var. tritici, causal agent of take-all. It produces both a yellow-green fluorescent siderophore and the antibiotic phenazine-1-carboxylate. Both compounds have been implicated as mechanisms of biological control. To determine the relative importance of each compound in take-all suppression, a set of 2-79 mutants deficient in production of the fluorescent siderophore (Flu^-Sid^-), the phenazine (Phz^-), or both factors ($Flu^-Sid^-Phz^-$) was constructed by recombinant DNA techniques. The parental strains and mutants were compared for ability to suppress take-all on wheat grown in natural or steamed Ritzville silt loam (pH 7.6) and Puget silt loam (pH 5.5). In all cases the phenazine was the predominant factor contributing to biological control by 2-79 and the fluorescent siderophore made only a small contribution.

75

ROLE OF PHENAZINE ANTIBIOTICS PRODUCED BY PSEUDOMONAS AUREOFACIENS 30-84 IN TAKE-ALL SUPPRESSION. L.S. Pierson III and L.S. Thomashow, USDA-ARS, Johnson Hall, WSU, Pullman, WA 99164

Pseudomonas aureofaciens 30-84 suppresses Gaeumannomyces graminis var. tritici (Ggt), causal agent of take-all in wheat. Strain 30-84 produces phenazine-1-carboxylate and 2-hydroxy-phenazine-1-carboxylate. Previous work with P. fluorescens 2-79 showed that phenazine-1-carboxylate is involved in suppression of take-all. Genetic studies were undertaken to determine if phenazines are also important in take-all suppression by 30-84. Tn5 mutants defective in phenazine production were noninhibitory to Ggt in vitro and were less suppressive of take-all in greenhouse tests. Complementation of mutants by a 30-84 DNA library fully restored phenazine production and fungal inhibition. These data indicate that phenazines are involved in take-all suppression by 30-84 as well as 2-79, and constitute additional evidence for the importance of these antibiotics in biocontrol of take-all.

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DETECTION OF PHENAZINE ANTIBIOTICS PRODUCED BY PSEUDOMONAS SPP. IN SOIL. L.S. Thomashow, L.S. Pierson III, D.M. Weller, and R.F. Bonsall, USDA-ARS, Johnson Hall, WSU, Pullman, WA 99164

Pseudomonas fluorescens 2-79 and P. aureofaciens 30-84 produce phenazine antibiotics inhibitory in vitro to Gaeumannomyces graminis var. tritici, causal agent of take-all. To obtain direct evidence for the production of phenazines in the rhizosphere, wheat seeds were treated with 2-79, 30-84, or phenazine-nonproducing (Phz^-) Tn5 mutants and sown in steamed or natural soils infested with Ggt. The benzene-soluble fraction of washings from roots of seedlings was then analyzed by HPLC and UV-VIS spectroscopy. Substances comigrating with and spectrally indistinguishable from phenazines produced in vitro were present in washings from roots colonized by Phz^+ strains, but not in those from Phz^- strains or noninoculated controls. These results indicate that phenazine antibiotics are produced in the rhizosphere of wheat and are consistent with previous evidence in support of their importance in take-all suppression.

77

INFLUENCE OF BACTERIAL SURFACE PROPERTIES ON COLONIZATION OF WHEAT ROOTS BY BIOCONTROL PSEUDOMONADS. David S. Heron and David M. Weller, USDA-ARS, Pullman, WA 99164-6430

Pseudomonas fluorescens strains 2-79 and R4a-80 suppress the take-all pathogen, Gaeumannomyces graminis var. tritici (Ggt) when applied to wheat seed before sowing, but root colonization by the bacteria is often inconsistent. Bacterial surface properties may influence root colonization ability. Two colony types of the above strains were selected in vitro and tested for ability to colonize seminal roots of wheat in natural soil with or without Ggt. Approximately 10^8 cfu of the bacteria were applied per seed. Seven days after sowing, populations of the introduced bacteria were determined on the segment of each seminal root extending from 3 to 5 cm below the seed. In soil with no Ggt, the frequencies of root colonization were 78% and 48% for 2-79 rough and smooth colony types, respectively, and 60% and 40% for R4a-80 mucoid and nonmucoid types, respectively. Similar trends were observed in experiments using soil amended with Ggt.

78

CONTROL OF RHIZOCTONIA CROWN AND ROOT ROT BY BIOCONTROL AGENTS COLONIZING SUGAR BEET SURFACES. L. J. Herr, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691

Greenhouse plants (≥6-wk-old) treated with binucleate Rhizoctonia spp. and Laetisaria arvalis biocontrol agents (BA), in soil either non-infested or infested with Rhizoctonia solani (RS), were assayed for BA and RS colonization by removing five 9-mm-dia disks from each crown and five disks vertically, crown to lower root, and plated on water agar (WA). Effective BA were isolated from 60-96% of crown disks, ineffective 0-27%; and effective BA were isolated in decreasing numbers vertically from crown to lower root, whereas ineffective BA were either not isolated or only from crowns. Disease ratings correlated negatively with BA and positively with RS isolations. Single 15-cm-dia disks from BA-treated crowns were not inhibitory to RS on WA. Further, when these disks were removed and re-plated on WA, RS grew from many disks indicating BA did not exclude RS colonization of disks on the antagonism test plates.

79

MICROBIAL ACTIVITY AND BIOMASS IN CONTAINER MEDIA PREDICTING SUPPRESSIVENESS TO DAMPING-OFF CAUSED BY PYTHIUM ULTIMUM. W. Chen, H. A. J. Hoitink, and A. F. Schmitthenner, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Predictive guidelines, based on general microbial activity and biomass, were developed for the formulation of container media suppressive to Pythium damping-off. Disease severity established in bioassays correlated negatively with microbial activity ($r=-0.78$, $P<0.01$), based on the rate of hydrolysis of fluorescein diacetate, and with microbial biomass ($r=-0.83$, $P<0.01$), based on extractible phospholipid phosphate content in the container media. A preliminary mathematic model for predicting Pythium damping-off severity, based on both microbial activity and biomass, was developed. The model was substantiated by determining Pythium root rot severity of various floricultural crops produced in a range of commercial container media.

80

INHIBITION OF FUSARIUM OXYSPORUM F. SP. APII IN VITRO AND IN VIVO WITH FUSARIMYCIN-PRODUCING PSEUDOMONAS FLUORESCENS STRAIN NP77A. L Ziegle and M. Davies Correll. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA 94608.

Pseudomonas fluorescens strain Hv37a has been shown to significantly inhibit *Pythium ultimum* infection and disease of seed and roots on cotton, celery and other crop seedlings. Strain NP77R is a rifampicin-resistant derivative of an NTG mutant of Hv37a found to have enhanced in vitro inhibition of *P. ultimum* and novel inhibiton of many *Fusarium oxysporum*, in particular f. sp. *apii*. The anti-Fusarium fraction has been tentatively termed fusarimycin for convenience. In paired greenhouse tests, the colonization of seed and roots by NP77R and Hv37aR2 did not differ significantly. NP77R was found to significantly reduce root infection by *F. oxysporum* f. sp. *apii* as compared to non-treated or Hv37aR2-treated celery. In separate tests, it was found that reducing *P. ultimum* infection, alone, significantly reduced *F. oxysporum* f. sp. *apii* infection of roots. Understanding and directing fusarimycin production in the rhizosphere will be required before significant reductions in Fusarium yellows of celery could be expected.

81

PREFERENTIAL FEEDING OF MYCOPHAGOUS COLLEMBOLA (INSECTA) ON RHIZOCTONIA SOLANI IN THE PRESENCE OF KNOWN FUNGAL BIOCONTROL AGENTS. R. T. Lartey, E. A. Curl, and C. M. Peterson, Departments of Plant Pathology and Botany and Microbiology, Auburn University, AL 36849.

Fungus-grazing collembolan insects (Proisotoma minuta and

Onychiurus encarpatus) have been cited as natural biological control agents which suppress Rhizoctonia solani. The biocontrol efficacy is further enhanced when insect populations are combined with known fungal biocontrol agents Trichoderma harzianum, Gliocladium virens, or Laetisaria arvalis. Laboratory feeding tests showed that this apparent "compatibility" between insects and beneficial fungi was due to food preference for the pathogen along with insect aversion to toxic metabolites produced by T. harzianum and G. virens. There was a highly significant migration of both insect species toward colonies of the pathogen and away from the fungal agents. Egg production and insect populations were greater in presence of R. solani.

82

INDUCTION OF INCREASED BENOMYL RESISTANCE IN A FUNGUS DEMONSTRATING POTENTIAL FOR NEMATODE BIOCONTROL.
S. L. F. Meyer, R. N. Huettel, and R. M. Sayre. Nematology Laboratory, USDA-ARS, Beltsville, MD 20705.

Selected fungi were assayed for antagonism to soybean cyst nematode eggs and for ability to grow in the presence of the fungicide benomyl. Further experiments were then conducted on one fungal species to induce mutants with increased benomyl resistance. To induce the mutants, conidial suspensions spread on potato dextrose agar containing benomyl were irradiated with ultraviolet light. The conidia were kept in the dark for 4-8 days following UV exposure. Colonies that grew more quickly than the majority of irradiated colonies were subcultured. To minimize genetic variability, single spore isolates were made from each of the fast-growing strains. The mutants are being studied to ascertain which are the most resistant to benomyl, whether the increased resistance is a stable character, and whether antagonism to nematode eggs has been affected.

83

A SYSTEM FOR SCREENING *TRICHODERMA* SPP. FOR POTENTIAL BIOCONTROL OF *PHYTOPHTHORA CACTORUM*. V. L. Smith, W. F. Wilcox, and G. E. Harman. Depts. of Plant Pathology and Horticultural Science, N. Y. State Agr. Exp. Sta., Cornell Univ., Geneva, 14456

Over 70 isolates of *Trichoderma* spp. were screened for ability to reduce crown rot of apple caused by *Phytophthora cactorum*. Candidate isolates were tested for ability to grow at 11 C, to survive and grow in saturated soil, and to sporulate on or inhibit growth of *P. cactorum in vitro*. Optimal concentration in the soil mix of *P. cactorum* was determined. Approx. 12 promising isolates were tested *in planta* on McIntosh apple seedlings, woody Mahaleb cherry seedlings, and MM106 apple rootstock liners. Plants were grown in soil mix containing both pathogen and biocontrol candidate; control was assessed after 72 hr flooding periods and subsequent infection by *P. cactorum*. To determine the efficacy range of isolates most active against *P. cactorum*, isolates were tested *in planta* against *Fusarium graminearum* (on wheat), *Rhizoctonia solani* (on radish), and *Sclerotium rolfsii* (on snapbean and cucumber). The system developed will aid in identification of isolates of *Trichoderma* with biocontrol potential against *P. cactorum* and other pathogens.

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COLONIZATION AND POPULATION DYNAMICS OF BACILLUS ISOLATE B153-2 -2 ON SOYBEAN ROOTS AND RHIZOSPHERE. Z. Liu and J. B. Sinclair, Dept. Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana, IL 61801-4709.

A potential biocontrol agent for Rhizoctonia solani on soybeans, Bacillus B153-2-2, isolated from soybean and containing a rifampicin-resistance marker was studied in the greenhouse & field. Colonization on soybean roots extended to 20 cm below and 5 cm above the soil line using bacterium-coated seeds. The highest population was 1.2×10^8 cfu/g fresh root at 5 cm. In the field the bacterium was recoverable at a distance at least 30cm horizontally and 40cm vertically from the application site. Maximum rhizosphere population recovered was 4×10^7 cfu/g soil. The population of B153-2-2 was high at the beginning and decreased toward the end of the growing season. It then remained stable and similar to that of the natrually-occurring Bacillus sp. throughout the winter. A population base of 8×10^4 cfu/g soil was needed for survival. The presence of R. solani did not affect colonization of B153-2-2 on roots or in the rhizosphere.

85

THREE-DIMENSIONAL MODELS FOR IDENTIFICATION OF BENEFICIAL RHIZOBACTERIA. A. J. Caesar, D. C. Hildebrand, O. C. Huisman, and M. N. Schroth. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Three-dimensional models of the phenotypic relationships of bacteria were prepared for use in the identification of beneficial rhizobacteria. Known beneficial rhizobacteria and random bacteria isolated from rhizospheres and nonrhizosphere soils were assayed for 70 phenotypic properties including standard biochemical tests, CH_2O utilization patterns, and such traits as PO_4 solubilization, in vitro antibiosis, and siderophore production. Three separate models consisting of strains of enteric spp., gram-positive, and Pseudomonas spp., distinguished beneficial strains from random rhizosphere and soil strains. Potentially important traits were determined by how they clustered or were associated with beneficial strains within a model. The models indicate that beneficial strains can be selected from natural enteric and Pseudomonas spp. populations by in vitro testing for key characters.

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IN VITRO INHIBITION OF *FUSARIUM OXYSPORUM* FF. SP. MICROCONIDIA WITH WATERMELON XYLEM EXUDATES. C. L. Biles and R. D. Martyn, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843.

Previous experiments detected differential xylem fluid proteins among watermelon (*Citrullus lanatus*) cultivars. Therefore, tests were conducted to determine if xylem exudates were inhibitory to the germination and growth of *Fusarium oxysporum f. sp. niveum* (FON) microconidia, causal agent of Fusarium wilt. Inhibitory effects of xylem exudates from cultivars differentially susceptible to FON and watermelon plants previously inoculated with various *F. oxysporum* isolates were compared. Results indicated that xylem fluid contained a highly inhibitory component(s) that was independent of the resistance status of the cultivar or previous inoculation with virulent or avirulent FON races. Incubation with proteinase K and lyzozyme suggested that the active component had proteinaceous properties with an oligosaccharide moeity. The xylem fluid mycotoxin(s) appears to act as a general defense mechanism against Fusarium wilt which virulent isolates can overcome upon infection.

87

ROLE OF CELL WALL MODIFICATIONS IN THE RESISTANCE OF BEAN LEAVES TO THE COWPEA RUST FUNGUS. C. Perumalla, and Michele C. Heath . Botany Department, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

The pre-inoculation application of inhibitors of various cellular processes revealed that the inhibition of callose deposition on mesophyll cell walls, without the inhibition of the accompanying deposition of silica and autofluorescent material, did not allow the cowpea rust fungus to form the first haustorium in the nonhost bean (Phaseolus vulgaris). Inhibition of silica deposition, with or without the inhibition of wall autofluorescence, usually increased the incidence of haustorium formation provided that the inhibitors used did not seriously affect fungal growth. Inhibitors that prevented wall autofluorescence invariably inhibited silica deposition, but not vice versa. The data support a significant role for silica deposition in preventing haustorium formation in this nonhost interaction.

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NECTRIA HAEMATOCOCCA MACROCONIDIA ATTACH TO PLANT SURFACES. **M. J. Hickman, and L. Epstein, Department of Plant Pathology, University of California, Berkeley, CA 94720.**

Spore attachment can be the first step in pathogenesis. We monitor adhesion of [^{14}C]-labeled macroconidia of Nectria haematococca mating population I (anamorph, Fusarium solani f. sp. cucurbitae race I). Macroconidia adhere to both hydrophobic and hydrophilic surfaces. Test surfaces include zucchini (Cucurbita pepo) hypocotyls and fruits (the usual infection courts), zucchini leaves and roots, glass, and polystyrene. On polystyrene, the attachment process occurs within 60 min and has two stages. The first stage requires active metabolism (adhesion is inhibited by 10 mM sodium azide) and is temperature dependent (adhesion occurs at 24 C, but is totally inhibited at 4 C). In the second stage when the spores bind to the substratum, adhesion is relatively temperature independent; it occurs at 24 C and at 4 C, although at a slower rate than at 24 C. Proteinase K (500 ug/ml), but not heat denatured Proteinase K, significantly reduces adhesion, which suggests that an extracellular protein is required for adhesion of macroconidia.

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MULTICOMPONENT ELICITOR OF PHYTOALEXIN BIOSYNTHESIS FROM PSEUDOMONAS CORRUGATA. D.L. Gustine, R.T. Sherwood, and F.L. Lukezic, USDA-ARS, Pasture Research Lab., and Dept. of Plant

Pathology, Penn State University, University Park, PA 16802.

Metabolites of P. corrugata isolated from 24-hr-old glucose-salts liquid culture medium elicited phytoalexin (medicarpin) biosynthesis (24-hour stimulation) in Ladino white clover callus. Active components were purified by the following steps: 80% ethanol precipitation of nutrients and inactive metabolites from the centrifuged medium, removal of components greater than 5,000 daltons by dialysis, and fractionation by preparative reversed-phase high performance liquid chromatography. Each of four fractions from HPLC did not elicit appreciable quantities of medicarpin in callus, but fraction 1 combined with fraction 4 elicited high concentrations of medicarpin. Elicitor activity was concentration dependent. Both active fractions were acidic in solution, but their elicitor activity was not dependent on pH. Fraction 1 was positive for reducing carbohydrate.

90

LIGHT AND SCANNING ELECTRON MICROSCOPY OF HYPERSENSITIVE LESIONS ASSOCIATED WITH PROTECTION OF PHASEOLUS VULGARIS FROM RHIZOCTONIA SOLANI INDUCED BY AN AVIRULENT, BINUCLEATE, RHIZOCTONIA-LIKE FUNGUS. C.G. Eayre and E. Echandi, Plant Pathology Dept., North Carolina State University, Raleigh, NC, 27695.

Colonization of bean (Phaseolus vulgaris) by an avirulent, binucleate, Rhizoctonia-like fungus (BNR) and R. solani was compared by light and scanning electron microscopy. Protection from Rhizoctonia solani induced in bean by BNR is associated with small hypersensitive lesions. BNR did not form infection cushions, but penetrated between epidermal cells, while R. solani formed branched infection cushions. BNR penetrated intercellularly through 2 to 3 cell layers, while R. solani penetrated intracellularly, up to 12 cell layers. Host cell collapse occurred in response to BNR. Colonization of the plant surface by BNR was sparse and only on hypocotyls at the soil surface, while colonization by R. solani was dense, extensive, and on roots, stems, hypocotyls, cotyledons, and leaves.

91

ACREMONIUM COENOPHIALUM DOES NOT EFFECT RHIZOCTONIA ZEAE GROWTH EITHER IN VITRO OR IN VIVO. K. D. Gwinn and S. C. Bernard. Department of Entomology & Plant Pathology, University of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1071.

Earlier reports have indicated that culture filtrates of Acremonium coenophialum inhibit growth of fungi. Rhizoctonia zeae was inhibited in vitro; however, addition of nutrients to filtrates restored growth to levels found in controls. Growth habit of R. zeae differed in filter sterilized and autoclaved filtrates, but dry weight was not significantly different. A. coenophialum was detected in fescue plants on three separate occasions by PAS-ELISA. Following artificial inoculation with R. zeae, disease severity did not differ significantly between plants with (E+) or without (E-) A. coenophialum. No significant differences were found between growth of R. zeae on malt agar amended with water extract from E+ and E- plants.

92 Withdrawn

93

LACCARIA BICOLOR ENHANCES PHENOLIC INFUSION OF CORTICAL CELL WALLS OF DOUGLAS-FIR PRIMARY ROOTS AND INHIBITS PENETRATION BY *FUSARIUM OXYSPORUM*. N. E. Strobel and W. A. Sinclair, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

The principal site of penetration of Douglas-fir primary roots by *Fusarium oxysporum* (FO) was the root tip region (distal 5mm). The walls of cortical but not stelar parenchyma cells in this region became infused with yellow-to-brown materials as a prepenetration response to the pathogen. Histochemical tests suggested that these wall infusions were formed by the polymerization of flavanols by FO-induced peroxidase activity. Roots exposed to but not infected by the ectomycorrhizal fungus *Laccaria bicolor* (LB) for 1 wk prior to challenge with FO developed more extensive wall infusions in response to FO and were less frequently penetrated by FO than were roots not exposed to LB. Root protection by LB was thus associated with an enhancement of cortical cell wall phenolic infusion in response to FO and may result from greater resistance of infused walls to enzymatic degradation. Root protection by LB was local rather than systemic in nature.

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USE OF DETACHED LEAVES IN VITRO FOR SELECTION OF RESISTANCE TO VENTURIA INAEQUALIS. L. M. Yepes and H. S. Aldwinckle, Dept. Plant Path., Cornell Univ., NYSAES, Geneva, NY 14456.

A simple method was developed to screen apple cultivars for resistance to *Venturia inaequalis*, using detached leaves from *in vitro* propagated shoots. Leaves of six cultivars with different levels of resistance to *V. inaequalis* were placed in petri plates containing water agar or wet filter paper, and were inoculated with a conidial suspension. Susceptible cultivars showed chlorosis three weeks after inoculation, and became necrotic after five weeks. Leaves from resistant cultivars showed no symptoms. Studies using fluorescence and electron microscopy indicated that the reaction of resistant and susceptible cultivars *in vitro* mimicked reactions in the greenhouse and field. The simplicity of this assay allows not only selection from much larger plant populations than is currently possible with greenhouse and field techniques, but also makes possible the identification of mutants for scab resistance in apple. This assay can also be useful for basic studies on host-pathogen interaction.

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TEMPERATURE AND LIGHT REGIME EFFECTS ON INCUBATON TIME AND LEAF MOVEMENTS OF SOYBEAN SEEDLINGS INOCULATED WITH TOBACCO STREAK VIRUS (TSV). J. Fetzer, B. Kennedy and R. Denny, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Seven day old soybean seedlings (Glycine max) were grown in controlled environments and mechanically inoculated with TSV every 4h for 20h. Following inoculation, plants were subjected to regimes of 25 C in 12h light followed by 12h dark (LD25), 25 C and continuous light (LL25), LD30 or LL30. Leaf movement and time of symptom appearance (crook at apex) were measured every 4h for 120h. Symptoms appeared sooner at 30C (47h in 50% of the plants) than at 25C (74h) and 5-8h sooner in LL than in LD. Time of inoculation had no significant influence on time of symptom appearance in continuous light (LL); however, plants grown in LD exhibited symptoms 4-16h sooner if inoculated at the transition between LD or DL. LL30 conditions resulted in a relatively rapid onset of symptoms; non-inoculated seedlings in this environment had reduced chlorophyll content and reduced amplitude of leaf movement.

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DIFFERENCES IN THE NUMBER OF INCLUSION BODIES PRODUCED BY MILD AND SEVERE CITRUS TRISTEZA VIRUS STRAINS IN FOUR CITRUS HOSTS. R. H. Brlansky, D. S. Howd, C. L. Davis and R. F. Lee, University of Florida, IFAS, CREC, 700 Experiment Station Rd., Lake Alfred 33850

Inclusion bodies produced in four susceptible citrus hosts with five biologically different strains of citrus tristeza virus (CTV) were observed using an azure A staining procedure. In two of the hosts, the number of the inclusions produced by the two more severe strains was consistently and significantly higher than those produced by the milder strains of the virus. There were no significant differences in the virus titers as related to either the severity of the virus strain or the numbers of inclusions. No differences were observed in the morphology of the inclusions produced in the various hosts.

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NON-HOST RESISTANCE: MOLECULAR ANALYSIS OF A GENE FROM XANTHOMONAS CAMPESTRIS PV. VESICATORIA DETERMINING RESISTANCE IN NON-HOST SPECIES AND A GENETIC ANALYSIS OF THIS RESISTANCE IN BEAN. Maureen C. Whalen and Brian J. Staskawicz, Department of Plant Pathology, University of California, Berkeley, 94720.

Xanthomonas campestris pathovars have highly specific relationships with particular hosts. We are studying the molecular basis of host range specificity in X. c. pv. vesicatoria tomato race 1 (XcvT). The inability of XcvT to cause disease on plant species other than tomatoes can be controlled by single genes. We have isolated a gene avrRxv from XcvT that upon mobilization into the bean pathogen X. c. pv. phaseoli confers avirulence activity on bean. The resistance in bean associated with avrRxv segregates as a single, incompletely dominant gene Rxv. avrRxv also confers avirulence activity in several other X. campestris pathovars: glycines on soybean, vignicola on cowpea, alfalfae on alfalfa, malvacearum on cotton, and holcicola on corn.

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CHITINASE AND β-GLUCANSE-RELEASED CHITOSAN INITIATES COMPLEX EVENTS IN FUSARIUM SOLANI FORMA SPECIALES (F.s.f.sp.)/PEA INTERACTIONS. L. A. Hadwiger, R. Buell, D. Christian, D. Horovitz, D. F. Kendra and S. Victory. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

In vitro, pure pea β-glucanase and chitinase release chitosan from F.s.f.sp. Chitosan, DNAase and pectic enzymes are released from F.s.f.sp. in the interaction with pea. These components may function in subsequent cytological developments. Cells in a small (resist. react.) or larger (susc. react.) radius of pea cells adjacent to a spore first experience loss of DNA-specific staining and then viability staining decreases. Subsequently there is increased callose accumulation, hypersensitive yellow-green coloration, lignin accumulation and tissue softening. A scenario of (1) gene activation by chitosan, (2) DNA complexing, nuclear distortion, and destruction and (3) lesion restriction (or its absence) via cellular deposition will be discussed.

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MAJOR GENE CONTROL AND DISEASE RESISTANCE RESPONSE GENES (DRRG). Lee A. Hadwiger, Chin C. Chiang, Catherine Daniels. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430

Disease resistance response genes (DRRG) of peas are differentially expressed in pea lines containing single dominant "Mendelian traits" (MT) in incompatible interactions, but are probably not MTs (Daniels, et al. Plant Mol. Biol. 8:309). We propose the MT control may involve the following features: (1) major structural changes in the host nucleus, in addition to the cis elements and trans transcriptional factors operating in regions adjacent to the DRRG structural gene; (2) special chromatin sites (e.g., attachments to nuclear membrane, nuclear matrix, lamina and chromosomal translocations; and (3) the chromosomal loop secured to a topoisomerase containing matrix. Elicitor action and the topoisomerase sites discovered in a DRRG 5' sequence will be discussed.

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THE EFFECT OF PLANT DENSITY AND KERNEL SINK LEVEL ON STALK ROT AND STRENGTH OF MAIZE. C. A. Martinson, D. C. Foley, and C. Marton, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011, Agricultural Research Institute, Martonvasar, Hungary.

Stalks of five lines grown as test crosses at three plant densities (87,719, 65,789, and 52,632 plants/ha) were measured for rot and strength. Kernel sink level was regulated by exposing silks for 2 days or indefinitely after silk emergence which gave 467 and 547 mean kernels/plant, respectively. Stalk rot was measured by parenchyma rot and stalk softness (crown rot). Stalk strength was measured by tensioning a rind section. Plant yield did not decrease when kernel number decreased as plants with fewer kernels had heavier kernels. Number of kernels did not affect rind strength but maximum fiber strength, an inverse measure of stalk rot severity, decreased with increased kernel numbers. Parenchyma rot and crown rot were more severe in plants with the greater numbers of kernels. Thus, number of kernels was more related to stalk rot severity than yield.

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IMAGE ANALYSIS AND VISUAL ASSESSMENT: A COMPARISON OF TWO METHODS FOR EVALUATING DISEASE REACTION OF CORN TO FUSARIUM STALK ROT. Laura R. Todd, Jacques Seed Co. and University of Minnesota, and T. Kommedahl, Univ. Minnesota, St. Paul.

Two field-grown hybrids (Zea mays) were evaluated for reaction to the stalk rot fungi F. moniliforme, F. graminearum and F. proliferatum using a visual assessment scale (1-4) and image analysis. Both rating methods showed hybrid W153RxA619 to be more susceptible than A632xA619 to all three pathogens. The difference between hybrids was greater when the image analyzer was used. Significant differences (P = 0.05) between the disease reactions caused by the three pathogens were found only between ratings made with the image analyzer: F. moniliforme (10.2% disease), F. graminearum (9.6%) and F. proliferatum (8.0%). F. proliferatum has recently been differentiated from F. moniliforme; this is the first indication that the two fungi may differ in pathogenicity. The image analyzer provides a more accurate means of evaluating corn for susceptibility to Fusarium stalk rot as compared to visual ratings.

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FUNGICIDE SEED TREATMENT EFFECTIVENESS FOR TILLETIA TRITICI CONTROL. Ervin Williams, Jr., Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-0285.

Common bunt, incited by Tilletia tritici, has reemerged as a significant production problem in southwestern Oklahoma. Commercially available fungicides have been inconsistent in providing adequate control at labeled rates in field tests. Tests conducted with inoculated seed in 1985-86 and 1986-87 showed that surface seed treatments of triadimenol and an experimental benzimidazole (WECO 965-85) significantly reduced common bunt infections compared to 13 and 34% in untreated check, respectively. These materials were tested in combination with TCMTB. Bunt control has been erratic for TCMTB at labeled rates. Evaluations were compared with PCNB (23.7%) as a standard. Triadimenol was also tested in combination with captan. Although triadimenol appears to be an excellent fungicide for bunt control, its phytotoxicity will limit applications to low rates (0.05-0.24g ai/k seed). Maneb at 0.8g ai/k provided total bunt control in the 1986-87 test.

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EFFECT OF DEFOLIATION AND *PUCCINIA RECONDITA* ON YIELD AND PREDICTION OF YIELD LOSSES IN WINTER WHEAT. K.V. Subba Rao, X.B. Yang, J.P. Snow, and G.T. Berggren. Dept. of Plant Path. and Crop Phys., La. Ag. Expt. Sta., LSU Ag. Center, Baton Rouge, LA 70803.

The contribution of each leaf to tiller grain yield of McNair 1003 and the effect of leaf rust on each leaf were determined by defoliation and inoculation. Treatments included removing/retaining F, F-1, F-2, and F-3 leaves in 10 combinations each from diseased and control tillers. Grain yield/tiller was not correlated linearly with the number of leaves retained/tiller indicating compensation among leaves. Maximum yield loss due to rust was 27%. Tiller yield was predicted by the model $Y_{ij} = B_0 + B_1 F_i + B_2(F\text{-}1)_i + B_3(F\text{-}2)_i + B_4(F\text{-}3)_i + E_{ij}$. The partial regression coefficients were the absolute contribution of stem and leaves towards the tiller yield. Relative AUDPC of each leaf was integrated into the model and the resultant partial regression coefficients were statistically not different in diseased and control conditions, indicating the additive effect of leaf rust. The model indicated only F, F-1, and F-2 and rust on these leaves were important in the prediction of yield losses.

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EFFECTS OF MANCOZEB IN THE MANAGEMENT OF GLUME BLOTCH OF WINTER WHEAT UNDER INTENSIVE AND CONVENTIONAL CULTIVATION STRATEGIES IN MARYLAND. C. E. Orth and A. P. Grybauskas, Dept. of Botany, University of Maryland, College Park, MD 20742.

Field experiments were conducted in Maryland in the 1986-87 and and '87-'88 growing seasons to set guidelines for managing glume blotch epidemics caused by Septoria nodorum (Berk). Winter wheat (Triticum aestivum L.) cultivars, Coker 916 and Florida 302, were grown under contrasting schemes, 20 cm row spacing with 53 Kg N ha^{-1}, verses 10 cm row spacing with 106 Kg N ha^{-1}. Different epidemics were generated by varying the inoculum level. Foliar disease severity ranged from 3-50% at growth stage 11 and was greatest on Florida 302. Fungicide application at growth stage 10 reduced disease severity from 50 to 18% at the highest inoculum level. Suppression of disease severity of the spikes was significantly greatest on Coker 916. Yield losses were not closely correlated to disease severity, but highest yields were obtained under intensive management using fungicides.

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EPIDEMICS OF WHEAT SOIL BORNE MOSAIC VIRUS IN FLORIDA ASSOCIATED WITH TWO CULTIVARS OF WHEAT. T.A. Kucharek, M. Griggs, R.E. Cullen, and S.R. Christie, Plant Pathology Dept. University of Florida, Gainesville, FL 32611. Second author P.O. Box 7154, Pensacola, FL 32514.

Epidemics of wheat soil borne mosaic virus (WSBMV) occurred in wheat in two adjacent counties in Florida from 1986 to 1988. The epidemics were limited to the recently popular cultivars, 'Florida 301' and 'Florida 302'. Grain yield of Florida 301 was reduced 32.7% ($P = 0.05$) at a site infested with WSBMV. In replicated field tests, Florida 301, Florida 302 and 'Coker 797' were the most susceptible based upon foliar symptoms while 'Hunter', 'Coker 983', and 'McNair 1003' were among the most resistant. Three cultivars of triticale were also susceptible. A correlation of -0.76($P = 0.01$) occurred between severity of symptoms and grain yields for 13 pedigrees of wheat. The fungal vector, Polymyxa graminis, was found in roots of susceptible and resistant cultivars. Rigid rods and cytoplasmic, amorphous inclusions were found consistently in leaf tissues of susceptible cultivars but not in highly resistant cultivars.

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THE INTERACTION OF MOISTURE AND DRYLAND ROOT ROT PATHOGENS ON BARLEY PRODUCTION AND DISEASE SEVERITY. W. E. Grey, D. E. Mathre, Dept. of Plant Pathology, and R. Engel, Dept. of Plant and Soil Science, Montana State University, Bozeman, MT 59717.

The interaction between varying moisture regimes (MR) and root pathogens, Cochliobolus sativus and Fusarium culmorum, as measured by 'Clark' spring barley grain yield and disease severity for 1986 and 87 growing seasons, was examined to explain increased crop losses under low moisture and uniform inoculation. The MR did not affect disease severity in inoculated and non-inoculated treatments. Plant emergence and harvestable tillers were reduced by C. sativus in 1986 and 87. In 1987, grain yield losses in C. sativus inoculated plants occurred in MR receiving < 209 mm growing season moisture (GSM), whereas in 1986, the plants compensated for reduced tillering by increased kernel weight. Grain yield was not reduced by F. culmorum. The timing of moisture during plant growth and the total GSM can influence the ability of barley to tolerate dryland root rot.

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ANALYSIS OF BLAST EFFECTS ON RICE GROWTH AND YIELD. C. Q. Torres and P. S. Teng, IRRI, P.O. Box 933 Manila, Philippines.

Leaf and panicle blast epidemics of Pyricularia oryzae were generated in two lowland field experiments at Los Banos, Philippines during 1987 by manipulating leaf-wetness duration, inoculum dose, time of inoculation, and application of tricyclazole. Blast severity, plant height, and biomass were measured weekly. Yield components and percent incidence panicle blast were determined at harvest. Path coefficient analysis showed that leaf blast had relatively stronger negative effects on plant height than on leaf, stem, and panicle biomass. Panicle blast had a stronger effect on panicle biomass than did leaf blast. Two equations were developed for estimating percent yield loss (Y) from percent leaf blast severity (L) and percent panicle blast incidence (P), i.e. $Y = 0.2101 + 1.0124L + 0.5102P$, $R^2 = 0.80$ and $Y = 13.2983 + 1.7717L + 0.4683P$, $R^2 = 0.73$.

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VARIATION IN PHYTOTOXIN PRODUCTION BETWEEN ISOLATES OF PYRENOPHORA TRITICI-REPENTIS. D. A. Brown and R. M. Hunger, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Seven isolates of P. tritici-repentis from wheat (Triticum aestivum) in Oklahoma and four isolates from smooth bromegrass (Bromus inermis) in North Dakota were evaluated for production of Ptr-phytotoxin in vitro. This phytotoxin elicits the chlorotic reaction on wheat foliage associated with tan spot disease caused by P. tritici-repentis. Ptr-phytotoxin was obtained from culture filtrates of the isolates and production was assessed by a seedling bioassay against wheat cultivars. The variation in toxin production observed between the 11 isolates was consistent between cultivars tested. Growth curves were established to determine whether observed variation was due to differential growth rates of the isolates in liquid culture. Phytotoxic activity per mg fungal dry weight was used to compare the specific activities of Ptr-phytotoxin produced by each of the isolates.

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VARIATION IN VIRULENCE AND AGGRESSIVENESS WITHIN THE *PYRENOPHORA TRITICI-REPENTIS* POPULATION IN NEW YORK. A. M. C. Schilder and G. C. Bergstrom, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Three-week-old plants of twelve wheat cultivars differing in resistance were inoculated with single-conidium isolates of *Pyrenophora tritici-repentis* (incitant of tan spot of wheat), collected from the predominant wheat-growing areas of New York, as well as from Maryland and Ontario. Analysis of variance showed significant isolate, cultivar, and isolate x cultivar effects on lesion length and percentage leaf area necrotic. Similar variation was observed after inoculating detached wheat seedling leaves with spore suspensions of the fungus. Isolates were placed into several virulence groups by cluster analysis. Reactions of the differential wheat cultivars to culture filtrates of isolates from different virulence groups will be discussed.

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AN IMPROVED METHOD FOR FIELD INOCULATION OF WINTER WHEAT WITH TILLETIA CONTROVERSA. H. S. Fenwick, S. V. Jones, and D. J. Eschen. Pl. Path. Div., Dept. of Plant, Soil, & Entomological Sci., Univ. of Idaho, Moscow, ID 83843.

Teliospores of T. controversa were preconditioned (PC) for germination by incubating them 2, 4, 6 or 8 wk in soil extract broth (SEB) under illumination at 5 C on an orbital shaker. They were then sprayed onto the soil surface of 1-m^2 replicated plots after seeding winter wheat in October 1985 and 1986. Noninoculated (CK) and nonpreconditioned teliospore (0-PC) inoculations were included. In August (1986 and 1987) plants were removed and the % bunted plants (BP) determined. The mean % BP in 1986 and 1987 for the CK, 0, 2, 4, 6 and 8 PC were: 27, 44, 72, 62, 64 and 62, and 11, 20, 42, 25, 37, and 46, respectively. The combined % of BP were: 19, 36, 56, 40, 48, and 53. These data indicate that preconditioning teliospores in SEB for at least 2 wk prior to field inoculation increases the incidence of dwarf bunt.

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THE EFFECTS OF PYTHIUM IRREGULARE AND SOIL PHYSICAL FACTORS ON WINTER WHEAT GROWTH. C. M. CARROLL and C. S. ROTHROCK, Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA 30223.

Pythium irregulare and soil physical factors were studied as part of research to determine the factors responsible for decreased growth of winter wheat following no tillage. A factorial experiment was conducted in a growth chamber with infested or noninfested soil at three different bulk densities and moisture levels. Pasteurized soil was artificially infested with oospores produced in wheat leaf broth. Top weight was significantly reduced by pythium infestation and increasing bulk density (BD). Emergence was lower in infested than in noninfested pots and emergence was also reduced by increasing BD and soil water content. Oxygen diffusion rates decreased as BD and soil water increased. Pythium irregulare and high soil bulk density and soil water content all contributed to decreased growth of wheat.

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INFLUENCE OF SOYBEAN AND SORGHUM SUMMER CROPS ON TAKE-ALL IN DOUBLE-CROPPED WHEAT. C. S. ROTHROCK, Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA 30223, and G. W. LANGDALE, USDA-ARS, Southern Piedmont Conservation Research Center, Watkinsville, GA 30677.

Sorghum and soybean were planted as summer crops in double-cropping systems with winter wheat at two sites. Site one developed moderate take-all severity and site two had a history of severe take-all. Wheat following sorghum had significantly less take-all than wheat following soybean over three years at site one and over two years at site two. At site one, sorghum summer crops in at least two of the last three years reduced take-all severity compared to disease severity following continuous soybean. Wheat yields were significantly greater following three summer crops of sorghum than following three summer crops of soybean. Wheat yields were negatively correlated with take-all damage in both studies. Suppressive soil microflora were partly responsible for the reduction in take-all following a sorghum summer crop.

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AGGRESSIVENESS OF PYRENOPHORA TRITICI-REPENTIS FROM ALTERNATIVE HOSTS. J. M. Krupinsky, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554.

Sixty-one isolates of Pyrenophora tritici-repentis obtained from diseased grass leaves (25 different host species) were tested for virulence on detached seedling leaves of wheat. Leaves were visually assessed for percent necrosis and lesion length was measured. While all isolates caused symptoms on wheat, isolates varied in their ability to cause disease symptoms. In the initial studies when isolates were randomly selected for comparison, the cultivar x isolate interactions were generally nonsignificant which would indicate a lack of specificity. In additional studies when selected isolates were compared, the cultivar x isolate interactions were rather small compared to the cultivar and isolate mean squares, and they were mostly nonsignificant.

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Purification of Formamide Hydro-Lyase from Gloeocercospora sorghi and Colletotrichum graminicola. P.Wang and H.D.VanEtten Plant Pathology Department, Cornell University, Ithaca, NY14853

Sorghum is a cyanogenic plant which releases cyanide upon fungal infection. The sorghum pathogens: G.sorghi and C.graminicola produce an enzyme, formamide hydro-lyase (FHL),that can detoxify cyanide by converting it to formamide. We are interested in determining whether FHL is required for pathogenecity on cyanogenic plants. We partially purified FHL from these two fungi. The enzyme was associated with microsomes and was not solubilized by a variety of detergents. However, an insoluble fraction highly enriched in FHL was obtained by solubilization of other proteins. The protein profile of the enriched FHL preparation showed a prominent band of ca. 47 kd polypeptide(s) on SDS-polyacrylamide gels. Two-dimensional gel electrophoresis resolved this band into several polypeptides. We believe that FHL is one of these polypeptides or the enzyme is made of several subunits. Currently, we are attempting to clone the gene (s).

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INDUCED RESISTANCE TO BLUE MOLD BY PERONOSPORA TABACINA AND TOBACCO MOSAIC VIRUS ELICITS A SIMILAR PATTERN OF B-PROTEINS. X. S. Ye and J. Kuč, Univ. of Kentucky, Lexington, KY 40546.

Tobacco plants, cv Ky 14, 2-3 months old, were either stem-injected with a spore suspension of P. tabacina or inoculated on 3-4 lower leaves with TMV. Injection resulted in expanding necrosis in the stem whereas TMV infection caused local necrotic lesions. Both treatments induced systemic resistance to blue mold (Ye & Kuč, 1988 APS abstract). A group of basic proteins (b-proteins) were extracted from induction sites and the upper protected leaves and separated by SDS-PAGE. The appearance of some b-proteins in the protected leaves coincided with the appearance of induced resistance. Upon challenge with P. tabacina, new b-proteins appeared and others intensified earlier in the induced plants than in the control plants. The b-proteins were also detected in H_2O and LiCl intercellular space washings of induced plants. The pattern of b-protein appearance following induction with P. tabacina and TMV appeared qualitatively similar.

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DOUBLE-STRANDED RNA MEDIATED ALTERATIONS IN NITROGEN METABOLISM IN HYPOVIRULENT STRAINS OF LEUCOSTOMA PERSOONII. Carolyn Pazur and Gerard Adams. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Virulent field isolates of L. persoonii are unable to grow on media containing 1.5% potassium perchlorate unless a spontaneous mutation confers chlorate resistance. Two hypovirulent strains of L. persoonii, containing 9 and 2 segments of double-stranded RNA (dsRNA) respectively, are chlorate resistant. When rendered dsRNA-free, the hypovirulent strains regain virulence and sensitivity to chlorate. Nitrogen utilization in dsRNA-containing and dsRNA-free strains are compared on thirteen representative organic and inorganic sources of nitrogen. The dsRNA-containing strains are unable to grow with $NaNO_3$, $NaNO_2$, cysteine or tryptophane as sole sources of nitrogen. DsRNA-free strains can utilize these sources. All three isolates were able to utilize $(NH_4)_2SO_4$ and hypoxanthine. The presence of dsRNA appears to be associated with alterations in nitrogen metabolism.

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CRITERIA FOR DETERMINING VIABILITY OF OOSPORES IN PHYTOPHTHORA. D. C. Erwin and J. Jiang. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Criteria were based on (i) morphology of oospores (*P. cactorum, P. megasperma*) categorized by oospores with a thick wall but without a pellucid body and an ooplast (nonviable), oospores with a thinner wall, one or more pellucid bodies, and an ooplast (viable); and (ii) plasmolysis of oospores. When oospores were plasmolyzed in 4 M sodium chloride or 3 M sucrose solution, the cytoplasm contracted in the center of the viable oospores but in non-viable oospores cytoplasm did not contract. When the plasmolyzed oospores were deplasmolyzed in distilled water and incubated in root exudate or soil extract, they immediately recovered and germinated normally in about 6 days. Significant positive correlations were obtained between % of morphologically viable oospores and % of oospores plasmolyzed in osmotica ($r=1.0$) and between % of morphologically viable oospores and % of germination ($r=0.985$).

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CHARACTERIZATION OF AN AVIRULENT FUNGUS CAUSING GROWTH PROMOTION. K.R. Narayanan, R.T. McMillan, Jr., L.J. Ramos, and J.M. Jeter, Tropical Res. & Educ. Center, Homestead, FL 33031

A fungus was isolated from flower buds of Callistemon which stimulates growth in vitro when the seedlings are infected in the roots. When compared with control, the root-infected plants (Callistemon, citrus) are 2-5 times taller with a greater root mass. This effect is also apparent, although to a lesser degree, in leaf-infected plants. Preliminary studies show that the fungus forms a sheath and its intercellular hyphae penetrate the outer most root cells like ectotrophic mycorrhize. Many factors may contribute to this growth promotion. These include (1) altered levels of phytohormones in host plant, (2) secretion of secondary metabolites and other growth promoting substances into the medium by the fungus, (3) better photosynthetic nutrient use efficiency by the infected host plant, and (4) effect of transformation of the host plant by the pathogenic fungus that has lost its virulence.

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COMPARISON OF INAX GENES FROM TWO ICE NUCLEATION ACTIVE, SPECTRALLY DISTINCT ISOLATES OF XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS. J. Zhao and C. S. Orser, Department of Bacteriology and Biochemistry, University of Idaho, Moscow ID 83843.

The threshold of ice nucleation of Xanthomonas campestris pv. translucens (Xct) strain X-56S is 6 C colder than that of strain X-40S. The ice nucleation genes (inaX) from the two strains were isolated and cloned into E. coli for restriction site mapping and sequence analysis to determine differences in the homologous genes. The loci encoding ice nucleation activity were found to span two contiguous EcoRI fragments of 2 and 5 kb for both strains. The complete nucleotide sequence of inaX was determined for strain X-56S and the 5'end for X-40S. Gene fusions were constructed to determine differences in the regulation of the two inaX alleles. Inactivation of ice nucleation activity by mutagenesis was consistently associated with loss of pigment in X-40S but not in X-56S.

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CORONATINE-PRODUCING STRAINS OF PSEUDOMONAS SYRINGAE PATHOVARS TOMATO, GLYCINEA AND ATROPURPUREA SHARE RELATED PLASMID DNA SEQUENCES. C. L. Bender and D. K. Malvick, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285.

The indigenous plasmid pPT23A is involved in production of the phytotoxin coronatine in P. syringae pv. tomato PT23. Regions of this plasmid which are thought to be involved in coronatine biosynthesis hybridize strongly to plasmid DNA isolated from coronatine-producing strains of P. s. glycinea and P. s. atropurpurea. Attempts to mutate coronatine genes in pathovars atropurpurea and glycinea were made using Tn*5*-inactivated coronatine sequences from P. s. tomato. Although these attempts were unsuccessful in P. s. atropurpurea, a bioassay indicated that coronatine production was reduced in P. s. glycinea. Experiments which will confirm this preliminary result and localize the site of mutation in P. glycinea plasmid DNA are in progress. These results may indicate that coronatine genes are strongly conserved between pathovars tomato and glycinea.

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ISOLATION AND CHARACTERIZATION OF THE TOXIN OF *PYRENOPHORA TRITICI-REPENTIS*, THE CAUSAL AGENT OF TAN SPOT OF WHEAT. G.M. Ballance, L. Lamari and C.C. Bernier, Dept. of Plant Science, Univ. of Manitoba, Winnipeg, MB, R3T 2N2, Canada.

A culture filtrate of *P. tritici-repentis* containing a cultivar-specific toxic activity was dialysed and concentrated by ultrafiltration prior to separation by gel filtration on a Sephadex G-100 column. A single, low molecular weight constituent peak contained the toxic activity. This peak was dialysed, concentrated and rechromatographed on a CM-cellulose column at pH 4.8. A linear salt gradient was used to elute several minor peaks as well as a single major protein peak shown to contain the toxic activity. Electrophoresis of aliquots of the pooled peak at pH 4.75 demonstrated that a single protein band was present and that the toxic activity corresponded to this protein band. SDS polyacrylamide gel electrophoresis confirmed the presence of a single protein band which had a relative molecular weight of 13,500. Amino acid analysis of the isolated protein constituent has been carried out.

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PSEUDOMICIN, A BROADRANGE ANTIMYCOTIC FROM PSEUDOMONAS SYRINGAE. L. Harrison, D. Kenfield, G. Bunkers, and G. Strobel, Dept. of Plant Pathology, MSU, Bozeman, MT 59717.

When grown in liquid culture, P. syringae MSU 174 produces an antimycotic that is inhibitory towards numerous pathogenic fungi in both plants and humans. After solvent extraction, this compound was isolated using Biogel P2 saturated with $FeCl_3$ followed by TLC. Preliminary FAB mass spectral and amino acid analyses indicate that pseudomicin is a peptide having a molecular weight in the range of 1300 containing ornithine (hydroxyornithine). Biological data and electron spin resonance indicates that iron as Fe^{+3} may be a molecular constituent by virtue of being chelated by pseudomycin.

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CHARACTERIZATION OF TOBACCO MOSAIC VIRUS MOVEMENT PROTEIN. C.M. Deom, K. Schubert, and R.N. Beachy. Biology Department, Washington University, St. Louis, MO 63130.

The putative movement protein (MP; syn. 30kD protein) of tobacco mosaic virus (TMV) has been shown to potentiate virus movement in transgenic tobacco plants (Deom et al., Science 237, 389, 1987). To further understand the function of MP, the protein was isolated and characterized from different organs of transgenic plants and from TMV-infected tobacco leaf tissue. In all tissues expressing the MP gene, the protein was found predominantly in cell wall fractions. These fractions contained at least 10-15 fold more MP than the respective soluble fractions. There was also a remarkable difference in the levels of expression between different tissues in transgenic plants. Cell walls from older leaves (>13 cm length) contained greater than 50-fold more MP than cell walls from young leaves (<13 cm), stems, and roots. When transgenic and TMV-infected leaf extracts were compared, MP was more abundant in transgenic tissues.

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CHEMOTAXIS OF AGROBACTERIUM TUMEFACIENS TOWARD SMALL, HEAT-STABLE MOLECULES FROM ISOLATED ROOT CAP CELLS. H. Lin, M. Hawes. Department of Plant Pathology, University of Arizona, Tucson 85721.

Motile A. tumefaciens cells are attracted to suspensions of isolated root cap cells (Hawes, 1987 Phytopathology 77:1693). We have initiated a study of the chemical nature of the attractant from root cap cells of pea. Preliminary results indicate that A. tumefaciens strain A348 is attracted to heat-stable substances from root cap cells. Thus, suspensions of root cap cells heated at 50° C for 25 min, or boiled for 10 min, remained attractive to A. tumefaciens. However, plant cells dialyzed against water for 5 hours lost the ability to attract the bacteria. Experiments designed to determine the size and chemical nature of the attractant molecule(s) are in progress.

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CYTOLOGICAL COMPARISONS OF CITRUS CANKER A AND CITRUS BACTERIAL LEAF SPOT INFECTED CITRUS. R. H. Brlansky C. L. Davis, E. L. Civerolo, and D. Achor, University of Florida, IFAS, CREC, Lake Alfred 33850 and USDA, ARS, Beltsville, MD 20705.

Comparative cytological studies of citrus canker A from Japan, citrus canker A from Florida, and citrus bacterial leafspot from Florida were performed using light, scanning electron (SEM), and transmission electron (TEM) microscopy. Greenhouse inoculated grapefruit (Citrus paradisi Macf.) and Mexican lime (C. aurantifolia (Christm.) Swing.) were sampled at various post-inoculation times and prepared for SEM and TEM. Both forms of canker A produced eruptent lesions that contained large numbers of bacteria, whereas the lesions of citrus bacterial leaf spot were flat. Differences were noted in hypertrophy and plasmolysis of cells and the response of cell layers.

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INDUCTION OF AN "ACTIVE OXYGEN" BURST IN TOBACCO CELL SUSPENSIONS BY PATHOVARS OF PSEUDOMONAS SYRINGAE. L.D. Keppler, M.M. Atkinson, & C.J. Baker, USDA-ARS, Microbiol. & Plant Path. Lab., Beltsville, MD 20705.

A burst of "active oxygen" production has been reported in plant tissue exposed to fungal pathogens. Here we have investigated possible "active oxygen" production in tobacco cell suspensions exposed to bacterial pathogens: Pseudomonas syringae pv. tabaci (compatible plant/pathogen combination); and Pseudomonas syringae pv. syringae (incompatible combination). "Active oxygen" production was monitored by measuring chemiluminescence produced by reaction of added luminol with the "active oxygen" specie(s), H_2O_2 and/or O_2^-. The results indicate that a transient increase in "active oxygen" is produced by plant cells in response to pathogenic bacteria. A similar burst of "active oxygen" production is induced by some, extracellular, heat-stable, component(s) present in the bacterial inoculum.

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IMMUNITY TO ERYSIPHE POLYGONI IN BETA MARITIMA ACCESSIONS FROM EUROPE. E. D. Whitney, 1636 E. Alisal St., Salinas, CA 93905

Powdery mildew caused by E. polygoni is a serious disease of sugar beet, B. vulgaris. In 1987 we reported some wild beets, B. maritima, visually free of mildew. Within crosses from mildew free plants, progenies were obtained that also were mildew free. Resistant progenies, susceptible beet, and a nonhost Chenopodium amaranticolor were inoculated, incubated in a growth chamber, and the fungus studied microscopically. Leaf discs taken at 1, 2, 4, and 6 days after inoculation were cleared by boiling in 70% ethanol for 30 min, stained for 5 min in lactophenol cotton blue, destained in water and semi-permanent mounts made with glycerin and water (1:1, v/v). Spore germination and appressorial initiation were similar in beet, B. maritima and C. amaranticolor. Mycelial initiation occurred in beet within 24 hrs. at 24C. E. polygoni appeared morphologically similar on B. maritima and C. amaranticolor with aborted appressoria and no mycelial growth. Only beet had mildew following incubation (1 mo) in a greenhouse suggesting immunity in B. maritima to E. polygoni from California.

(See page 1618 for Abstract 128)

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MODELING SUGARBEET-POWDERY MILDEW INTERRELATIONS. S. S. Adams & R. S. Loomis, Department of Agronomy, Univ. of California, Davis, CA 95616. Present address of first author: Department of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706.

This study focused on modeling the dynamic interrelations of a powdery mildew (Erysiphe betae) population and its sugarbeet (Beta vulgaris) community. Physiologically-based models of host and pathogen growth were coupled to form a combined, simulation model (SUBMIL) for crop loss and ecological energetics assessments. Regressions of simulated vs. observed yields for 9 California location-years indicated that SUBMIL accounted for 90% of observed year-to-year variation in healthy crop yields, 80% of diseased-crop yield variation, and 70% of yield loss variation. In simulations, 40-60% of yield loss accrued from pathogen consumption of host carbohydrate; photosynthetic decline accounted for the rest. Consumption ranged from 5 to 15% of host net primary productivity (NPP) and was temperature- and site-dependent. Yield losses (% basis) were twice as great as NPP losses.

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HISTOPATHOLOGY AND ULTRASTRUCTURE OF VASCULAR RESPONSES IN PEAS INFECTED WITH FUSARIUM OXYSPORUM F. SP. PISI. B.J. Tessier and W.C. Mueller, Department of Plant Sciences, University of Rhode Island, Kingston, RI 02881.

A histopathological and ultrastructural examination of resistant and susceptible suscept-pathogen interactions was conducted in peas following inoculation with race 1 and race 2 of F. oxysporum f. sp. pisi. Responses were characterized and compared with healthy controls. No qualitative differences were found to explain resistance or susceptibility. No differences in response were observed between resistant or susceptible interactions up to 4 days after infection. After 4 days the pathogen, in susceptible interactions, moved laterally from initially infected vessels into adjacent vessels and parenchyma cells until the vascular bundle was completely colonized, while in resistant interactions the pathogen was confined to vessels of initial distribution. An increase in cytoplasmic activity of vascular parenchyma cells was detected in both resistant and susceptible interactions.

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EFFECT OF NONSPECIFIC PATHOGENICITY GENES ON HOST AND PATHOGEN FITNESS IN THE *USTILAGO HORDEI-HORDEUM VULGARE* SYSTEM. D.D. Pope, C.F. Wehrhahn, and C.O. Person, Department of Plant Pathology, University of Georgia, Athens, GA 30602

A population of 20 F_3 dikaryons of *Ustilago hordei* was constructed from a parental cross between single teliospores of race 7 and race 11. The F_3 population was homozygous for a dominant gene conferring virulence on barley cultivar Trebi and was segregating for up to 5 nonspecific pathogenicity genes. Cultivars Trebi and Odessa were inoculated with each dikaryon and quantitative measurements for 58 host and pathogen fitness related variables were taken. Statistically significant differences among dikaryons were found for several of the fitness related variables. Significant differences among the dikaryons were attributed to nonspecific pathogenicity factors with pleiotropic effects. One factor of large effect was found to be tightly linked to the mating locus. ANOVA revealed significant inter- and intralocus interactions for several of the fitness related variables.

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Search for race-specific interactions between bacterial pathogens of crucifers and the plant host ***Arabidopsis thaliana***. G. R. Lazo and R. A. Ludwig. Thimann Laboratories, University of California, Santa Cruz, CA 95064.

The small crucifer, ***Arabidopsis thaliana***, has been described as a convenient laboratory plant for molecular genetic studies. In the interest of investigating plant-microbe interactions, 15 bacterial pathogens of crucifers and five ecotypes of ***A. thaliana*** were tested for race-specific interactions. Strains from a diverse collection of crucifer pathogens, ***Xanthomonas campestris*** pvs. ***campestris*** and ***armoraciae***, and ***Pseudomonas syringae*** pv. ***maculicola***, were grown in liquid culture suspensions and spray-inoculated on small patches of one-month-old, vegetatively grown ***A. thaliana*** plants. Several of the bacterial strains appeared to be pathogenic, yet differential responses among related pathogens on a susceptible host were observed. Likewise, the virulence of a pathogenic strain on different ecotypes of ***A. thaliana*** varied. The possibility of devising a system to study race-specific interactions appears plausible, thus the development of a system to identify genes involved with this interaction is in progress.

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POPULATION STRUCTURE OF NATURAL *RHYNCHOSPORIUM SECALIS* (OUD.) DAVIS POPULATIONS BASED ON VARIATION IN ISOZYME, RIBOSOMAL DNA, COLONY COLOUR AND PATHOGENICITY MARKERS. J. M. McDermott[A], B. A. McDonald[B], R.W. Allard[A], and R.K. Webster[C] [A]Department of Genetics, [C]Department of Plant Pathology, University of California, Davis CA 95616. [B]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

The genetic structure of two *Rhynchosporium secalis* populations naturally infecting Composite Cross II and V barley populations were compared using four types of markers. Frequencies of the different multilocus phenotypes and genetic markers taken separately were significantly different in the two populations. Hierarchical diversity analysis revealed the diversity was distributed on a fine scale; most of the diversity was within subplots (86%). There was a significant amount of differentiation between populations and among subplots within populations. There was a significant difference in the average number of differentials infected by isolates collected from Composite Cross II (mean=8.95) than in isolates collected from Composite Cross V (mean=6.44). The results are consistent with interactions between host resistance and pathogen virulence significantly influencing the genetic structure of the pathogen populations.

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LACK OF CORRELATION BETWEEN INCIDENCE AND SEVERITY OF BLACK ROOT ROT OF COTTON CAUSED BY THIELAVIOPSIS BASICOLA AND YIELD. M.A. Chapman, and R.B. Hine, Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Soil inoculum density and incidence of black root rot of cotton were monitored in two adjacent fields planted mid-April to Acala 1517 at Duncan, Arizona (1160 m elev.). Forty soil cores (3.5 x 15 cm) were taken from the root zone and 80 plants were collected biweekly in the 2 fields from 5/7/87 to 9/28/87. The inoculum density (cfu/g air dry soil) was determined by plating soil dilutions onto a selective medium. Disease severity was rated on a scale of 1 (slight cortical decay) to 4 (severe cortical decay). Mean inoculum density in Field 1 soil was 65 cfu/g and 20% of the seedlings were infected with a severity rating averaging 1.6. In Field 2 the inoculum density, percentage of infected plants and disease rating were 225 cfu/g, 93, and 3.2, respectively. No cortical decay was noted after June 6 in either field. Yields were similar in both fields.

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EFFECTS OF DETASSELING AND COCHLIOBOLUS CARBONUM RACE 3, ON YIELD AND YIELD COMPONENTS OF THREE MAIZE INBREDS. D. C. Michels and W. L. Pedersen, Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801.

The effects of detasseling and infection by Cochliobolus carbonum race 3 were evaluated on yield and yield components of three susceptible maize inbreds. Female inbreds were detasseled either by cutting or hand-pulling. Propiconazole was applied to half of the inoculated plots and to uninoculated plots to provide different levels of disease. Detasseling by cutting removed 30% of the leaf area compared to 8% for pulling. Cutting significantly reduced yield and produced a higher percentage of small, round seed as compared to the hand-pulled method for all disease levels. Plots inoculated with C. carbonum and unsprayed had the highest level of disease and significantly lower yield and smaller seed sizes than plots treated with propiconazole for both detasseling methods. The combination of cutting, inoculation, and no fungicide had the lowest yield and smallest seed.

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EFFECTS OF FRUIT INFECTION BY VENTURIA INAEQUALIS ON APPLE QUALITY AND CROP VALUE. K. L. Reynolds, R. C. Seem, and L. M. Henecke. Dept. Plant Pathology, Cornell University, NYS Agricultural Experiment Station, Geneva, NY 14456.

Semidwarf trees were subjected to fungicide spray programs or were left untreated to obtain a range of scab incidence. Six primary infection periods occurred between 4/7/87 and 6/9/87. Fruit were harvested, weighed, and graded according to USDA standards and the presence of apple scab lesions was recorded. Yield was unaffected by scab incidence, except for that of cv. Jonathan which was significantly reduced. Treatment means of scab incidence on harvested fruit were 4%, 19% and 42% for cv. Jersey Mac and 9%, 12% and 27% for cv. Summerland Mac, and ranged from 1% to 10% in four other varieties. Increasing incidence of scab resulted in significantly fewer Extra Fancy fruit and more culled fruit but had little net effect on the proportion of fruit in the Fancy, Utility and U.S. #1 classes. The decrease in proportion of highest quality fruit did not result in a significantly lower crop value.

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MODELING THE INFLUENCES OF TEMPERATURE AND PLANT INFECTION RATE ON POTATO FOLIAGE AND YIELD LOSSES CAUSED BY Verticillium dahliae. K. B. Johnson, Dept. of Plant Path., University of Minnesota, St. Paul, MN 55108.

A submodel was coupled to a potato crop growth simulator to describe effects of V. dahliae infection on loss of leaf area and tuber yield. This was accomplished by accelerating the leaf tissue aging rate as a function of V. dahliae incidence in stems and high temperature stress. Model parameters were estimated from sequential samples of crop biomass and V. dahliae incidence data from five inoculated crops of cv. Russet Burbank grown in three seasons. In two crops with similar infection rates, modeled and observed yield loss were 4.6 and 6.5%, respectively, when conditions after onset of infection were cool (20.0 C), and 17 and 15%, respectively, when conditions were warmer (23.3 C). Simulation analysis indicated that air temperature after infection, number of stems infected, and timing of infection are important considerations for interpreting yield losses caused by this pathogen.

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POWDERY MILDEW ASSESSMENT IN RELATION TO GRAIN YIELD OF WINTER WHEAT CULTIVARS. P. E. Lipps and L. V. Madden, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Powdery mildew severity in relation to grain yield was determined on three to six cultivars over a 3-yr period in Ohio. Four assessment systems based on leaf area covered by lesions and a 0-10 scale were evaluated for estimating disease severity. All were highly correlated with grain yield, although correlations varied with cultivar (highest r=0.95 for 'Becker' and 0.57 for 'Caldwell'). Yield was rarely correlated (P<0.05) with disease severity before Feekes growth stage (GS) 10, but disease severity at GS 10.3 was most consistently correlated with yield for all cultivars and years (r ranged from -0.40 to -0.93). Coefficients of determination for linear regression equations describing the relationship between yield and disease severity for five of the six cultivars were

relatively high (R^2=0.66-0.87 in 1986 and 0.50-0.83 in 1987), indicating that disease assessments using the 0-10 scale at GS 10.3 were adequate for predicting yield.

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Quantification of Barley Yield Losses Caused by Rhynchosporium secalis Using Visual versus Remote Sensing Assessment Methods. F. W. Nutter, Jr. and R. M. Cunfer, University of Georgia, Athens 30602

A multispectral radiometer was used to measure the amount and quality of sunlight reflected from barley canopies differing in the amount of green leaf area (GLA). To quantify relationships between GLA, disease severity, and yield, Tilt was applied to barley at different growth stages to obtain a range of different disease levels of barley scald (caused by Rhynchosporium secalis). Plots were assessed visually for disease proportion (x) and by using a CROPSCAN radiometer to measure GLA (1-x). Percent reflectance at 800 nm was found to increase linearly as GLA increased. Critical, multiple and area under the curve models were developed from these data to compare assessment methods. Over four location-years, yield loss models developed from reflectance data explained 30 to 63% more of the variation in yield compared to models based on visual disease assessments.

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EFFECTS OF CERCOSPORA BLIGHT ON ASPARAGUS YIELD. K. E. Conway, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Proportions of disease, incited by Cercospora asparagi, on asparagus ferns of cultivars 'UC 157 F_2' and 'Mary Washington' were used to calculate area under the disease progress curve (AUDPC). Various treatments (fungicide, cultural and none) had been applied either to fern residue prior to spring harvest or to ferns after harvest in individual blocks of each cultivar. Variations in disease incidence due to treatment effects resulted in different amounts of AUDPC. Blocks were 4 rows wide and 25 m long. Disease incidence and yield were assessed (determined) each year from the inner 2 rows during a four year period (1984-1987). AUDPC was compared to yield using linear regression analysis. Regression equations and coefficients of correlation were determined for each cultivar. The correlation (P=0.05) was negative between the amount of disease on ferns during the summer months and yield of asparagus during the following spring for both cultivars.

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EFFECT OF THE RATE OF PLANTING SUGARCANE ON YIELD LOSS FROM RATOON STUNTING DISEASE. M.P. Grisham, Sugarcane Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 470, Houma, LA 70361.

Five cultivars of sugarcane (L 62-96, CP 65-357, CP 70-321, CP 74-383, and CP 76-331) either affected with ratoon stunting disease (RSD) or healthy were planted at a standard, hand-planting rate of approximately 1175, 1.8-m sugarcane stalks per hectare or at a double rate of approximately 2350 stalks per hectare. Yields were obtained for the plant crop and two ratoons. The double planting rate, irrespective of disease, increased yield (kg sugar per hectare) of the plant crop, but not the ratoon crops. Percent loss over the three-crop cycle from RSD (28, 12, 44, 17, and 9% for the five cultivars listed, respectively) was not affected by the rate of planting, and only in the plant crop was the yield of the RSD treatments planted at the double rate equal or greater than the yield of the healthy treatments planted at the standard rate.

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RESPONSE OF PINK ROOT RESISTANT AND SUSCEPTIBLE ONION CULTIVARS TO DIFFERENT PYRENOCHAETA TERRESTRIS POPULATIONS AND SOIL FUMIGATION. D. L. Lindsey, S. V. Ornelas and J. N. Corgan, Entomology, Plant Pathology and Weed Science Department, New Mexico State University, Las Cruces, New Mexico 88003.

A study was conducted to evaluate the effect of Pyrenochaeta terrestris populations on pink root disease severity and growth of spring planted onions. A split-split plot experimental design utilized high and low pink root incidence fields, Cholorpicrin fumigated and non-fumigated plots and pink root resistant ('86-62') and susceptible ('Golden Cascade') cultivars. Preplant population of P. terrestris in low pink root incidence field was 0.4 cfu/g soil and at harvest populations in fumigated and non-fumigated plots were 57.0 and 32.3 cfu/g soil, respectively. Disease severity ratings for '86-62' were significantly less and bulb weights were significantly greater than "Golden Cascade" in both high and low incidence fields. Soil fumigation with Chloropicrin (24 l/ha) increased bulb weight of both cultivars 30% in the low pink root incidence field and 25% in the high incidence field.

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EFFECT OF SINGLE VERSUS MIXED INOCULUM OF HV ISOLATES OF XANTHOMONAS CAMPESTRIS pv MALVACEARUM ON DISEASE REACTION OF THREE COTTON GENOTYPES. T. P. Wallace and K. M. El-Zik, Dept. of Soil and Crop Sciences, Texas A&M Univ., College Station, TX 77843

Cotton germplasm with resistance to Xanthomonas campestris pv malvacearum has been successfully identified using artificial inoculation with a mixture of USA races 1,2,7, and 18. New isolates of the pathogen from Africa, virulent on previously resistant cultivars, were used singularly and in mixtures as inoculum to determine disease reactions on cotyledons of resistant and susceptible genotypes. When the HV1 isolate was used in mixtures with isolates HV3, HV7, Sudan, or USA race 18, disease grades were significantly reduced compared to those obtained with HV1 alone, suggesting an antagonistic or competitive effect. Screening for resistance to the new virulent isolates will require inoculation with a single isolate versus a mixture of isolates as has been the practice with the USA races of the blight pathogen.

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BEAN EPIPHYTE ASSAY FOR BACTERICIDE EFFICACY. H. F. Schwartz, M. S. McMillan, and L. M. Wierdsma, Dept. of Plant Pathology and Weed Science, Colorado State Univ., Fort Collins CO 80523.

Copper-based bactericides were applied 40 hours after epiphytic establishment (10^4 to 10^5 cfu/ml) of a pathogenic isolate of Pseudomonas syringae pv. phaseolicola (PSP) on fully expanded unifoliate leaves of the dry bean cultivar 'Olathe' in the greenhouse. Leaf disks, collected from plants from each replicated treatment, were shaken in phosphate buffer for 1 hour and enumerated on Kings B medium after incubation at 22 C for 3 days. Leaves treated with products such as Agtrol AF, Agtrol CP, Agtrol NF, Bravo C/M, and SDS 64216 had significantly lower (40 to 60%) PSP populations than did the control. Populations of bean bacterial epiphytes were effectively reduced and illustrate that the timely application of bactericides can reduce populations required for initiation of a bacterial disease such as halo blight of beans. Assay procedures and epiphyte management strategies will be discussed.

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AN IN VITRO ASSAY TO EVALUATE LATE BLIGHT RESISTANCE K.L. Deahl, S.L. Sinden and R.W. Goth, USDA:ARS:Vegetable Laboratory, Beltsville, MD 20705

Sterile stock plants of 15 Solanum tuberosum clones were propagated in "Plant Cons" containing a modified MS medium for 45 days at 24 C under cool-white fluorescent light (80μ E/m^2s, 16-hr photoperiod). Expression of resistance to Phytophthora infestans by the tissue cultured plants was assessed by inoculating leaves with bacteria-free sporangial suspensions from six different races. Disease reactions of the clones were compared with those obtained from inoculating detached leaflets and greenhouse plants. With the in vitro assay, we could detect differences in R-gene resistance to the various races by differential hosts. Slower disease development in compatible combinations and less defined hypersensitive reactions were observed in detached leaflet assays because of more rapid senescence. Use of this in vitro system provides an effective evaluation technique for assaying Solanum germplasm for late blight resistance.

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IDENTIFICATION OF TILLETIA INDICA TELIOSPORES BY ISOZYMES. M. R. Bonde, G. L. Peterson, USDA-ARS, Frederick, MD and T. T. Matsumoto, Calif. Dept. Food Agric., Sacramento, CA

Wheat from foreign countries where Karnal bunt is present is of quarantine significance in the USA because the disease is not known to be present. Teliospores of Tilletia indica are difficult to differentiate from those of Neovossia barclayana (causal agent of kernel smut of rice), which are frequently present as a contaminant in wheat shipments. Single-

teliospore isolates (58) of T. indica from India, Pakistan, and Mexico were compared by isozyme analysis on starch gels. Average coefficient of similarity (CS), comparing any two isolates, was 0.85 (85% of alleles in common) and never lower than 0.73 based on 15 putative isozyme loci. When comparing isolates of T. indica with N. barclayana, the average CS was low (0.07). Isozyme analysis easily differentiated these two pathogens, and the technique is currently being used for routine identification of the pathogens for regulatory purposes.

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TECHNIQUE FOR CYCLING FUNGICIDE-RESISTANT BOTRYTIS CINEREA POPULATIONS ON GERANIUMS. G. W. Moorman, The Pennsylvania State University, Department of Plant Pathology, 211 Buckhout Lab., University Park, PA 16802.

The fifth leaf from the apical meristem of 12 wk-old seed geraniums (Pelargonium X hortorum cv. Red Elite) was removed. Leaf no. 1 was arbitrarily defined as at least 1 cm. in diameter. Ten, 1 cm-diameter disks were removed with a cork borer from along the leaf margin and placed in a petri plate on moistened filter paper. Inoculation was accomplished by pipetting 0.025 ml of liquid containing 100 spores of B. cinerea onto each disk. The petri plates were sealed and incubated at 20C in a lighted incubator (16 hr light;8 hr dark). Ten days after inoculation, the number of infected leaf disks was recorded and spores were collected using a vacuum apparatus. Spores were quantified, assayed to determine the ratio of fungicide resistant:sensitive individuals in the population and used to inoculate new leaf disks as above. Selected fungicide regimes are being tested for their influence on the dynamics of Botrytis populations containing known numbers of fungicide-resistant individuals.

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SEM PREPARATION OF *TUBAKIA DRYINA*. G. Munkvold and D. Neely, Illinois Natural History Survey, Champaign, IL 61820

Leaves of northern red oak (*Quercus rubra* L.), inoculated with the fungus *Tubakia dryina* (Sacc.) Sutt., were prepared for scanning electron microscopy by several methods. Specimens were fixed in either 4% aqueous glutaraldehyde (pH 7.29, osm. =748 milliosmoles) with phosphate buffer, or aqueous Karnovsky's fixative (pH 7.09, 2010 milliosmoles) with phosphate buffer, or in vapor of 2% aqueous Osmium tetroxide. Fixed specimens were then either dehydrated with an ethanol series and dried at the critical point of Carbon dioxide, or frozen in liquid freon and vacuum dried in an Edwards Pearse tissue drier. Vapor fixed specimens were air dried. Other specimens were freeze dried in freon without prior fixation. The methods were then compared in terms of convenience and effectiveness in preserving fungal structures. No differences were observed between the two liquid fixatives, although glutaraldehyde was quicker to prepare. Vapor fixation followed by air drying revealed that a matrix surrounds the conidia. Hyphae and conidia consistently collapsed if air dried. Freeze drying fixed specimens often precipitated fixative salts onto the specimen. Ethanol dehydration followed by critical point drying was effective in preserving fungal structures, but was the most time consuming method. Freeze drying fresh, unfixed specimens was also effective, and was the quickest method.

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METHANOL STRESS - A NEW METHOD FOR ARTIFICIAL DETERIORATION OF COTTONSEED. V. H. Hernandez, K. M. El-Zik, and J. M. Halloin, Dept. of Soil and Crop Sciences, Texas A&M Univ., College Station, TX 77843, and USDA-ARS, East Lansing, MI 48826.

Reliable laboratory tests are needed to monitor and evaluate cottonseed quality. Methanol treatments were evaluated as a method for deterioration of cottonseed. In laboratory and field tests in 1986 and 1987 seed-seedling disease incidence increased, and germination, emergence, and stand decreased, as exposure time to 20% methanol increased from 0 to 6 h. The effects of methanol on disease incidence were more pronounced with Pythium ultimum than with Rhizoctonia solani. Methanol stress simulates the effects of field weathering relative to seed deterioration, germination, reaction to seed-seedling pathogens, and stand establishment. Immersing cottonseed in 20% methanol for 6 h is a reliable technique to alter seed viability and vigor, and may prove useful in predicting potential field performance.

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APPLICATION OF THE MOST PROBABLE NUMBER METHOD TO THE ENUMERATION OF SOILBORNE FUNGAL PROPAGULES. M. L. Courtney, Department of Plant Pathology, E. E. Gbur, Jr., Agricultural Statistics Laboratory, and J. C. Rupe, Department of Plant Pathology. University of Arkansas, Fayetteville, AR 72701.

The most probable number (MPN) method is commonly used to enumerate bacteria in wastewater and Rhizobia in soils, but it has rarely been used to estimate populations of soilborne fungi. The population of Fusarium solani propagules in a field soil was estimated by the MPN method and the dilution plate count (DPC) method. With the MPN method, the population was estimated to be 238,833 propagules/g whereas the DPC method gave an estimate of 236,000 propagules/g. No significant difference between methods was found. These results suggest that the MPN method could be of value in the determination of soilborne pathogen densities.

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EVALUATION OF SPRAY DEPOSITION OF A MODIFIED MISTBLOWER FOR BLACK SIGATOKA CONTROL IN PLANTAINS. J. M. Rivera and J. P. Krausz, Honduran Foundation for Agricultural Research (FHIA), Apartado 2067, San Pedro Sula, Honduras.

A modified commercial mistblower was compared with a conventional unit of the same model at two swath widths (2.1 and 4.3 m) and a uniform output of 1.2 L/min. Modifications were an inversion of the fan housing so that air was expelled directly upwards and a replacing of the pleated flexible air outlet extension tube by a rigid, smooth plastic tube aiming upwards. The replicated trial was repeated twice using a fluorescent tracer as spray marker. Samples from the first fully expanded leaf of adult, non-flowering plants were evaluated for spray deposition (percentage of leaf area covered and density of spray droplets) under UV light. The modified mistblower consistently outperformed the conventional one at both swath widths, giving twice the spray deposition when used at the narrower swath. The narrower swath resulted in better coverage although the difference was not statistically significant.

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A GENERAL METHOD FOR THE DETECTION OF POTYVIRAL GENE PRODUCTS IN PROTOPLASTS AND INTACT TISSUE. C.S. Luciano, K.S. Gibb and P.H. Berger, Department of Plant Pathology, University of Kentucky, Lexington, KY, 40546.

We describe a rapid and effective immunostaining method for the detection of potyviral proteins in infected protoplasts or cells. With this method, antibodies directed against viral proteins reacted specifically with material present in infected epidermal strips, mesophyll cells or protoplasts, but absent from mock-inoculated samples. Although any of the antibodies tested detected potyviral infections, clearest results were obtained when anti-cylindrical inclusion antibodies (anti-CI) were used. In protoplasts, a positive reaction with anti-CI was observed as early as 24 hrs after inoculation, and the amount of immunoreactive material increased, in parallel with the accumulation of coat protein as measured by ELISA, for an additional 48 hrs. Positive reactions observed with epidermal strips and mesophyll cells suggest the potential utility of the method in studies of cell-to-cell movement of potyviruses.

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RELATIONSHIPS AMONG CLIMATE, PRIMARY INOCULUM SOURCE, DORMANT AND POST-EMERGENCE CONTROL SPRAYS, AND GRAPE POWDERY MILDEW IN CALIFORNIA. **J. J. Stapleton[+], W. D. Gubler[#], D. Fogle[#], D. Chellemi[#], L. Bettiga[+], G. Leavitt[+], P. Verdegaal[+], R. Smith[+], and K. Kelley[+], Cooperative Extension[+], and Department of Plant Pathology[#], University of California, Davis, CA 95616.**

Experiments were done in 1987 and 1988 to evaluate the role of ascospores as primary inoculum for grape powdery mildew (PM) (Uncinula necator) in California. Numbers of cleistothecia and levels of bud perennation were determined in several vineyards in different climatic regions and correlated with incidence and severity of PM. Dormant and post-emergence sulfur and copper spray trials were conducted to determine effects on PM. Results indicated that PM-infected grapevines in coastal growing areas generally had higher population densities of cleistothecia and higher levels of early-season PM than those in interior valleys or the Sierra foothills. Higher incidence of bud perennation was found in the interior valleys. Dormant sprays sometimes reduced PM incidence and severity on foliage and berry clusters.

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RUSSET SCAB CORRELATED WITH NONWAXY AREAS OF PRUNE FRUITS AND ITS CHEMICAL CONTROL. **Themis J. Michailides and J. M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, 95616.**

Russet scab of prune (*Prunus domestica*) has an unknown etiology and has caused significant losses of fruit in California orchards. Symptoms develop as shiny areas on the stylar end of green fruit and become brown and scabby on ripe fruit. These areas have a thin cuticle and no epicuticular wax. The disease

has been most severe when rainfall has occurred during flowering. Russet scab symptoms were induced by hand-spraying entire trees with distilled water until runoff on hours 1800, 2400, and 600 for 4 days or by intermittent misting (20 sec every 10 min) with distilled water for 28 hrs during full bloom. This suggests that moisture is a factor associated with russet scab of prune. The severity of russet symptoms on green fruit was correlated ($r > 0.90$) with the incidence of russet scab on dehydrated ripe fruits. Fungicides (captan, captafol, quintar, folpet, and chlorothalonil) applied at full bloom, reduced the severity of russeting and the percentage of fruit with russet scab, although fungi were not isolated from scab.

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SCLEROTIUM ROLFSII CONTROL ON BULBOUS IRIS AND LILIES WITH IN FURROW FUNGICIDE APPLICATIONS. G. A Chastagner, J. M. Staley and K. L. Riley, Washington State Univ., Puyallup, WA 98371.

Fungicides were applied at planting on field-grown 'Ideal' iris and 'Enchantment' lilies to control S. rolfsii. After 9 mo., 2% of the check iris and 98-100% of iris treated with benodanil and flutolanil each at 8.1-32.6 kg ai/ha, quintozene (48.8 kg ai/ha) and Nor-Am SN-596 (4.1-8.1 kg ai/ha) were healthy. All fungicides significantly increased bulb yields, but SN-596 was least effective and reduced number and height of flowers. On lilies, benodanil (0.3-2.2 kg ai/ha), flutolanil, myclobutanil and diniconazole each at 0.6 kg ai/ha, quintozene (13.5-53.8 kg ai/ha), quintozene + benomyl (3.4+1.1 kg ai/ha), flutolanil + Nor-Am SN-596 (0.6+0.04 kg ai/ha) and Nor-Am FBC39865 (0.14 kg ai/ha) significantly increased healthy plant numbers and flowers. Myclobutanil and dinconazole were less effective in providing season-long (17-wk) disease control; dinconazole reduced plant height. All fungicides increased bulb yields, but myclobutanil was least effective.

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SOIL FUNGICIDE EFFICACY AND TREATMENT COSTS FOR COMMERCIAL PRODUCTION OF SYNGONIUM PODOPHYLLUM. P. F. Colbaugh and A. Heidel. Texas Agricultural Experiment Station, Texas A&M University Research and Extension Center at Dallas.

Greenhouse trials with potted Syngonium plants were used to determine efficacy and treatment costs for singly applied or combination soil fungicide treatments during the cropping cycle. Seventy treatments were individually applied at recommended rates on day 1 and 55 during a 130 day growth period. Measurements of plant growth and visual assessments indicated 30% of the treatments were superior to others used. Combination treatments generally gave higher numbers of healthy leaves and a better visual appearance than singly applied fungicides. Treatment costs for production of superior quality pot plants ranged from $2-11 for treatment of 100 6-inch pots depending on chemical cost, required rates and application method. These observations suggest production efficiency can be improved and treatment costs can be reduced significantly by the judicious selection of soil fungicide treatments.

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IR-4 ORNAMENTALS PROGRAM - PESTICIDE REGISTRATION STATUS. J.E. Elson, IR-4 Project, Cook College, Rutgers University, New Brunswick, NJ 08903

The IR-4 Project was initiated in 1963 to assist growers in obtaining the pesticides needed for efficient production of "minor" crops. The main emphasis was on food uses until 1977 when the Ornamentals Program was initiated to meet the pesticide needs of the ornamentals industry. Since 1977, IR-4 has sponsored over 11,000 research trials and over 2,600 ornamental registrations for fungicides, herbicides, insecticides, nematicides and plant growth regulators have been granted based partially or fully on the data developed. These registrations cover a variety of ornamentals treated with the following fungicides and nematicides: chlorothalonil, copper hydroxide, dodemorph, etridiazole, etridiazole plus thiophanate methyl, fosetyl Al, iprodione, mancozeb, metalaxyl, oxamyl, streptomycin, triadimefon, triforine, and vinclozolin.

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OCCURRENCE AND CONTROL OF *PUCCINIA ASPARAGI* IN DESERT IRRIGATED ASPARAGUS. E. L. NIGH, JR., Dept. Plant Pathology, Yuma Valley Agriculture Center, University of Arizona, 6425 W. 8th Street, Yuma, Arizona 85364.

Asparagus is harvested January-March in southern California, Arizona and northwest Mexico. The growing season is from April through November when dormancy is initiated by suspension of irrigation. Developing fields established by direct seeding, seedlings or crown transplants may grow the entire year. Foliage is available each month for infection by *P. asparagi*. Rust epidemics occur during the hot June-August summer months but most frequently from October-February. Irrigation increases humidity required for infection and disease development. Rank growth in these geographic areas favors epidemics and makes control difficult. Contact fungicides have limited effect while systemic compounds are more effective if applied correctly. Triadimefon, triforine and bitertanal give satisfactory control especially when applied by chemigation.

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EFFECTS OF CGA-453 AND FOLICUR ON TOMATO EARLY BLIGHT AND ON TOMATO. P. B. Shoemaker, Dept. of Pl. Path., N.C. State Univ., 2016 Fanning Br. Rd., Fletcher, NC 28732-9216

Experimental fungicides CGA-453 3.5E and Folicur 1.2E (terbutrazole) were sprayed on tomato plants to run-off in the greenhouse at 0.5, 1.0 and 2.0 ml/l 1 day before (protectant) and 2 days following (curative) inoculation with Alternaria solani. Both materials at all rates provided complete protectant and curative control of early blight. The standard, 2.4 g/l mancozeb 80W, gave control as a protectant only. The two higher rates of CGA-453 caused some leaf necrosis and slight leaflet distortion. Folicur significantly ($P=0.05$) reduced plant height at all rates and caused leaflet distortion and reduced flower development and fruit set at the two higher rates. In a second greenhouse test, applications of CGA-453 and Folicur at 0.5 ml/l up to 7 days following inoculation significantly ($P=0.05$) reduced sporulation in early blight lesions.

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TOWARD OPTIMAL TIMING OF METALAXYL APPLICATION FOR CONTROL OF POTATO LATE BLIGHT VIA COMPUTER SIMULATION AND FIELD EXPERIMENTATION. M. A. Doster and W. E. Fry. Cornell University, Ithaca, NY 14853.

The optimal timing for both disease control and limiting selection for metalaxyl resistance in Phytophthora infestans of a single metalaxyl spray during a season was investigated using computer simulation and field experimentation. Computer simulations using a complex validated model showed that the optimal timing varied throughout the season and depended on several factors such as the density of the fungal population, amount of host tissue available for infection, and the presence of metalaxyl resistance. In a field experiment with a rapid epidemic of a metalaxyl-sensitive isolate, an early metalaxyl spray resulted in the smallest area under disease progress curve (AUDPC) (15.9), highest yield (31 kg/plot), but the most tuber blight (8%), while the late season spray resulted in the highest AUDPC (20.8) and lowest yield (19 kg/plot), but also the least tuber blight (3%). Computer simulations gave similar results for the conditions of the field experiment.

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SUPPRESSING THE RHIZOCTONIA DISEASE COMPLEX OF POTATO THROUGH SEED TREATMENTS. S.S. LEACH, USDA/ARS, N.E. Plant, Soil and Water Laboratory, University of Maine, Orono, ME 04469.

One biological and fifteen chemical seed treatments were evaluated for the control of Rhizoctonia solani on white potato over a two year period. The biological agent (Laetisaria arvalis) provided control equal to a standard chemical treatment (PCNB+thiabendazole). The experimental fungicides CGA-449 and CGA-142705 + Apron significantly reduced infections on stems and stolons, but did not reduce the amount of sclerotia on tubers. The latter, at the 2#/CWT rate, produced a significantly higher yield of US # 1 tubers over all other treatments. Flutolanil, applied at 0.2 and 0.4 oz ai/CWT significantly reduced all phases of the disease and were the only treatments that resulted in sclerotia-free tubers. Tops, captan and Rizolex were not effective in 1987. Dip treatments were generally more effective than dusts.

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EFFECT OF SOIL-BORNE PATHOGEN CONTROL METHODS ON THE PRODUCTION OF WHITE PINE NURSERY SEEDLINGS. S. A. Enebak[1], M. A. Palmer[2], and R. A. Blanchette[1], Univ. of Minn., Dept. of Plant Path.,[1]

U.S.D.A. Forest Service,[2] St. Paul, MN 55108.

Treatment with 100% Methyl bromide (MB), 100% chloropicrin (CHL), 67% MB/33% CHL, 33% MB/67% CHL or dazomet reduced populations of Fusarium, Pythium and Rhizoctonia species in soil 4 weeks after application; captan, thiram, captan and thiram, or silica sand had no effect compared to nontreated plots. Nine months after application, populations of these pathogens remained significantly lower (p=0.05) in plots with MB and/or CHL, while populations in dazomet treated soils were not different from those in nontreated plots. Seedlings growing in plots treated with MB and/or CHL had the least amount of damping-off and root rot. At the end of the first growing season, seedling stand densities averaged 464/m^2 in plots with the various MB and/or CHL treatments and 250/m^2 in dazomet treatments. Other treatments produced an average of 106 seedlings per m^2.

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PROGRESSIVE STAGES OF CANKER FORMATION AND DECAY BY CERRENA UNICOLOR IN ACER AND BETULA. S. A. Enebak and R. A. Blanchette, University of Minnesota, Deptartment Plant Pathology, St. Paul, MN 55108.

Cerrena unicolor (Bull:Fr) Murr. (Daedalia unicolor) was found to be a pathogen on two northern hardwood tree species, (Acer saccharum Marsh) and (Betula papyrifera Marsh) causing a canker-rot. Canker development was determined using inoculation studies and examination of cankers 6 months, 1.5 and 2.5 years after the fungus was introduced. Histological examination revealed that the host response consisted of cell wall lignification and formation of a suberized impervious tissue. Movement past host barriers in the phloem was via a thick-walled hyphal wedge that circumvented the periderm and killed the cambium. Cerrena unicolor is an aggressive decay organism moving throughout the wood causing extensive white rot. Multiple zones of decayed and discolored wood were present on inoculated trees which are characteristic of cankers found in the field.

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CARBOHYDRATE AND NITROGEN CONTENT OF ROOTS OF DECLINING RED SPRUCE TREES. P.M. Wargo, USDA Forest Service, Hamden, CT 06514, D.R. Bergdahl, C.W. Olson and D.R. Tobi, School of Natural Resources, Univ. of Vt., Burlington, VT 05405.

Second order woody roots from 3 health classes of red spruce (Picea rubens Sarg.) growing above 900 m on Mt. Abraham, Lincoln, VT were randomly selected and evaluated for total soluble carbohydrate (TSC), total starch (S) and soluble nitrogen (N). Health classes were based on the percentage of crown dieback and rated as either healthy, initially declining, or severely declining trees (<10, >10-50 and >50 percent dieback, respectively). A 10 cm section was removed from the proximal end of each of 3 roots from 9 trees per health class. All samples were frozen, freeze dried, separated into bark and wood, ground and then assayed for TSC, S and N. Severely declined trees had less TSC, S and N in wood and less S and N in bark compared to healthy trees. Healthy and initially declining trees had similar concentrations of TSC, S and N in the wood but declining trees had less S and N in the bark.

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SCREENING FOR BLIGHT RESISTANCE AND PATHOGENICITY IN CHESTNUT SEEDLINGS. F. V. Hebard and L. Shain, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

One- and two-yr-old chestnut seedlings, ca 1 cm in diam at 10 cm, were acclimated for 1 week to a growth chamber with 14 hr, 23 C days and 10 hr, 18 C nights. Light intensity was 27 μE m^{-2} s^{-1} at 1 m from cool-white fluorescent tubes. Plants were inoculated with agar disks of Endothia parasitica 2 mm in diam. They were incubated at 25 C for 5 days to ensure success of inoculation, then returned to 23/18 C. American chestnut were encircled in ca 40 days by elliptically shaped, sunken cankers which extended to the vascular cambium and fruited heavily in ca 35 days. Cankers on Chinese chestnut were non-encircling, irregularly shaped, not sunken, superficial, and showed little fruiting for at least 60 days. In another assay, stem segments from American chestnut produced significantly more ethylene as compared to controls than did segments from Chinese chestnut, when placed on agar disks of E. parasitica. Thus both assays are promising as screens for blight resistance.

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WHITE FIR CHARACTERISTICS ASSOCIATED WITH WOUND RESPONSE TO TRICHOSPORIUM SYMBIOTICUM, FUNGAL SYMBIONT OF THE FIR ENGRAVER BEETLE. G. T. Ferrell, W. J. Otrosina, and C. J. DeMars, Jr. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA.

White firs in 6 stands in northern California were inoculated with T. symbioticum and phloem wound reactions related to tree characteristics. Mean vertical reaction length (RL) varied among stands and among sampled clusters of trees within stands. RL was greater in large diameter dominant, than in small diameter suppressed, trees. RL was directly related to percentage of crown dead, dying or missing, and indirectly related to percentage of tree height occupied by living crown. Results will be discussed in relation to wound concentrations of resin monoterpenes, xylem starch reserves, growth efficiency, and host resistance, of trees.

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OPERATIONAL CONTROL OF WHITE PINE BLISTER RUST BY PRUNING. R.S. Hunt, Pacific Forestry Centre, Victoria, B.C. V8X 1M5

Surveys prior to pruning and pre-commercial thinning in eight western white pine (Pinus monticola) stands indicated that 26.5% had blister rust (Cronartium ribicola) stem cankers of which 20% and 30% were on trees pruneable to 1.5 m and 3.0 m respectively, 22% could be protected by removing threatening branches and cankers, and 51% were healthy. Post-treatment surveys 4-5 years later indicated that 9% of the trees still had threatening branch cankers and 25.5% still had stem cankers. Poorly trained crews failed to fall stem-cankered trees and to prune infected branches above the designated pruning height of short trees. Most trees were protected from future attack, although some new cankers originated on short trees not pruned to 3 m. Pruning small trees to 1.5 m will protect more of them from stem cankers than delaying pruning. If infected branches between 1.5 and 3 m are removed, followed by a second pruning to 3 m a few years later, even greater survival can be obtained.

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ROOT VITALITY AND MYCORRHIZAL STATUS ON DIFFERENT HEALTH CLASSES OF RED SPRUCE TREES. P.M. Wargo, USDA Forest Service, Hamden, CT 06514, D.R. Bergdahl, C.W. Olson and D.R. Tobi, School of Natural Resources, Univ. of Vt., Burlington, VT 05405

Roots from healthy, declining and severely declined (<10, >10-50 and >50% crown dieback, respectively) red spruce (Picea rubens Sarg.) growing above 900 m on Mt. Abraham, Lincoln, VT were evaluated to determine root vitality and mycorrhizal status. Root systems were excavated by hand and judged either living or dead by color and texture. Number of root tips with mycorrhizae and number of mycorrhizal types were recorded. Woody roots of declining trees had a greater percentage of dead length and discolored wood and greater number of wounds than healthy trees. Declining trees had fewer mycorrhizal tips and mycorrhizal types, a greater percentage of dead fine roots and more dead length per total length of fine root than healthy trees. Severely declined trees had a greater percentage of dead root tissue for both woody and fine roots compared to declining trees.

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FUSARIUM DISEASES OF CONTAINERIZED CONIFER SEEDLINGS IN NORTHERN ROCKY MOUNTAIN NURSERIES: INFECTION, SYMPTOM PRODUCTION AND PATHOGENICITY OF ASSOCIATED FUSARIA. R. L. James, R. K. Dumroese and D. L. Wenny. USDA Forest Service, TCFPM, P. O. Box 7669, Missoula, MT 59807 and University of Idaho Research Nursery, Moscow, ID 83843.

Production of containerized conifer seedlings in northern Rocky Mountain nurseries is often seriously affected by disease incited by Fusarium spp. Investigations were conducted to elucidate epidemiological characteristics of these diseases. Diseases included pre-and post-emergence damping-off and seedling root diseases. Most losses occurred early in the crop. Losses were also substantial whenever seedlings were stressed. Infected seedlings often did not display external disease symptoms. Infection and disease occurred randomly throughout greenhouses. Secondary spread of disease was not detected. Fusarium spp. isolated from Douglas-fir seed and seedlings in order of prevalence were F. acuminatum, F. oxysporum, F. avenaceum, F. sambucinum, and F. tricinctum. Pathogenicity varied widely.

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ASSOCIATION OF INTRODUCED HYPOVIRULENCE IN THE CRYPHONECTRIA PARASITICA POPULATION AND IMPROVED CONDITION OF CHESTNUT TREES IN CONNECTICUT. S. L. Anagnostakis, The Connecticut Agricultural Experiment Station, 123 Huntington St., Box 1106, New Haven, CT 06504.

Hypovirulent (dsRNA containing) strains of C. parasitica were inoculated into chestnut blight cankers in two forest plots in Connecticut, at least yearly, from 1978 to 1981. In 1987 the chestnut trees in and adjacent to these treated experimental plots$^{(T)}$ had a larger size index than the chestnut trees in control areas$^{(C)}$ some distance from the experimental plots. T trees had several abnormal cankers per stem whereas C trees were either free of blight or had single, normal (killing) cankers. About half (37/70) of the isolates of C. parasitica from cankers adjacent to the T plots (25 to 75 m away) contained large molecules of dsRNA. Among isolates from the C plots three of the 11 checked contained dsRNA. There appears to be an association between prior hypovirulence introduction and improved tree condition.

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RELATIONSHIPS BETWEEN ISOZYMES AND INTERSTERILITY GENOTYPES IN HETEROBASIDION ANNOSUM FROM WESTERN UNITED STATES. W.J. Otrosina, T.E. Chase, F.W. Cobb, Jr., and J.W. Taylor. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA; and University of California, Berkeley, CA.

Dikaryotic and monokaryotic cultures of H. annosum were obtained from sporophores collected in pine and true fir (Abies) stumps in northern California and southern Oregon. Intersterility (IS) genotypes for the isolates were determined by mating experiments with appropriate tester strains. Isozyme analyses were conducted on dikaryons with several enzyme systems yielding distinct mobility differences between intersterility groups. Two MDH alleles appeared to be diagnostic for IS groups, while other enzyme systems such as ADH and GOT were highly polymorphic within each IS group and have different mobilities between the groups. These data suggest considerable genetic divergence between IS groups of H. annosum in the western United States.

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ANTIBODIES TO HYPOXYLON ATROPUNCTATUM AND IMMUNOHISTOLOGICAL STUDIES OF LATENT INFECTIONS IN OAK TISSUE. Patrick Fenn, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Immunohistology is being used to examine the nature and tissue location of latent infections of H. atropunctatum and to study fungus development in stressed trees. Rabbit antibodies were raised to extracellular antigens collected by cold ethanol precipitation of culture filtrates of two isolates grown on a modified Fries medium. Isolates were selected based upon peptide banding (SDS-PAGE) of the antigen preparations, growth rates and colony morphologies. Double diffusion tests showed serological similarity between the isolates. Indirect immunofluorescence and enzyme conjugate (alkaline phosphatase) techniques are being used to determine specificity of the antisera for H. atropunctatum and other fungi isolated from living oak tissues. Sections from nodal tissues of seedling sprouts are being examined to identify latent infections.

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EVALUATION OF CERATOCYSTIS FIMBRIATA PLATANI TOXIN HOST SPECIFICITY AND AS AN INDICATOR OF SYCAMORE CANKER STAIN RESISTANCE. F. I. McCracken, Southern Hardwoods Laboratory, USDA Forest Service, P.O. Box 227, Stoneville, MS 38776

Cultures of C. fimbriata platani were removed from the media, homogenized, and extracted with water, ethanol, or acetone. Solvents were filtered, flash evaporated and the residue suspended in water. Seedling leaves of Phaseolus vulgaris, Cucumis sativus, Glycine max, Triticium aestivum, Fraxinum pennsylvanica, Populus deltoides, Magnolia grandiflora, and 6 select sources of Platanus occidentalis were bioassayed with uniform toxin amounts in a controlled environment. Necrotic zones were significantly greater in diameter (P = 0.05) on sycamores than on all other species tested. The diameter of necrotic zones did not differ significantly among susceptible and tolerant sycamore seed sources.

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SOURCE OF AECIOSPORES FOR GREENHOUSE INOCULATIONS AFFECTS PREDICTIONS OF FUSIFORM RUST. C. H. Walkinshaw and R. L. Anderson, USDA, Forest Service, Southern Forest Experiment Station, Gulfport, MS 39505, and Resistance Screening Center, Asheville, NC 28804.

Effect of inoculum was measured with 30-gall composites of aeciospores of Cronartium quercuum f. sp. fusiforme which were collected from loblolly and slash pines. Control- and open-pollinated pine seedlings were inoculated with 20,000 or 50,000 basidiospores per milliliter. Significant differences due to source and concentration were found. Separation of slash pine families which were chosen on the basis of large differences in resistance to inocula from slash pines was poor with inocula from loblolly pines. Family rank for resistance of slash pines was dependent on inoculum source. Inocula collected from loblolly pines in South Carolina, Georgia, Alabama, Mississippi, and Louisiana did not separate susceptible, moderately resistant, and highly resistant slash pines.

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NUCLEAR CYCLE, TAXONOMY, AND NOMENCLATURE OF AUTOECIOUS PINE STEM RUSTS Y. Hiratsuka, Northern Forestry Centre, Canadian Forestry Service, Edmonton, Alberta T6H 3S5, Canada

In 1969, the endocyclic genus Endocronartium was established to include E. harknessii (=Peridermium harknessii) in North America and E. pini (=P. pini) in Europe, two autoecious pine stem rusts, but the reasons for establishing this genus have been questioned. Morphology and cytology of spores and germlings of these rusts and several new pine-to-pine forms reported from Asia and Europe have been examined and evaluated. Number of nuclei and relative DNA contents in various stages of spore germination, number and nature of septa and branches, and mode of initial host penetration suggest that the germlings of the two species function as metabasidia with nuclear fusion and meiosis, rather than as aeciospore germ-tubes. It is concluded that the recognition of the endocyclic genus Endocronartium is justified and desirable.

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K.J. Lewis and E. M. Hansen. Old growth stumps as an inoculum source for Inonotus tomentosus root disease centres in second growth stands. Oregon State University, Corvallis, Oregon.

Inonotus tomentosus causes a serious root disease of mature spruce and pine in northern British Columbia. Stump and tree excavations on regenerated clearcuts indicate that disease centres in the second growth stands can be established from inoculum in the stumps. Viable mycelium persists in the stumps for at least 26 years. Stain and decay is confined to the centre of the root in young stumps then expands longitudinally and radially throughout most of the root system by 15 years. Root contacts between stumps and regenerated trees are common; I. tomentosus mycelium was observed on 40% of contacted tree roots and was often accompanied by a root lesion.

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EXPRESSION OF THE syrB GENE REQUIRED FOR SYRINGOMYCIN PRODUCTION BY PSEUDOMONAS SYRINGAE PV. SYRINGAE DURING PHYTOPATHOGENESIS. Y.-Y. Mo and D. C. Gross. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

The syrB gene required for syringomycin production by Pseudomonas syringae pv. syringae B301D was associated with the formation of an iron-regulated protein (~360 kDa) thought to function as a syringomycin synthetase. Transcriptional fusions of a promoterless lacZ gene to syrB were obtained by Tn3HoHo1 mutagenesis, and constructs were then introduced into the genome of B301D by marker exchange. Certain gene fusions expressed high levels of ß-galactosidase activity (>800 units) when cultured on media conducive to syringomycin production. Iron concentrations ≥2µM induced maximum levels of ß-galactosidase activity. A syrB::lacZ fusion was used to monitor gene expression in planta and to determine whether the syrB gene is expressed early, late or throughout disease development.

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PHYSICAL AND FUNCTIONAL ANALYSIS OF THE syrA GENE REQUIRED FOR SYRINGOMYCIN PRODUCTION BY PSEUDOMONAS SYRINGAE PV. SYRINGAE. N. B. Quigley, G.-W. Xu, and D. C. Gross. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

The syrA gene, necessary for syringomycin production and pathogenicity in Pseudomonas syringae pv. syringae B301D, was shown to control the expression of large proteins (≥360 kDa) associated with syringomycin synthesis. Moreover, introduction of syrA::Tn5 into the genome of the syringotoxin-producing strain B457 by marker exchange yielded a nontoxigenic phenotype. Mapping analysis of the syrA function showed it to lie in a 2.3-2.8 kb DNA segment. Tn3HoHo1 mutagenesis was used to generate transcriptional fusions to lacZ for determining the direction of transcription of syrA and factors that modulate its expression. Genetic analysis of other syr genes will also be summarized.

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THE ROLE OF TRANSCRIPTIONAL REGULATION OF ICE E OF ERWINIA HERBICOLA IN THE EXPRESSION OF ICE NUCLEATION. D. Gurian-Sherman, N. J. Panopoulos and S. E. Lindow, Dept. of Plant Pathology, 147 Hilgard Hall, Berkeley, CA 94720

The level of ice nucleation activity in Erwinia herbicola, as well as other ice nucleating bacteria, is substantially affected by several cultural factors. Random insertion of the promoterless Tn3-lac transposon, Tn3HoHo1, into iceE created fusions which were used to examine the regulation of iceE under various cultural conditions. The amount of IceE protein present under several of these conditions was also estimated from Western blots. Of the cultural factors tested, only growth cycle had a clear effect on transcription, with an increase in β-galactosidase activity of about ten fold beginning in late log phase. The effect of other cultural factors on ice nucleation probably occurs post translationally. For instance, while ice nucleation activity of cells grown in salt-containing media was about 1000 fold higher than in media without salt, β-gal activity was about the same in both cases.

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CLONING OF ADDITIONAL GENES FOR OOMYCIN A BIOSYNTHESIS IN P. FLUORESCENS STRAIN Hv37A. N. Gutterson, K.S. Greisen and D.U. Leong. Advanced Genetic Sciences, 6701 San Pablo Avenue, Oakland, California, 94608.

The afuE locus of P. fluorescens strain Hv37a apparently encodes one or more enzymes of the biosynthetic pathway for oomycin A. When the afuE locus is expressed in E. coli or a P. putida strain, no oomycin A is produced, suggesting that there are other genes encoding enzymes of the biosynthetic pathway. Additional gene(s) have now been isolated by mutant complementation using an improved assay for oomycin A biosynthesis. A single locus has been identified after isolation of 4 overlapping clones from a pRK7813 cosmid library. This locus, designated afuX, has been subcloned and we are pursuing its expression constitutively using a strong E. coli promoter.

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CHARACTERIZATION AND EXPRESSION OF A NOVEL PECTATE LYASE GENE FROM *ERWINIA CAROTOVORA* EC153. D. Trollinger, W. Belser, and N. T. Keen, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

A pectate lyase (*pel*) gene was cloned from *E. carotovora* EC153 and expressed in *E. coli*. The gene could only be expressed in the anti-orientation relative to the *lac* promoter of pUC vectors, indicating that the *Erwinia pel* gene carried its own promoter and that the protein was toxic to *E. coli*. Sequence analysis disclosed that the *pel* gene contained an open reading frame of 1706 bases and encoded a 58,000 dalton protein. *E. coli* cells overexpressing the gene produced a new protein at ca. 56,000 daltons, in close agreement with the value from sequence data. The *E. carotovora pel* gene shares 85% amino acid homology with the *pelY* gene previously sequenced from *Yersinia pseudotuberculosis*. These genes represent a third family of *pel* genes in addition to the two structural families previously observed in *Erwinia* spp. The *E. carotovora pel* gene macerated plant tissue less efficiently than the *pelE* gene product from *E. chrysanthemi*.

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SEQUENCE COMPARISON OF AVIRULENCE GENE D FROM PSEUDOMONAS SYRINGAE PV. TOMATO AND A PUTATIVE RECESSIVE ALLELE FROM P.S. PV. GLYCINEA. D. Kobayashi, S. Tamaki and N. T. Keen, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

We previously characterized the avirulence gene, *avr*D, cloned from *Pseudomonas syringae* pv. *tomato*. This gene modulates a unique pattern of hypersensitive reactions on various soybean cultivars when expressed in *P. syringae* pv. *glycinea* (*Psg*). *avr*D resides on a 5.6 kb *Hind*III fragment and sequence analysis indicates a 933 bp open reading frame (ORF) associated with the avirulence gene activity. Southern blot analyses revealed that all *Psg* races examined contained a conserved 5.6 kb *Hind*III fragment homologous with *avr*D, although none of them expressed the *avr*D phenotype. The homologous fragment was cloned from *Psg* race 4 and sequenced. An ORF of 933 bp homologous to *avr*D was present and the predicted amino acid sequences of the ORFs indicated 86% identity. The total conservation in all *Psg* races suggests they contain a recessive allele to *avr*D which may have an important pleiotropic function in the bacteria.

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CHARACTERIZATION OF THE ANTIBIOTIC BIOSYNTHESIS LOCUS afuE OF PSEUDOMONAS FLUORESCENS STRAIN HV37A. D. U. Leong and N. Gutterson. Advanced Genetic Sciences, Inc., 6701 San Pablo Avenue, Oakland, California, 94608.

The afuE locus appears to encode one or more enzymes of the biosynthetic pathway for the antibiotic oomycin A in P. fluorescens strain Hv37a. In order to localize the genes involved in oomycin A synthesis, a series of deletions of this locus was constructed. Five DNA fragments of the region extending from 2.2 to 6.6 kb from the 3' end of the 9.0 kb afuE locus were each inserted downstream of the tac promoter in E. coli plasmid pKK223-3. E. coli maxicell analysis of this deletion series allowed the tentative assignment of the 5 proteins observed to particular regions of the locus. The 5 afuE DNA fragments, along with the tac promoter were cloned into the broad host range vector pRK767. P. fluorescens strains containing these afuE deletions have been assayed for antibiotic production to determine which of the afuE proteins is involved in oomycin A synthesis.

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GENETIC ANALYSIS OF ANTI-FUNGAL ACTIVITY OF ALCALIGENES SP. STRAIN MFA1. G. Martinetti and J. E. Loper, USDA, ARS, Horticultural Crops Research Lab., Corvallis, OR 97330

Alcaligenes sp. MFA1 exhibits antagonism against Fusarium oxysporum f. sp. dianthi in culture and reduces the severity of Fusarium wilt of carnation, presumably due to its production of a siderophore (Yuen and Schroth, Phytopathology 76:171-176). Nine derivative strains of MFA1, deficient in antagonism against F. o. dianthi and in iron-limited growth, were obtained by Tn5 mutagenesis. Southern analysis of EcoR1-digested genomic DNA from these derivative strains confirmed the presence of a single Tn5 insertion in each strain. Marker exchange mutagenesis of strain MFA1 with cloned EcoR1 fragments, containing Tn5 and flanking sequences from representative mutants, confirmed the association of single Tn5 insertions with the loss of anti-fungal activity and iron-independent growth of MFA1. These results are consistent with the involvement of siderophore biosynthesis by MFA1 in the antagonism against F. o. dianthi.

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A *RECA* MUTANT OF *AGROBACTERIUM TUMEFACIENS* STRAIN C58. S.K. Farrand, S.B. O'Morchoe and J. McCutchan. Department of Plant Pathology, University of Illinois, Urbana, IL. 61801.

We report here the engineering of a *recA* mutation into strain NT-1, a Ti plasmidless derivative of *A. tumefaciens* strain C58. The C58 *recA* gene was isolated from a clone bank by complementation in *E. coli*. The *Agrobacterium* gene complements *E. coli recA* mutations for repair and recombination, and also the Rec⁻ mutation in the Ach5 *A. tumefaciens* strain, LBA4301. The gene was mapped by Southern analysis with an *E. coli recA* probe, by subcloning and by Tn3-HoHo1 mutagenesis. The C58 *recA* gene was mutagenized by insertion of an erythromycin resistance cassette and marker exchanged into strain NT-1 resulting in the *recA* mutant, UIA143. The mutant is sensitive to UV irradiation and MMS but remains transformable and proficient as a plasmid donor or recipient. The mutation is stable and reversion could not be detected. Southern analysis showed strong hybridization with other *A. tumefaciens* biovar 1 strains although polymorphisms are the rule. Hybrization with DNA from biovar 2 and 3 isolates was detectable but less intense.

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ISOLATION OF A XANTHOMONAS CAMPESTRIS PV. ORYZAE AVIRULENCE GENE. Segenet Kelemu and Jan E. Leach, Department of Plant Pathology, Kansas State University, Manhattan, KS. 66506.

Races of Xanthomonas campestris pv. oryzae, causal agent of rice bacterial blight, with specificity to host genotypes have been described. To identify genes involved in race-specificity, a genomic library of a race 2 X. c. pv. oryzae isolate was constructed and mobilized into a race 6 isolate by conjugation. Two cosmid clones (approximately 35 kb) were identified which altered the specificity of the race 6 isolate on rice cultivar Cas 209 containing Xa-10 resistance gene. The avirulence gene is being characterized by subcloning and restriction mapping. This is the first report of a cloned avirulence gene from a monocot pathogen.

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THE ROLE OF PHYTOTOXIN PRODUCTION BY *PSEUDOMONAS CORRUGATA* IN TOMATO PITH NECROSIS. W. Chun and J. V. Leary, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Pseudomonas corrugata constitutively produces a phytotoxin which is apparently involved in pathogenicity. The toxin was extracted with methanol and an active fraction was isolated using anion exchange chromatography. Purified toxin in concentrations ranging from 1 ng/ml to 10,000 ng/ml was inoculated into the stems of 5-week-old tomato plants. After 5 days, brown to black water-soaked lesions in the pith with a mean lesion length of 3 mm were observed. Controls displayed no discoloration in the pith. No linear correlation between toxin concentration and lesion length was observed. Coinoculations of toxin and a non-pathogenic, Tox$^-$ mutant, EMS1-1, significantly increased the mean lesion length to 9 mm. Restoration of toxin production by mobilization of the cosmid clone pLAFR561-9-2B into EMS1-1 resulted in symptoms nearly identical to that of the wild type parent. Thus it is believed that the phytotoxin plays a crucial role in pathogenicity.

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SEROLOGICAL STUDY OF TOMATO SPOTTED WILT VIRUS ISOLATES M. Wang and D. Gonsalves, Plant Pathology Department, Cornell University, NYSAES, Geneva, NY 14456

Tomato spotted wilt virus (TSWV) has one nucleoprotein (26K) and three membrane proteins (52, 56 and 78 K). Serological relatedness of different isolates of the virus was studied to determine the usefulness of polyclonal antibodies produced to a Hawaiian isolate for routine diagnosis of TSWV by enzyme-linked immunosorbent assay (ELISA). Polyclonal antibodies that were produced to whole virus, to purified nucleoprotein, and to 78K membrane protein of the virus were used in characterization of TSWV isolates by ELISA and western blotting. Twenty-four isolates from different regions of the US and Canada were tested in various ELISA assays and eleven of them were tested in western blotting. All isolates were consistently detected in double sandwich direct ELISA using antibody against whole virus. Different levels of reactions occurred with antibodies produced against the nucleoprotein of the virus in indirect ELISA. However, all eleven isolates reacted with anti-nucleoprotein or anti-membrane protein antibodies in western blotting. Our data indicate that antibodies produced specifically to whole virions are useful for diagnosis of TSWV in double sandwich ELISA.

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CELL-FREE TRANSLATION PRODUCTS DERIVED FROM HIGH SALT, HIGH PH BUFFER-PURIFIED SOIL-BORNE WHEAT MOSAIC VIRUS IN WHEAT GERM EXTRACTS. Yukio Shirako, Div. of Biology, California Inst. of Technology, Pasadena, CA 91125.

Incubation of crude polyribosome preparations isolated from SBWMV-infected wheat leaves in wheat germ extracts stimulated the synthesis of RNA I-coded 220K polypeptide in addition to the host plant proteins. In order to determine whether the 220K product was translated from viral RNA in polyribosomes, virions were isolated in a buffer of the same ionic strength and pH as used for polyribosome isolation (0.2M Tris-HCl, 0.4M KCl, pH 8.6) but with 25mM EDTA instead of $MgCl_2$. With this virion preparation only 220K, 25K and 19K polypeptides were produced in wheat germ extracts. Virion preparations made in neutral pH or low salt buffer did not stimulate any viral protein synthesis but reincubation of the preparations in high salt and high pH buffer prior to translation stimulated the viral protein synthesis and vice versa.

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INTERACTIONS BETWEEN TWO CUCURBIT GEMINIVIRUSES UNDER FIELD CONDITIONS. J. E. Polston[1], T. M. Perring[2] and J. Allan Dodds[1], [1]Dept. of Plant Pathology, [2]Dept. of Entomology, Univ. of California, Riverside, CA 92521.

Two geminiviruses have been recognized which cause squash leaf curl disease in southern California. Like other whitefly-transmitted geminiviruses, they have a bipartite DNA genome, and are antigenically indistinguishable. However, they have unique genome restriction sites and one has a broader host range (bean, squash and melon) than the other (bean, squash). A nucleic acid spot hybridization assay was developed which could distinguish among the genome components of these viruses, and interactions of these viruses at the component level were examined. We found only the broad host range virus components in melon plants collected from the field. In squash plants from the field various combinations of two and three viral components as well as complete mixed infections were found. This was similar to the results of mechanical inoculations from mixed infections to bean, another common host.

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TRIPLE NATURAL INFECTION OF WHEAT WITH THE WHEAT SPOT MOSAIC AGENT, WHEAT STREAK MOSAIC AND WHEAT STRIATE MOSAIC VIRUSES IN ALBERTA. C. Hiruki and M.H. Chen, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada.

In October 1987, volunteer wheat plants growing in Lethbridge, Alberta were found to be infested with mites (Eriophyes tulipae) and showed severe streak mosaic symptoms. Transmission electron microscopy of thin sections of infected leaf and root cells revealed the following intracellular structures: 1) membrane-bound, ovoid bodies, 0.1-0.2 μm in diameter, 2) flexuous, elongated particles, 700 nm long and 15 nm in diameter, and pinwheel inclusions, 3) bacilliform or bullet shaped particles, mostly 200-250 x 75 nm. Of these, only the agent of 1) was transmitted by E. tulipae to a Triticum x Agropyron hybrid that was immune to wheat streak mosaic virus. This agent was identified as the wheat spot mosaic agent (WSpMA). The agents of 2) and 3) were identified as wheat streak mosaic virus and wheat striate mosaic virus, respectively.

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NATURE OF RESISTANCE OF TOMATO SOMACLONES TO TOMATO MOSAIC VIRUS (ToMV). S. S. Smith and H. H. Murakishi, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Six tomato somaclones regenerated from a fully ToMV-susceptible line (GCRI-26) were selected for resistance to ToMV. The inheritance of this somaclonal resistance appears to be complex, involving multiple nuclear genes and a maternally inherited factor in each case. Somaclonal resistance has been characterized and compared to resistance conferred by the genes Tm-1, Tm-2, and Tm-2^2 for the following: temperature sensitivity, virus strain response, and response to other viruses. To gain a better understanding of the role of resistance in systemic movement of virus, plant grafting experiments were done using resistant and susceptible material. Detection of virus in protoplasts after protoplast infection or release from previously inoculated plants has helped to elucidate the resistance mechanism, which appears to involve limited virus multiplication and movement.

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MAGNETIC MICROSPHERE ELISA FOR DETECTION OF POTATO VIRUS X AND POTATO LEAFROLL VIRUS. E. E. Banttari, D. L. Clapper, S. P. Hu, and S. M. P. Khurana, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Magnetic microsphere ELISA (MM-ELISA) detected potato virus X (PVX) in infected sap from potato foliage serially diluted to 1 x 10^{-5} in either healthy potato sap or in 1.0% glycine buffer, pH 9.6 plus 0.1% T20. Purified PVX at 1-3ng/ml of buffer was detected. Potato leafroll virus (PLRV) was detected at dilutions of 1 x 10^{-3} to 2 x 10^{-3} of infected sap from potato in 0.1% Tris-HCl buffer pH 9.6 + 0.1% T20. Purified PLRV was detected at 3-10 ng/ml in the same buffer. Bio-Mag 4100 beads with covalently immobilized IgGs provided the immunosorbent solid phase and were separated from sample and washing buffer using a Corning Magnetic Separator. Alkaline phosphatase Ig conjugates and 5-bromo-4-chloro-3 indolyl phosphate and

nitroblue tetrazolium generated color that was quantitated at 562 nm. The test can be completed in approximately 1 hr and sensitivities were comparable to those obtained by dot-ELISA.

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INDUCTION OF IMMUNOLOGICAL TOLERANCE TO ENHANCE THE PRODUCTION OF VIRUS-SPECIFIC HYBRIDOMAS. H. T. Hsu, Y. C. Wang, and R. H. Lawson. USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, Md. 20705.

Suppression of immune response to normal host antigens was induced by injecting BALB/c mice 4 times with healthy plant extracts on days 1, 3, 5, and 7 after birth. Subsequently, 3 groups, 2 mice each, were injected once with partially purified tomato spotted wilt virus (TSWV) at age 5, 7, and 9 weeks, respectively. Mice not injected neonatally with host antigens but immunized once with TSWV at corresponding ages served as controls. Splenectomy and fusion of splenocytes with myeloma cells were performed 4 days after TSWV immunization for each group. Percentages of TSWV-specific hybridomas were 83%, 50%, and 39% for groups of mice immunized at 5, 7, and 9 weeks of age, respectively; those for controlled mice at 5 and 7 weeks were 0%, and 16%, respectively. Conventional immunization of adult mice (25- to 30-g size) with 3 TSWV injections resulted in only 20% virus-specific hybridomas.

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PRODUCTION OF RADIO-LABELLED PROBES AGAINST TOMATO RINGSPOT VIRUS (TomRSV). S. Mohan and T. A. Chen, Dept. of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903.

RNA and complementary DNA (cDNA) probes to Tomato Ringspot Virus (TomRSV) were synthesized and successfully used to detect the virus infection in diseased plants. Viral RNA was purified using a single phase phenol-SDS procedure and used for the preparation of cDNA and 5'-end labelled RNA probes. The viral RNA was dephosphorylated with bacterial alkaline phosphatase and 5'-end labelled with polynucleotide kinase and gamma 32P ATP. Complementary DNA to the virus RNA was synthesized using reverse transcriptase and calf thymus DNA-derived random primers. Instead of dCTP, alpha 32P dCTP was used in the reverse transcription. Both the probes detected the virus infection when partially purified RNA from the infected plants was used in dot blot hybridization.

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REACTIVITY OF A MONOCLONAL ANTIBODY TO THE AMORPHOUS INCLUSION PROTEIN OF PAPAYA RINGSPOT VIRUS-TYPE W (PRSV-W). C. A. Baker and D. E. Purcifull, Department of Plant Pathology, University of Florida, Gainesville, 32611.

A monoclonal antibody (MCA) to the amorphous inclusion protein (AIP) of PRSV-W was produced by injection of SDS-PAGE purified AIP into BALB/c mice and subsequent fusion of spleen cells to SP2/0 cells. The MCA is of the subclass IgG1 and reacts with purified AIP and sap of tissue infected with PRSV-W in both plate-trapped and antibody-trapped indirect ELISA. It reacted positively with 17 isolates of PRSV including 15 isolates of PRSV-W (one each from California, New York, Jordan, Greece, the ATCC, and 10 isolates from Florida), an isolate of PRSV-T from Guadeloupe, and an isolate of PRSV-P from Hawaii. All viruses tested were in pumpkin Cucurbita pepo L. ('Small Sugar'). There was no reaction with sap from healthy pumpkin, with purified PRSV-W capsid protein, or with purified PRSV-W cylindrical inclusion protein. This MCA appears to react with an epitope of a non-structural protein common to PRSV isolates with broad geographical and biological diversity.

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MONOCLONAL ANTISERA FOR DETECTION AND DIAGNOSIS OF LUTEOVIRUS INFECTIONS. C. J. D'Arcy, R. R. Martin and L. Torrance. Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C. V6T 1X2 and SCRI, Dundee, Scotland.

Twenty-five monoclonal antisera produced in Canada, the U.K. and the U.S. to 4 luteoviruses were tested for their ability to detect 7 luteoviruses in triple antibody sandwich ELISA. Antisera were to BYDV-PAV (3), BYDV-MAV (3), BYDV-RPV (5), BWYV (7), PLRV (5) and SDV (2). Plates were coated with polyclonal IgG homologous to the virus to be trapped and blocked with nonfat dry milk in PBS. Leaf samples ground in PBS + Tween + milk were incubated overnight. Monoclonal antisera, alkaline phosphatase conjugate and substrate were then added. The mimimum numbers of epitopes identified on each virus were 5 for PLRV, 4 for RPV and BWYV, 3 for PAV and SDV and 2 for MAV and BLRV. Common epitopes were identified on the following: MAV-PAV, PAV-PLRV, RPV-BLRV, RPV-PLRV, RPV-BLRV-BWYV, BWYV-PLRV, BWYV-SDV and PLRV-SDV. Antisera specific for MAV, PAV, RPV, BWYV, PLRV and SDV were found. None of the antisera tested detected BYDV-RMV, CRLV or SMYEV.

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PROPERTIES AND IN VITRO TRANSLATION OF MAIZE DWARF MOSAIC VIRUS RNA. P. H. Berger, C. S. Luciano, D. W. Thornbury, H. I. Benner, J. H. Hill, and R. J. Zeyen. University of Kentucky, Department of Plant Pathology, Lexington 40546.

The RNA of the Johnsongrass strain of maize dwarf mosaic virus (MDMV-A) was characterized and compared with RNA of strain B (MDMV-B), which does not infect Johnsongrass. Glyoxal-treated MDMV-A RNA has a M_r of 3.32 x 10^6 measured in agarose gels, compared with MDMV-B RNA at 3.41 x 10^6, under the same conditions. Translation products of both RNAs in a rabbit reticulocyte cell-free system ranged from 23 K to 121 K. Anti-virion antisera immunoprecipitated polypeptides, from the strain-specific translation products, which co-migrated with the authentic coat protein for each strain. Specific subsets of products reacted with tobacco etch virus anti-49K and anti-54K nuclear inclusion antisera; but not with antisera to helper component or cylindrical inclusion protein from either potato virus Y or tobacco vein mottling virus.

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NATURE OF RESISTANCE OF TN 86 TOBACCO TO TWO POTYVIRUSES. K. S. Gibb and T. P. Pirone. University of Kentucky, Lexington, KY 40546.

Comparative studies were done on the reaction of a "resistant" (Tn 86) and a susceptible (Ky 14) cultivar of tobacco to tobacco vein mottling virus (TVMV) and tobacco etch virus (TEV). In Tn 86, TVMV does not spread to uninoculated leaves, but can be recovered from inoculated leaves, while TEV infects systemically, but symptom expression is delayed and reduced. Using an immunostaining procedure to detect TVMV cylindrical inclusion protein (CI) in individual mesophyll cells, CI was detected in Ky 14 cells between 1 and 3 days after mechanical inoculation. With Tn 86, there was no evidence of virus in mesophyll cells up to 15 days following inoculation, indicating that the lack of movement of TVMV from inoculated leaves is due to restricted cell-to-cell movement. With TEV, virus movement into Tn 86 mesophyll cells occurred 2 days later than Ky 14 cells and fewer cells were infected, indicating that movement of TEV in Tn 86 is retarded.

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DEGRADATION IN SITU OF THE BOTTOM COMPONENT RNA OF BEAN POD MOTTLE VIRUS AS THE CAUSE OF DECLINE IN SPECIFIC INFECTIVITY. S. Kartaatmadja and O.P. Sehgal, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Bean pod mottle virus (BPMV) progressively loses infectivity in relation to the age of infection. The specific infectivity of virions isolated 20 days post-infection (p.i.) is only about 5% that of virions isolated 3 days p.i. Slight differences exist in the electrophoretic behavior and the mw of the small coat protein subunit from preparations having high and low infectivity, but these do not contribute directly to the decline in the infectivity. Gel electrophoresis reveals that RNA of the bottom BPMV component, but not that of the middle component, undergoes degradation in situ with increasing age of infection. Infectivity can be fully restored by replacing the bottom component in preparations from late infections with that from early infections.

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CYTOPATHOLOGY AND ULTRASTRUCTURE OF MAIZE CHLOROTIC MOTTLE VIRUS IN MAIZE LEAVES. E. D. Ammar and D. T. Gordon, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Maize leaves systemically infected with maize chlorotic mottle virus (MCMV), an isometric, beetle-transmitted, unclassified virus, were processed for light and electron microscopy 3 wk following mechanical inoculation. Aggregates of MCMV-like particles (VLP), frequently in crystalline array, were commonly observed in the cytoplasm and vacuoles of epidermal, mesophyll, phloem and bundle sheath cells. Large quasi-spherical inclusions, containing dense granular and/or fibrous material with rows or clusters of VLP at the periphery, were found in cell vacuoles. Elaborate membranous structures, apparently connected with the tonoplast and studded with VLP, frequently

protruded into cell vacuoles, sometimes connecting inclusions within the vacuole. This combination of cytopathological features appears unique among isometric plant viruses, suggesting that MCMV may be a member of an as yet undescribed plant virus group.

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MONOCLONAL ANTIBODIES AGAINST BARLEY YELLOW DWARF VIRUS INHIBIT CIRCULATIVE TRANSMISSION AT DIFFERENT SITES WITHIN APHID VECTORS. Stewart M. Gray, USDA/ARS, Ithaca, NY 14853.

Aphid vector specific transmission of barley yellow dwarf virus (BYDV) isolates is determined by the virus capsid protein (CP). Receptors on the aphid hindgut and accessory salivary gland membranes recognize CP and allow virus to be transported across the membranes. Monoclonal antibodies against the MAV and RPV isolates were used to define surface epitopes on CP involved in vector specificity. Homologous and heterologous combinations of virus and antibody were acquired by aphids through parafilm membranes or aphids were injected with an antibody prior to acquiring virus. One of 2 anti-MAV monoclonal antibodies reduced MAV transmission, but only when injected into the hemocoel of the vector. Two of 3 anti-RPV monoclonal antibodies reduced RPV transmission when injected. One was also effective when ingested. Heterologous combinations of antibody and virus did not reduce transmission. The differential effect on transmission suggests different epitopes are involved for each membrane receptor.

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PATH COEFFICIENT ANALYSIS OF EPIDEMIOLOGICAL DATA. J.H. Bowers and D.J. Mitchell. Dept. of Plant Pathology, Univ. of Florida, Gainesville, 32611.

Path coefficient analysis was conducted to determine the direct and indirect effects of rain variables and time on disease progress in the Phytophthora capsici-pepper pathosystem. Path analysis partitions the correlations among variables into the direct effect of each variable plus indirect effects through other variables to determine the relative influence of a variable on the variation observed in disease progress. Field plots were established in Delray Beach, FL in the spring and fall of 1984, the spring of 1985, and the fall of 1986. Rainfall was recorded daily and variables describing amount of rainfall in cm, days of rainfall, and intensity of rainfall between assessment dates and adjusted for latent periods of 1-7 days were calculated. Disease proportion was transformed by the logistic transformation before analysis. Each rain variable was highly correlated with all other rain variables and with disease progress (r_{ij}>.9). Cumulative cm of rain had a large direct effect on disease progress in all years and was a large component of the indirect effects of the other variables. Cumulative number of raindays and cumulative rain intensity values had lesser effects and were not consistent over time. Calendar time had relatively lesser effects. Rainfall data adjusted for a latent period of 5 days gave consistently high degrees of determination of disease progress.

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VIRUS DISEASES OF CHICKPEA IN CALIFORNIA. N. A. Bosque-Perez, I. W. Buddenhagen and J. E. Duffus, Dept. Agron. & R.S., University of California, Davis, and USDA, ARS, Salinas.

Winter planting of chickpeas in California has revealed their vulnerability to several aphid-transmitted viruses. Virus incidence of 95% with complete yield loss occurred in plots at Davis. Incidence was less in plots in the Central San Joaquin Valley and even less at Salinas. Both high and low incidence were recorded in large commercial plantings. Beet Western Yellows Virus was most frequent at all sites. Two other luteoviruses were also found: Legume Yellows in the Central Valley and Subterranean Clover Red Leaf, at Salinas and Davis. Mechanically transmissible viruses, not yet fully characterized, were also found. Virus transmission began in early March, increasing through April. Only two aphid species colonized the crop, Aphis craccivora, the most abundant, and Acyrthosiphon pisum, rarely. Other aphids found feeding on the crop were Myzus persicae and Hyperomyzus lactucae. Chickpeas, however, were shown to be a poor aphid host; experimental virus transmission proved difficult.

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FOREST PATHOLOGY RESEARCH & MANAGEMENT IN CHINA. M. M. Chen, Department of Plant Pathology, University of California, Berkeley, California USA 94720.

China and North America have similar flora and, since the recent establishment of plantations in China, have similar forestry practices as well. Therefore, many pathogens are of interest to pathologists on both continents. Research in silviculture, management and protection has been an important concern of forestry institutions in China, and has focused on detection, distribution, impact assessment and management of major pests causing conifer, poplar, and paulownia diseases, rusts, nematode problems, etc. Recently, we proposed research on the biology, evolution, biogeography and management of these pests in order to establish a network of scientific communication permitting development and evaluation of integrated pest management strategies and their transferability between continents.

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COMPARATIVE EPIDEMIOLOGY OF NET BLOTCH AND LEAF SCALD OF BARLEY. B. J. Steffenson, D. L. Grasmick, and R. K. Webster, Dept. of Plant Pathology, University of California, Davis, CA 95616.

Infection and spore release periods of Pyrenophora teres (the net blotch fungus) and Rhynchosporium secalis (the leaf scald fungus) were assessed in the field using mobile nursery plants (MNP). After a 48 hr exposure period in the field, half of the MNP were exposed to 48 hr of mist (under conditions conducive for infection by both pathogens) and half were placed directly in the greenhouse. This allowed for the separation of infection periods from spore release periods. The number of leaf scald lesions on MNP increased markedly during rain episodes, whereas the number of net blotch lesions decreased. The converse was true during periods of no rain when there was sufficient dew for infection by P. teres. Data on the number of net blotch lesions on MNP and the airborne spore concentration (ASC) of P. teres were subjected to time series analysis. Values for the cross correlation function indicated a high correlation (at lag zero) between the ASC and the number of lesions on MNP given a mist incubation.

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EFFECT OF FREE MOISTURE AND PLANT GROWTH STAGE ON DISEASE FOCUS DEVELOPMENT IN RHIZOCTONIA AERIAL BLIGHT OF SOYBEAN. X.B. Yang, G.T. Berggren, and J.P. Snow. Dept. of Pl. Path. & Crop Phys., La. Ag. Expt. Station, LSU Ag. Center, Baton Rouge, LA 70803.

Soybean aerial blight caused by Rhizoctonia solani spreads in the canopy by mycelial growth from leaf to leaf. To quantify disease focus expansion as affected by free moisture and plant growth stage, soybeans were planted in polyethylene chambers in a greenhouse. An inoculum source was introduced into the canopy at growth stages V2, V4-5, or V9. Free moisture treatments consisted of 12 h/day, 24 h/day, and 24 h/day followed by two periods of 12 h/day. Focus diameter was highly correlated with accumulated free moisture hours (AFMH). Dry interruption greatly slowed focus expansion. Number of diseased plants (DP) was predicted by the model $DP=3.142(BT)^2$, where T is AFMH and regression coefficient B is the focus expansion rate which differed in each moisture treatment and increased with growth stage.

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ASCOSPORE GERMINATION AND INFECTION OF *VITIS* BY *UNCINULA NECATOR*. David M. Gadoury and Roger C. Pearson, Dept Plant Pathology, Cornell Univ., N. Y. Agric. Exp. Stn., Geneva 14456.

Ascospores of *Uncinula necator* were mechanically released from ascocarps periodically from November (leaf fall) to May (bud break). Osmotic potential of ascospore cytoplasm decreased continuously during this period. Ascospores did not germinate in distilled water before December. Thereafter, until April, ascospores germinated only in the presence of free water, but frequently burst due to high turgor pressure. After April, ascocarps dehisced naturally when wet, the released ascospores rarely burst, and germinated in free water and at VPD 10.9 mm Hg (RH 54%) at 25 C. The percentage of germinated ascospores, ascospores with appressoria, and germ tube length increased as temperature increased from 10 to 25 C. Infection of *Vitis* tissue culture plants occurred at temperatures from 10 to 25 C. Ascospores germinated within 12 hr at 20 C, formed lobate appressoria, and occasionally formed multiple germ tubes. *U. necator* is generally considered to be a xerophyte whose anamorph is adversely affected by free water. However, free water is required for ascocarp dehiscence, ascospore discharge, and has no deleterious effect on ascospore germination.

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RELATIONSHIP OF INOCULUM SIZE AND DENSITY TO FUSARIUM SEEDLING BLIGHT OF CORN. L. G. Skoglund and W. M. Brown, Jr. Dept. Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523

Inoculum size (small = <1.0 mm, medium = 1.0-2.3 mm, large = 2.4-6.4 mm) of three Fusarium spp. did not affect percent infection of or lesion formation on 7-day-old corn seedlings. Inoculum density (0.1, 0.3, 1.0, 3.0, 10.0 mg/g air-dried soil) and Fusarium spp. (F. moniliforme, F. subglutinans, and F. graminearum) significantly affected percent seedling infection and lesion formation. Percent infection was high, reaching 100% at 3 mg with F. moniliforme and F. subglutinans. With F. graminearum, infection did not occur with the lowest inoculum density but reached 80% with the highest inoculum density. Lesion formation on mesocotyls and roots was low (<10%) with F. moniliforme and F. subglutinans at all inoculum densitities. F. graminearum caused more lesion formation on mesocotyls and roots than F. moniliforme and F. subglutinans even at low inoculum densities.

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INFLUENCE OF MOISTURE AND TEMPERATURE ON SHOT HOLE DISEASE OF ALMOND LEAVES. D.A. Shaw, Coop. Ext., Univ. of California, San Diego, CA 92123; J.E. Adaskaveg, and J.M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

Temperature and free-moisture period influenced the development of shot hole disease on almond leaves caused by *Stigmina carpophila*. In controlled-environment studies, a 14-hr moisture period resulted in an average of 0.1 and 45.0 lesions/leaf at 8 and 22 C, respectively. At 8-25 C, extended moisture periods increased the number of lesions. Temperature after the infection period influenced symptom expression, rate of lesion development, and lesion abscission but not the total number of lesions. Lesions were lighter in color and developed faster at 15-22 C than at 8 C. Lesion abscission was significantly greater at 22 C than at 8 or 15 C. Additional moisture after an initial infection period resulted in the formation of sporodochia after 16 days at 22 C. Results were verified in the field under controlled-moisture periods. A predictive model for the disease was developed based on temperature and moisture duration when inoculum was present.

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USE OF SIMULATION MODELS TO DEVELOP A THEORY FOR A LOW RISK STRATEGY TO SUPPRESS BOTH EARLY BLIGHT AND LATE BLIGHT IN POTATOES. Shtienberg, D., Doster, M.A., Pelletier, J.R., and Fry, W.E. Cornell. Ithaca, NY 14853.

Simulation models describing potato early blight development, potato late blight development and chlorothalonil dynamics were used to evaluate the relative contribution of each fungicide application in a weekly schedule (common grower practice). For early blight, early fungicide applications (3-5 weeks after planting) made no substantial contribution to disease suppression. Applications beginning at 6-7 weeks after planting were the first to make a positive contribution to disease suppression. For potato late blight, regardless of crop age, sprays applied at or within 2-3 weeks after inoculation, were most important. The date at which sprays can be safely terminated (date of last application) was similar for both diseases: approximately three weeks before vine kill. Based on these results, we developed a theoretical fungicide use strategy which should reduce the number of sprays without affecting risks.

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INFLUENCE OF LEAF REMOVAL AROUND FRUIT CLUSTERS ON EVAPORATIVE POTENTIAL WITHIN GRAPEVINE CANOPIES. J.T. English, A.M. Bledsoe, and J.J. Marois, Department of Plant Pathology, University of California, Davis 95616.

Removal of leaves around grape berry clusters controls Botrytis cinerea in California vineyards. In controlled experiments growth and reproduction of B. cinerea was inhibited by high values of evaporative potential. The relationship between evaporative potential, wind, temperature, and vapor pressure deficit in canopies with and without leaf removal was evaluated. As determined by multiple regression and path analysis, evaporative potential outside canopies was influenced most strongly by vapor pressure deficit and wind speed. In contrast, significant increases in evaporative potential in canopies with leaves removed depended upon large increases in wind speeds; vapor pressure deficits were very similar in canopies with and without leaf removal. Atmometers were shown to be effective in quantifying evaporative potential in grapevine canopies.

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A SIMULATION MODEL FOR S. NODORUM-APPROACHES AND IMPLEMENTATIONS. A. M. Djurle and J. E. Yuen, Department of Plant and Forest Protection, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden and Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A.

A simulation model for Septoria nodorum was developed by adding routines to a wheat growth model. The model simulates disease on each of 10 leaf levels and the ear. The progression of lesions through different development stages was done with a series of delay functions. Lesion growth and fusion was simulated by increasing the size and decreasing the number of lesions between the different stages. An exponential function was used to distribute inoculum in the vertical direction. The driving variables of the model are temperature, rain, evaporation, solar radiation and relative humidity. Since the disease simulation is based on a crop growth model, it can also be used to study the effect of different epidemics on yield loss.

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MODIFICATION OF A WHEAT GROWTH MODEL FOR FOLIAR DISEASE SIMULATION. J. E. Yuen and A. M. Djurle. Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A. and Department of Plant and Forest Protection, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden.

An existing growth model for winter wheat was modified in order to develop a disease simulation model. The original model was based on phasic development, operated on a daily timestep and was driven by temperature, rain, evaporation and solar radiation. This model was modified by representing the leaf canopy as individual leaves. Leaf area and age were simulated for each of 10 leaves by coupling leaf initiation and dying to fixed development stages of the crop. Assimilate partitioning was also modified in order to mimic canopy development. Yield prediction was retained in the modified growth model enabling studies of disease development and yield loss caused by the disease.

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CORRELATION OF ENVIRONMENTAL VARIABLES WITH POTATO YIELD REDUCTION CAUSED BY VERTICILLIUM DAHLIAE AND PRATYLENCHUS PENETRANS. L. J. Francl, L. V. Madden, R. C. Rowe and R. M. Riedel. USDA/ARS, Beltsville, MD, and OSU/OARDC, Wooster, OH.

Variation in 8 yr of absolute and relative yields (RY) of potato cv. Superior in microplots was expressed as residuals from a regression equation that used preplant pathogen densities as predictors. Degree days (DD), number of days with a mean temperature > 24 C, and ratio of precipitation (PPT) to DD were calculated for intervals of length 6, 12, 18,...84 d, beginning 2 d after planting (DAP); then, interval onsets were advanced by successive increments of 3 d. Yields of all treatments were negatively correlated ($P<0.05$) with DD 50 to 67 DAP. V. dahliae alone and V. dahliae + P. penetrans treatments extended this to 82 DAP. RY from these latter treatments were also negatively correlated with DD 17 to 43 DAP. Correlations with the > 24 C stress index corroborated these results. Correlation of RY with PPT/DD suggested a negative effect of high ratios 29 to 58 DAP for plants with early dying syndrome.

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MAIZE CHLOROTIC DWARF VIRUS (MCDV) SPREAD IN MAIZE FROM POINT SOURCES. L. V. Madden, J. K. Knoke, and R. Louie, Dept. of Plant Pathology, Ohio State Univ., and USDA/ARS, Wooster, 44691.

Viruliferous Graminella nigrifrons leafhoppers were released in the center of May- and July-planted maize plots in 1984-86. Disease incidence (y) was assessed at least twice after insect release and represented as the proportion of plants infected by MCDV for 80-cm wide annuli from the source. Disease gradients were best described by the log-logistic model, i.e., logit of y versus ln(distance) was a straight line. The model indicated that the rate of spread was proportional to y, $1-y$, and 1/distance. The spread parameter (b), a measure of the gradient steepness, ranged from 1.33 for the early planting of 1985 to 2.0 for the late planting of 1984. At ~21 days after release, the distance at which y declined to 10% ranged from 124 cm to 494 cm. MCDV spread, therefore, can be substantial when relatively low numbers of leafhoppers are introduced into a field of susceptible maize.

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PRODUCTION AND CHARACTERIZATION OF ANTISERA SPECIFIC FOR SPIROPLASMA CITRI SURFACE PROTEINS. J. Fletcher, C. Wijetunga, and P. Zuber. Departments of Plant Pathology and Microbiology, Oklahoma State University, Stillwater, OK 74078

Spiroplasma citri strain BR3 surface membrane proteins of 89, 77, 58, and 29 KDa, were electrophoretically purified and individually injected into rabbits for antiserum production. Reactivity of antisera was assayed on protein extracts of whole cells by Western blotting, and anti-spiralin (p29) activity was removed from the first three sera by cross-absorption to spiralin. The resulting monospecific sera, and anti-whole cell serum, were compared for effects on S. citri. Anti-spiralin serum deformed spiral morphology, inhibited growth on solid medium and metabolism in liquid medium. The activity of the anti-spiralin serum was less than that of anti-whole cell serum. Anti-p89, -p77, and -p58 sera had no effect in any of the tests, compared to preimmune sera, suggesting that antibodies to these proteins do not contribute to the inhibition activity of the anti-whole cell serum.

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CLAVIBACTER XYLI SUBSP. CYNODONTIS: PRELIMINARY DISTRIBUTION STUDIES IN THE UNITED STATES AND FRANCE. S.J. Kostka, P.W. Reeser, J-P. Prunier*, and J. Flynn. Crop Genetics International, Hanover, MD 21076 and *INRA, Montfavet, France.

Clavibacter xyli subsp. cynodontis (Cxc) was isolated from bermudagrass from 9 of 14 states surveyed in the United States (Maryland, Georgia, Florida, Alabama, Mississippi, Louisiana, Tennessee, and Illinois) and two departments in France (Alpes-Maritimes and Var). Bermudagrass stems were cut, sap expressed, and isolations madeon appropriate media. Identity of isolates was based on media specificity, serological cross-reactivity to C.xyli subsp. xyli, (using immuno-fluorescent antibody staining and/or radio-immunoassay) colony color, cell morphology, physiological and biochemical tests, and fatty acid profiles. Isolates were indistinguishable from the type culture (TB1A). Bacterial populations as high as 1 x 10^{10} cells/ml of extracted xylem sap were observed. None of the sampled clones showed any visual evidence of stunting.

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EFFECTS OF CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) COLONIZATION ON GROWTH AND YIELD OF CORN. G. Johnson, S.F. Tomasino, and J. Flynn. Crop Genetics International, Hanover, MD 21076.

Field studies were conducted in Maryland, Louisiana and Florida in 1987 to determine the effects of Cxc on height and yield of corn. Seedlings of several corn varieties were wound inoculated with 1 x 10^9 cfu of Cxc in phosphate buffered saline (pH7). An average of 70% of the plants were confirmed colonized by Cxc 5 weeks after inoculation. No consistent differences in plant height occurred between Cxc colonized and control plants. No significant differences in yield between Cxc inoculated and control plants were seen in 20/27 trials.

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FIELD RELEASE OF A TRANSFORMED STRAIN OF CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) CONTAINING A DELTA-ENDOTOXIN GENE FROM BACILLUS THURINGIENSIS SUBSP. KURSTAKI (BT). S.J. Kostka, S.F. Tomasino, J.T. Turner, and P.W. Reeser. Crop Genetics International, Hanover, MD 21076.

Two field tests are being conducted in Maryland to test the environmental fate of a Cxc/Bt recombinant (CG525). Each site is less than 0.8 ha in size. The plot consists of a central area planted in corn (<0.6 ha.) surrounded by a plant-free barren zone, a parameter of trap plants (corn and bermudagrass), a dike and a security fence. Test paremeters include i) colonization studies, ii) evaluation of natural and mechanical dispersal in corn and weeds, iii) persistence in plant residues, soil and run-off water, and iv) yield. Sensitivity of detection methods range from 1 x 10^2 to 1 x 10^4 cfu/g of sample and depend on sample type. Detailed sanitation procedures have been implemented to preclude movement of the recombinant from the test site on personnel or equipment. Preliminary results will be presented.

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EXPERIMENTAL HOST RANGE OF CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) AND A CXC/BACILLUS THURINGIENSIS RECOMBINANT (CXC/BT). S. J. Kostka, P. W. Reeser, and D. P. Miller. Crop Genetics International, Hanover, MD 21076.

The only known natural host of Cxc is bermudagrass (Cynodon dactylon L.). The ability of Cxc and Cxc/Bt to colonize some common horticultural and agronomic plants and weeds was tested under greenhouse conditions. Seedlings were inoculated with up to 1 x 10^{10} cfu of the bacterium and harvested 30-45 days post-inoculation. Degree of colonization was rated according to incidence and cell counts from expressed sap. Degree of colonization varied widely among experimental hosts. The experimental host range included representatives of at least 83 species in 26 families. Non-hosts included 52 species in 26 families. No pathogenic effects were observed. In further studies, quantitative methods were employed to determine the population dynamics of Cxc in host plants, and to categorize hosts which support growth of Cxc and hosts in which Cxc may persist but not replicate.

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POPULATION DYNAMICS OF CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) AND A CXC/BACILLUS THURINGIENSIS SUBSP. KURSTAKI (BT) RECOMBINANT IN CORN (ZEA MAYS). P. W. Reeser and S. J. Kostka, Crop Genetics International, Hanover, MD 21076.

To serve as biopesticides, genetically-engineered, endophytic micro-organisms must achieve sufficient populations in appropriate plant parts to produce effective doses when the target insects are active. Ten day-old corn seedlings were inoculated with 1 x 10^7 cfu of Cxc or a Cxc/Bt recombinant. Populations of Cxc or a Cxc/Bt recombinant reached 1 x 10^7 cfu/gm fresh weight in the whorl within 7 - 11 days after inoculation, and 1 x 10^7 to 1 x 10^8 cfu/gm fresh weight in the basal internode 21 days after inoculation. In mature plants, basal internode populations were in excess of 1 x 10^9 cfu/gm fresh weight, and stem, leaf, and husk segments throughout the plants had populations between 1 x 10^7 and 1 x 10^9 cfu/gm fresh weight. All corn varieties tested were similarly colonized.

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SEED TRANSMISSION OF CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) IN CORN. P.W. Reeser and M.L. Sommerfeld. Crop Genetics International, Hanover, MD 21076.

Studies were conducted to determine if efficient seed transmission of Cxc occurs in corn. Progeny from Cxc colonized plants grown in 1986 were harvested and assayed for Cxc. Cxc colonized 4 out of 2082 (0.2%) plants tested. Examination of a large seedlot from the variety (Doebler 89XC) with the highest incidence of Cxc colonization did not yield colonized progeny (0/654). In the sweet corn cultivar, Seneca Horizon, none of the 419 progeny from known colonized plants (1 x 10^7 to 5 x 10^6 cfu/g) were colonized by Cxc. To detect colonization of seeds by Cxc, seeds from colonized plants were washed and rinsate assayed. The seeds were then homogenized and assayed. Cxc recovery from seed surfaces ranged from 9.8 x 10^2 to 2.6 x 10^3 cfu/seed, and populations from homogenates were less than 1.3 x 10^0 cfu/seed.

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MECHANICAL TRANSMISSION OF A CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) AND A CXC/BACILLUS THURINGIENSIS SUBSP. KURSTAKI (BT) RECOMBINANT. S.F. Tomasino, G. Johnson, P.W. Reeser, and S.J. Kostka. Crop Genetics International, Hanover, MD 21076.

Mechanical transmission studies involving Cxc and a Cxc/Bt recombinant were performed under greenhouse conditions. Cutting tools contaminated by trimming through colonized corn were used to trim uninoculated corn, bermudagrass, and marigolds. In the field, corn plants colonized by Cxc were used as inoculum foci to determine transmission to seven weed species via trimming and cultivation. Natural dispersal of Cxc was also examined in the field. In greenhouse studies, both Cxc and the Cxc/Bt recombinant were transmitted efficiently on cutting tools. No differences in transmission were observed between Cxc and the Cxc/Bt recombinant. Under field conditions, mechanical transmission by repeated trimming and/or cultivation was very limited. Incidental spread of Cxc in the field was rarely observed with only 3 of 2,264 total test plants sampled outside the inoculation foci being colonized.

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PERSISTENCE OF CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC) AND A CXC/BACILLUS THURINGIENSIS RECOMBINANT (CXC/BT) IN SOIL, WATER, AND PLANT DEBRIS. J.T. Turner, and S.F. Tomasino, Crop Genetics International, Hanover, MD 21076.

Populations of marked strains of Cxc and a Cxc/Bt recombinant were monitored in soil, water, and colonized plant debris. Neither Cxc nor the Cxc/Bt recombinant were detected beyond 42 days at 20°C, or beyond 7 days at 30°C in water collected from any of 5 sources. In growth chamber experiments neither was detected in soil after 28 days at 5°C; higher temperatures resulted in more rapid decline with populations reaching undetectable levels by 7 days at 30°C and by 24 hours at 40°C. Under field conditions Cxc was not detected 2 weeks after its addition to the soil. Cxc did not persist beyond 18 days in green corn residues chopped and incorporated into the soil. Cxc populations declined in corn plants during senescence; no Cxc was recovered beyond early November in stalks either standing or lying on the soil surface.

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CLONED DNA AND RNA PROBES FOR DETECTION OF ASTER YELLOWS DISEASE MYCOPLASMALIKE ORGANISM (MLO). I.-M. Lee and R. E. Davis, USDA, ARS, Microbiology and Plant Pathology Laboratory, Beltsville, Maryland 20705.

A method was developed for enriching MLO DNA in extracts from diseased plants of Catharanthus roseus. Using enriched extracts, DNA of the aster yellows (AY) MLO was cloned in sp6 plasmid vectors. ^{32}P labeled and biotinylated DNA and RNA probes were prepared from the cloned recombinant plasmids and used for MLO detection by dot blot hybridization. The probes all hybridized specifically with nucleic acid from AY MLO-infected, but not healthy plants. Some probes hybridized also with nucleic acid from plants infected by other MLOs. Both DNA probes and riboprobes were useful for MLO detection, but riboprobes were more useful in differentiating AY from other MLO diseases.

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OCCURRENCE OF CLAVIBACTER XYLI SUBSP. CYNODONTIS IN CALIFORNIA AND RECOVERY FROM XYLEM-FEEDING LEAFHOPPERS. Alexander H. Purcell and Karen G. Suslow. Department of Entomological Sciences, University of California, Berkeley, CA 94720.

C. xyli subsp. xyli (Cxc) was isolated from Bermuda grass from two of nine California localities sampled: Indio and Bakersfield. Identifications of Cxc were based on cultural characteristics on Davis' SCMS medium and by radio-immunoassay. Cxc was readily transmitted to Bermuda grass by needle puncture. Attempts to transmit Cxc from Bermuda grass to maize or Bermuda grass using the xylem feeding leafhoppers Carneocephala fulgida and Draeculacephala minerva were negative, but Cxc was regularly isolated from both leafhopper species after they had fed on Cxc-colonized Bermuda grass.

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MULTIPLICATION AND TRANSLOCATION OF THE PIERCE'S DISEASE BACTERIUM IN GRAPEVINES. S. M. Fry and R. D. Milholland, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Petioles of 6-mo-old plants of the bunch grapevine French Colombard (susceptible), and the muscadine vines Carlos (tolerant) and Noble (resistant) were inoculated with a strain of the Pierce's disease bacterium (PDB). The number of PDB per cm petiole and leaf vein was determined over time. The mean number of PDB in petioles of French Colombard ($\sim 10^5$ cfu/cm) was significantly higher than that of Carlos and Noble ($\sim 10^4$ cfu/cm). PDB rapidly colonized leaf veins of all cultivars. Two strains of PDB were used to inoculate stems of 5-wk-old plants. The number of PDB per cm section of stem taken 10, 20, and 50 cm above the inoculation point was determined over time. Either strain was detected in French Colombard at populations of $\sim 10^5$-10^6 cfu/cm stem after 8 wk. The C strain was not detected in Carlos and Noble, but the FC strain was recovered from both cultivars at $\sim 10^2$-10^4 cfu/cm stem after 8 wk.

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IMPROVED MEDIA FOR THE CULTURE OF CLAVIBACTER XYLI SUBSP. CYNODONTIS. J.J. Anderson, and Lisa Flaherty. Crop Genetics International, Hanover, MD 21076

C. xyli subsp. cynodontis can be cultured on solid (SC) and liquid (S8) medium as described (Davis, et al. Science 210: 1365, 1980). We have produced simplified versions which eliminate bovine serum albumin and hemin chloride and are optically clear. The plate medium (GC) uses Gelrite™ and employs a tryptone-soytone base. Glucose is required as well as L-cysteine; efforts to replace L-cysteine with L-methionine, L-cystine and reducing agents such as ascorbic acid were unsuccessful. The growth of nine Cxc isolates on the new broth (S27) equalled or exceeded the growth rate in the original medium. The GC plate's optical clarity allowed the visualization and enumeration of 0.1 mm Cxc colonies. A K-maleate buffered version of S27 was used for ^{32}P labelling studies.

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BIOTINYLATED CLONED DNA FRAGMENTS FROM AN UNKNOWN MYCOPLASMALIKE ORGANISM (MLO) DETECT INFECTION IN SEVERAL PLANT DISEASES. R. E. Davis, I.-M. Lee, S. M. Douglas, and E. L. Dally. USDA, ARS, Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705, and Connecticut Agricultural Experiment Station, New Haven, CT 06504.

DNA of an unknown mycoplasma (ORCHl MLO), discovered in trap plants of Catharanthus roseus in peach orchards affected by eastern X (EX) disease, was cloned in Escherichia coli using sp6 plasmid vectors. Fifteen biotinylated probes prepared from recombinant plasmids were used in dot blot hybridizations for MLO detection. All hybridized with nucleic acid extracted from ORCHl MLO-infected, but not healthy or EX-diseased, C. roseus. Also detected were MLOs of other diseases including aster yellows, western X, clover proliferation, potato witches broom, and tomato bid bud in C. roseus, as well as alfalfa witches broom in alfalfa (Medicago sativa L.), aster yellows in China aster (Callistephus chinensis), celery (Apium graveolens L.), and Macrosteles fascifrons, and ash yellows in ash (Fraxinus spp.). The results indicate partial homology among a broad array of MLOs, which permitted detection of MLOs whose DNA has not yet been cloned.

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CLONING AND PARTIAL SEQUENCE OF THE 16S RIBOSOMAL RNA GENE FROM THE WESTERN X-DISEASE MYCOPLASMA-LIKE ORGANISM. B. C. Kirkpatrick and J. D. Fraser, Department of Plant Pathology, University of California, Davis, CA 95616.

The sequence of the 16s ribosomal RNA (rRNA) which is widely used to study procaryotic phylogeny, is being determined for the western X-disease mycoplasma-like organism (WX-MLO). Southern blots of healthy and WX-MLO-infected celery DNA were probed with labelled fragments of pKK3535 which contains the 16s rRNA gene of E. coli. A 3.1 Kb probe containing the entire E. coli 16s rRNA gene hybridized with two unique DNA fragments, 9.5 and 1.9 Kb, of EcoRI/HindIII digested, WX-MLO-infected celery DNA. A smaller, 0.7 Kb fragment of pKK3535, containing most of the 3' end of the E. coli 16s rRNA gene hybridized with a WX-MLO-specific, 0.8 Kb EcoRI/BamHI fragment but did not hybridize with the E/H 1.9 Kb WX-MLO fragment. These results suggest the 1.9 Kb E/H and the 0.8 Kb E/B WX-MLO-specific fragments should contain most of the WX-MLO 16s rRNA gene. The 0.8 and 1.9 Kb fragments have been cloned in E. coli and the partial sequence of the 1.9 Kb E/H fragment determined.

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PROTOPLAST RELEASE AND FUSION IN USTILAGO HORDEI. Caroll E. Henry, E. Steward-Clark. Chicago State University, Ninety-Fifth Street at King Drive, Chicago Illinois 60628

Heterozygous diploids of Ustilago nordei are necessary for mitotic recombination and linkage studies. Diploids were obtained by protoplast release and fusion. Twenty-four hour shake cultures of auxotrophs I_4A and E_3a mating types, grown in YEG (yeast extract glucose), were centrifuge at 2000g for 20 min. and the pellets obtained contained 10^8 cells/ml. Pellets were then resuspended in 100mM Tris HCl, 5mM EDTA and 5mM DTT(dithiothreitol) incubated for 30 min. After centrifugation at 2000gfor 20 min the pellets were resuspended in 1.2M sorbitol, 5mM DTT, 2mg/ml -glucoronidase and 2mg/ml Novozym 234 and incubated for 60 min. at 30C, 98% protoplast release was obtained. The protoplasts were freed from debris by centrifygation and resuspended in 0.3M $CaCl_2$. Fusion of compatible auxotrophs was obtained by treatment with 4500 mwt. PEG (polyethylene glycol). Diploids were detected by plating on osmotically stabilized minimal medium. This technique is effective for obtaining diploids in U. hordei

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INHERITANCE OF RESISTANCE TO FOOT ROT (PSEUDOCERCOSPORELLA HERPOTRICHOIDES) IN THREE CULTIVARS OF WINTER WHEAT. C. A. Strausbaugh and T. D. Murray. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Inheritance of foot rot resistance was studied in parental, F1, F2, and backcross populations of all possible crosses between the cultivars VPM-1 (resistant), Cappelle-Desprez (moderately resistant), and Daws (susceptible). Epidermal cell responses were used to determine the percent successful penetrations in the first-leaf-sheath. One semidominant gene for resistance with narrow-sense heritability (H) = 0.34 segregated in the Cappelle X Daws cross; one dominant gene for resistance with H = 0.35 segregated in the VPM X Daws cross; and two genes for resistance exhibiting overdominance with H = 0.77 segregated for resistance in the VPM X Cappelle cross. Maternal effects were not evident. Ratings derived from these epidermal cell responses are correlated with field resistance of the parents, genetically associated with resistance in segregating progeny, and may be useful for screening potential cultivars.

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INHERITANCE OF RESISTANCE TO RACES 2 AND 33 OF DOWNY MILDEW IN SOYBEAN. S. M. Lim, USDA-ARS and Department of Plant Pathology, University of Illinois, Urbana, IL 61801

The objectives of this study were to determine the inheritance of resistance to Peronospora manshurica races 2 and 33 in PI 88.788 a soybean line previously identified as resistant to the two races and to determine whether the genes conferring resistance in PI 88.788 and cv. Union which carries the gene Rpm resistant to race 2, are allelic or nonallelic. The F_2 seedlings of Union x PI 88.788 segregated 3 resistant : 1 susceptible when inoculated with race 33 and 15 resistant : 1 susceptible when inoculated with race 2 indicating that the gene for resistance in PI 88.788 segregates independently from the Rpm gene of Union. The F_3 families from the cross PI 88.788 x Union segregated in the ratio of 7 homozygous resistant, 4 segregating in a ratio of 15 resistant : 1 susceptible, 4 segregating in a ratio of 3 resistant : 1 susceptible, and 1 homozygous susceptible. The gene symbol Rpm_2 was assigned to the gene in PI 88.788.

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INHERITANCE OF RESISTANCE IN SOME EUROPEAN AND WORLD DIFFERENTIAL CULTIVARS TO NORTH AMERICAN RACES OF PUCCINIA STRIIFORMIS. Xianming Chen, and Roland F. Line. Dept. of Plant Pathology, Washington State Univ. Pullman, WA 99164-6430.

Inheritance of stripe rust resistance in Clement, Compair, Heines Kolben, and Heines Peko was studied in parental, F1, BC1, and F2 seedlings using seven races of Puccinia striiformis. Each cultivar has two resistance genes. The results confirm that Clement has Yr9, Compair has Yr8, Heines Kolben has Yr6, and Heines Peko has Yr2 and Yr6. The second genes in Clement and Heines Kolben are not Yr2, as previously suggested. The additional genes in Clement, Compair, and Heines Kolben are different from one another and have not been previously reported. Yr8 and Yr9 were dominant in all tests. The second gene in Clement was dominant or recessive depending upon race. The second gene in Compair was dominant to most races, but incompletely dominant to one race. Yr2, Yr6, and the second gene in Heines Kolben were dominant or recessive depending upon race and cross.

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pH AND ACIDITY IN GUMMING DISEASE AFFECTED RED SPANISH PINEAPPLE FRUITS. L. J. Liu, E. Rosa-Marquez and E. Lizardi, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P.R. 00927

Gumming disease, incited by Bratrachedra sp. has become an increasingly important problem on pineapple in Puerto Rico. The principal cultivar, Red Spanish which occupies more than 90% of the total pineapple acreage, is highly susceptible to the disease. The cultivars, PR1-67 and Smooth Cayenne which were relatively resistant are now being affected in the humid areas of Manati. Results of pH studies using dyes indicate that the average pH of the Red Spanish fruits with gumming disease is slightly lower than that of affected fruits. Studies made by the Central Analytical Laboratory of the Agricultural Experiment Station confirmed our findings that Red Spanish pineapple fruits with the gumming disease have a higher acidity than those without the malady.

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VIRULENCE AND RACE DYNAMICS OF PUCCINIA RECONDITA IN CANADA FROM 1956 THROUGH 1987. J.A. Kolmer, Agriculture Canada, Winnipeg MB. Canada, R3T 2M9.

The changes in virulence and UN race composition since 1956 of wheat leaf rust in three regions of Canada were examined. Levels of virulence with respect to specific resistance genes were generally variable in the Pacific and Eastern regions, even though susceptible wheats have been grown in these areas. In the Prairies, virulence levels on lines with specific resistance genes increased in a manner consistent with directional selection, after the release of resistant wheat cultivars. UN races were found at variable frequencies in the Eastern and Pacific regions, without strong directional trends. In the Prairies, changes in the race composition could generally be related to changes in the individual virulences, and the presence of specific resistance genes in the host population.

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DIFFERENTIATION OF STRAINS AND PATHOGENIC RACES OF FUSARIUM OXYSPORUM F.SP. NIVEUM BASED ON VEGETATIVE COMPATIBILITY. R. P. Larkin, D. L. Hopkins, and F. N. Martin, Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611.

Over 250 isolates of Fusarium oxysporum were tested for pathogenicity on watermelon and used to determine vegetative compatibility groups for F. oxysporum f.sp. niveum. Isolates were collected from infected watermelon plants, soil samples from a wilt-infested field, and known f.sp. niveum strains imported from various locations around the world. Vegetative compatibility was assessed by pairing complementary nitrate-nonutilizing mutants on a nitrate medium. All pathogenic isolates belonged to one of three distinct vegetative compatibility groups (VCG's) and were not compatible with any nonpathogenic isolates. Race 1 isolates from North America, Taiwan, and Australia were all contained within a single VCG. Race 2 isolates from Texas were compatible only with highly virulent Florida isolates, corroborating pathogenicity tests and confirming the presence of race 2 in Florida. A third VCG was comprised only of isolates from Florida, with race designations pending results of pathogenicity tests. Additional relationships between race and vegetative compatibility groups will be discussed.

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MITOCHONDRIAL DNA POLYMORPHISM IN *Phytophthora infestans*. S. B. Goodwin and W. E. Fry, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Phytophthora infestans isolates from Europe, Israel, Mexico, and the United States were tested for mitochondrial DNA (mtDNA) polymorphisms. A polymorphism was found among the Israeli isolates. The aberrant phenotype consisted of an addition of over 1.5 kb to the mitochondrial genome. Because the increased genome size was accompanied by the loss of an Eco RI site and altered migration distances of three different Eco RI fragments when compared to wild type, it does not appear to be a simple insertion, but involves some type of rearrangement of the mitochondrial genome. The *P. infestans* mtDNA restriction map is being extended to include additional enzymes; this information will be used to further elucidate the nature of the mutant mtDNA phenotype. Cloned fragments of the mitochondrial genome are being tested for use as probes to study the population genetics of this polymorphism.

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ISOZYME ANALYSIS OF RACES AND VEGETATIVE COMPATIBILITY GROUPS OF FUSARIUM OXYSPORUM F. SP. LYCOPERSICI. K.S. Elias and R. W. Schneider. Dept. Plant Path. and Crop Physio., La. Agric. Exp. Sta., LSU Agric. Center, Baton Rouge, LA 70803.

Isozyme analysis of mycelial proteins of Fusarium oxysporum f. sp. lycopersici (FOL) was employed to estimate genetic diversity among and between isolates previously characterized as to formae speciales, race, geographic origin, and vegetative compatibility group (VCG). A worldwide collection of 120 isolates of FOL, other f. spp., and the recently isolated FOL race 3 from California were included. Proteins were extracted from mycelia, electrophoresed on a starch gel, and stained to visualize specific enzymes. The presence or absence and relative electrophoretic mobility of all enzyme loci were recorded for each isolate. Isolates of different geographic origin, race and VCG showed few isozyme polymorphisms. Phylogenetic analyses to determine genetic diversity within and among races, VCGs and formae speciales were conducted and these findings will be discussed relative to the origin of races within FOL.

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PHYTOPHTHORA DISEASE REACTIONS IN SOMATIC EMBRYO REGENERANT SOYBEAN LINES. A. F. Olah and A. F. Schmitthenner, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691

During 1986 immature field-grown embryos were excised from 30 public and private cultivars of field-grown Group III soybeans and placed into tissue culture using procedures to induce somatic embryos. A total of 84 regenerant lines (Ro) were established and R1 seed of these was produced during 1987. Progeny testing for culture-induced stable resistance changes to Phytophthora megasperma f.sp. glycinea, races 1,3,4,7,12 and 16, showed 12 regenerant lines, representing five cultivars had changed in at least one resistance allele. Eight changes were from susceptible to resistant for races 1 or 3. Four lines became susceptible to race 3. Changes in reaction to races 12 and 16 were noted in 10 lines, but these were variable and may represent continuing segregation.

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INHERITANCE OF PATHOGENICITY OF MELAMPSORA LINI culture X82. G. D. Statler, Dept. of Plant Path., NDSU, Fargo, ND 58105.

Races 1 and 400 of Melampsora lini, the causal agent of rust of flax (Linum usitatissimum), were crossed and the resulting F_2 progeny evaluated on near isogenic flax lines. The infection types of the parental cultures, the F_1, and the segregating F_2 progeny were used to evaluate the inheritance of pathogenicity. Races 1 and 400 as well as the F_1 and all the F_2 progeny were avirulent on the N gene indicating cross X82 to be homozygous avirulent. The F_1 and all the F_2 progeny were virulent on flax lines containing L^9, M^1, M^4, and P indicating cross X82 to be homozygous for virulence. Segregation ratios of the F_2 cultures fit theoretical monogenic ratios on flax lines with host genes K, L^2, L^5, L^6, L^7, L^{11}, M, M^2, M^3, N^1, P^1, P^2 and P^3. Race 1 was avirulent, race 400 virulent and the F_1 avirulent on these lines. Several linkage groups were indicated including P^1, P^2 and P^3. Segregation on lines L, M^5 and cultivars Flor, Dufferin, Linott, and Wishek fit a digenic model.

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THE GENETIC CONTROL OF VIRULENCE IN PHYTOPHTHORA INFESTANS AGAINST POTATO AND TOMATO RESISTANCE GENES. L.J. Spielman, B.J. McMaster, and W.E. Fry, Cornell University, Ithaca, NY 14853.

The genetic control of virulence (specific compatibility) was investigated in crosses between Mexican isolates of Phytophthora infestans. A detached-leaflet assay method was used to score parents and progeny for the presence of compatible or incompatible reactions. Potato cultivars carrying single genes for resistance to late blight (R1, R2, R3, or R4) and a tomato cultivar carrying a single resistance gene, Ph1, were used as testers. Virulence/avirulence against tomato gene Ph1 segregated in progeny of two avirulent parents, indicating that avirulence against Ph1 is dominant. Virulence/avirulence against potato gene R2 segregated in two different crosses in which both parents were virulent. Virulence/avirulence against R4 behaved similarly in a cross between two virulent parents. Therefore, for both R2 and R4, virulence is dominant. In a cross involving a virulent parent and an avirulent parent against R3, all the progeny were avirulent, indicating that virulence against R3 is recessive.

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GENETIC RELATIONSHIPS OF *PSEUDOMONAS SYRINGAE* PATHOVARS BASED ON RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS. Timothy P. Denny. Dept. of Plant Pathology, UGA, Athens, GA 30602.

Total DNA was isolated from 63 strains of *Pseudomonas syringae* that represented 22 pathovars. The DNAs were completely digested with *Eco*RI, electrophoresed through 0.6% agarose gels and transferred to nylon membranes. Eight different cosmid clones of *P. syringae* DNA were used as hybridization probes of the DNA blots to detect restriction fragment length polymorphisms (RFLPs). A preliminary analysis suggested the following grouping of the pathovars: group 1-*glycinea, hibisci, lachrymans, savastanoi, tabaci, mori,* and *morsprunorum*; group 2 -*papulans, pisi,* and *syringae*; group 3-*atropurpurea* and *coronafaciens*; and group 4-*antirrhini, berberidis, maculicola, tomato,* and *persicae.* Pathovars *delphinii, passiflorae, striafaciens* and *tagetis* did not appear to be related and did not fit in any of the four groups. Cluster analysis of distance matrices constructed from the RFLPS will be used to create dendrograms that reveal the genetic relationships.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) MAPPING OF A GENE FOR RESISTANCE TO MAIZE DWARF MOSAIC VIRUS (MDMV). M.D. McMullen and R. Louie, USDA-ARS and the Depts. of Agronomy and Plant Pathology, OARDC, The Ohio State Univ., Wooster, OH 44691.

Genetic analysis with chromosome translocation stocks indicated a major gene(s) for resistance to MDMV on chromosome 6 of selected maize lines including the inbred Pa405. RFLP analysis of individual backcross plants of the crosses (Pa405 x yM14) x yM14 and (Pa405 x K55) x K55, inoculated with MDMV-strain A, mapped this resistance gene to a region near the centromere of chromosome 6. This region is tightly linked to the RFLP probe UMC-85. In yM14 backcross plants this gene both delayed symptom appearance and reduced symptom severity. However, in K55 backcross plants this gene only delayed the appearance of mosaic symptoms, indicating that the effect of this gene on symptom development can be influenced by the genotype of susceptible parents. (RFLP probes were kindly provided by David Hoisington of the Univ. of Missouri and Tim Helentjaris of NPI.)

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Effects of potato cultivars susceptible and resistant to common scab on populations of Streptomyces spp. producing melanoid pigments. A. P. Keinath and R. Loria, Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908.

Populations of Streptomyces spp. that produce melanoid pigments, including S. scabies, were sampled in field plots planted to Chippewa (susceptible to common scab) or Superior (resistant) potatoes and in fallow plots. Indices of diversity, based on proportions of the most frequently recovered morphological types, were calculated for populations in soil, the rhizosphere, and on tuber surfaces. Populations recovered from soils planted to potatoes were more diverse than those from fallow soil in 1986 and 1987 ($P \leq 0.09$). Relative numbers of three Streptomyces spp. differed in the rhizospheres of Chippewa and Superior ($P < 0.05$). More colonies of Streptomyces spp. morphologically and physiologically similar to S. scabies were recovered from Chippewa than Superior tubers ($P < 0.10$) as the tubers aged. This is the first report of differences between potato cultivars for populations of Streptomyces spp.

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THE EFFECT OF NITROGEN ON CORKY ROOT OF LETTUCE. A.H.C. van Bruggen and P.R. Brown., Department of Plant Pathology, University of California, Davis, CA 95616.

In field experiments at Davis, sidedressing with NH_4NO_3 (150 kg/ha) increased susceptibility of lettuce cv Salinas to corky root (CR). There was a signifant interaction between nitrogen fertilization and soil-infestation with CR bacteria in their effects on lettuce yield: sidedressing with NH_4NO_3 increased yield in uninfested plots, but decreased yield in infested plots. In a growth chamber experiment, there was a curvilinear relationship between level of NH_4NO_3 and CR severity with a maximum severity at 400 kg/ha as sidedressing. Nitrogen injury was observed at 300 kg/ha and above, and was not affected by CR. In a field experiment at Salinas, sidedressings with 150 kg/ha of $(NH_4)_2SO_4$, NH_4NO_3, urea, or $Ca(NO_3)_2$ were compared with a control (no sidedressing). Sidedressing with nitrogen increased CR over the control, but there were no significant differences between the forms of nitrogen. Control plants were visibly N-deficient and had significantly lower fresh weights.

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SUPPRESSING EFFECT OF RYE COVERCROP ON INCIDENCE AND SEVERITY OF BLACK ROOT-ROT CAUSED BY Thielaviopsis basicola. M. S. Reddy and Z. A. Patrick. Department of Botany, University of Toronto, Toronto, Canada, M5S 1A1.

Field observations show that rye (Secale cereale, L.) as a covercrop reduces incidence and severity of root-rot by T. basicola in subsequently planted susceptible crop but the specific disease-suppressing mechanisms are controversial. Studies showed that the major mechanisms involved in the disease-suppressing effect of rye include reduction in populations of infective propagules of T. basicola in soil during the time the nonhost is growing. There was also an increase in the indigenous soil bacteria during the decomposition of rye residues of which 68% were antagonistic to T. basicola when tested in culture. Also, some of the bacterial isolates enhanced the growth of tobacco seedlings while others inhibited melanization of chlamydospores of T. basicola.

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Damage thresholds for flooded and nonflooded avocado with Phytophthora root rot. R.C. Ploetz and B. Schaffer. University of Florida, IFAS, TREC, 18905 SW 280th Street, Homestead, 33031.

Damage thresholds were determined in greenhouse studies for flooded (1 wk) and nonflooded avocado (Persea americana) with different severities of Phytophthora root rot (caused by P. cinnamomi). Percent root necrosis was used as a measure of disease severity and host gas exchange characteristics were used as threshold parameters. Net CO_2 assimilation, stomatal conductance, and transpiration were each reduced to nondectable levels when plants with root necrosis $>15\%$ were flooded; in contrast, nonflooded plants tolerated root necrosis $\leq 90\%$. Although reductions in assimilation and conductance were highly correlated, a concomitant increase in internal concentrations of CO_2 suggests that nonstomatal factor(s) reduce assimilation under these conditions.

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FIELD TESTING OF LacZY-MARKED RECOMBINANT SOIL BACTERIA. D. Drahos, G. Barry, B. Hemming, and E. Brandt, Monsanto Co., St. Louis, MO 63198; E. Kline, H. Skipper, D. Kluepfel, T. Hughes, and D. Gooden, Clemson University, Clemson, SC, 29634.

The longevity, dissemination, and genetic exchange potential of a recombinant soil bacterium are being assessed under actual field conditions as part of an 18 month study at the Edisto Research Center, South Carolina. The test, initiated Nov. 2, 1987, is evaluating the competitive performance and ecological behavior of a microorganism intended for future use as a biocontrol agent. The Pseudomonas aureofaciens strain in the study was engineered to carry the E. coli lacZ and lacY genes, which have been permanently inserted into the host bacterial chromosome. These genes permit the metabolic selection of the marked strains at a sensitivity of <10 CFU/gram soil. Initial results have demonstrated: 1) Equal colonization ability of the recombinant strain and the non-engineered parent; 2) Efficacy of the lacZY tracking system; 3) Very limited movement of the recombinant bacterium from the rows of inoculated plants.

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THE POTENTIAL FOR SELECTED STRAINS OF RHIZOBIUM TO INHIBIT THE ROOT ROT PATHOGEN, RHIZOCTONIA SOLANI. Serita Frey and Linda K. Blum. Department of Environmental Sciences, University of Virginia, Charlottesville, Va. 22903.

34 strains of Rhizobium phaseoli were screened for antagonistic activity toward the root rot pathogen Rhizoctonia solani. Four screening techniques were used: streak plates, double-layer plates, autoclaved suspensions, and filter sterilized suspensions. Results were consistent between methods, although the techniques might detect different types of antagonisms. The autoclaved suspension technique was determined to be a rapid and reliable method for screening large numbers of organisms which yields quantitative results. Nine Rhizobium strains were found to inhibit R. solani biomass production by at least 50%. These strains are potential biological control agents.

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INFLUENCE OF CULTURAL PRACTICES ON POPULATIONS OF THIELAVIOPSIS BASICOLA AND INCIDENCE OF BLACK ROOT ROT OF COTTON. P.A. Mauk and R.B. Hine. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Populations of Thielaviopsis basicola were monitored over three seasons in a field with a history of black root rot of cotton. In the first cropping season of Pima cotton there was 100% disease incidence and the population was 596 ± 52 cfu/g of air dried soil 2 wk after planting March 28, 1986. Populations gradually decreased to 149 ± 35 cfu/g in July and stabilized at 142 ± 36 cfu/g. During the second season, 1987, the field was split into 2 plots: one plot rotated to wheat and the other fallowed. In February 1988, one year after rotation and fallowing, populations dropped to 87 ± 18 cfu/g in the wheat rotation and 10 ± 3 cfu/g in the fallow treatment. In the third season, March 1988, Pima cotton planted in the wheat rotation plot had a disease incidence of 71% with an average cortical decay of 47% on diseased plants. In contrast, cotton planted in the fallow treatment had a disease incidence of 43% with an average cortical decay of 23% on diseased plants.

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FUSARIUM OXYSPORUM, CAUSAL AGENT OF ROOT ROT OF SUGARBEETS IN THE TEXAS PANHANDLE. R. D. Martyn[1], C. M. Rush[2], C. L. Biles[1], and E. M. Baker[2], Texas Agricultural Experiment Station, [1]College Station 77843, and [2]Bushland 79106.

Sugarbeets (*Beta vulgaris* L.) in the Texas Panhandle affected by a root rot disease had foliar symptoms resembling those of Fusarium yellows [*F. oxysporum* f. sp. *betae* (F.o.b.)]. Symptoms included interveinal chlorosis, wilt, and eventual collapse of the leaves. Cross sections of infected root and crown tissue showed extensive vascular discoloration. Additional symptoms not typically associated with Fusarium yellows occured, however, and included a rot of the tap root that generally began at the distal end and proceded proximally. Lateral roots were also affected and served as the primary infection site. Isolations from diseased roots consistently yielded an atypical *F. oxysporum*. Greenhouse inoculations of sugarbeets (cv. TX9) with each of two *F. oxysporum* isolates from diseased, field-grown sugarbeets reproduced all of the field symptoms, including root rot after 5 months. Comparison of morphological features and isozyme patterns of several enzymes of the Texas isolates with F.o.b. indicated that the two organisms were distinct.

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RELATIONSHIP OF CULTIVAR SUSCEPTIBILITY, FUSARIUM SOLANI, AND SOYBEAN CYST NEMATODE TO SOYBEAN SUDDEN DEATH SYNDROME OF SOYBEAN (SDS). John C. Rupe Univ. of Arkansas, Fayetteville 72701.

Cultivar susceptibility, F. solani (FS), soybean cyst nematode (SCN) and areas known to have high, moderate, and low SDS disease pressure were related to disease development. FS was first detected in roots 3 wk after planting and isolation frequency increased throughout the season. Cultivar susceptibility and infection were unrelated but FS root infections and soil populations were greatest in areas of high, followed by moderate and then low SDS pressure. In all three areas, foliar symptoms were greatest on the cultivar with high susceptibility followed by the cultivar with moderate susceptibility. No foliar symptoms occurred on the cultivar with low susceptibility. SCN populations at the end of the season were positively related to SDS intensity for cultivars with high and moderate, but not low, susceptibility to SDS.

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TILLAGE AND ROOTWORM EFFECTS ON MAIZE ROOTS INFECTED WITH Fusarium graminearum IN MINNESOTA. T. Kommedahl, P.M. Burnes, W.C. Stienstra, C.M. Ocamb-Basu, D.A. Andow, K.R. Ostlie, and J.F. Moncrief, University of Minnesota, St. Paul.

At Waseca (clay loam) in 1987, incidence of roots infected with F. graminearum, varied from 28% in chisel plowed soil to 58% in no-till, while incidences under ridge and mold-board tillages were 30 and 35%, respectively, in roots infested with the rootworm. Without rootworm, the same relationships held but incidence of infected roots was 10-20% lower per tillage. At Goodhue (silt loam), there were almost no differences per tillage and rootworm infestation. In 1986 and 1987, F. graminearum was usually <10% of total Fusarium colony-forming units (cfu) isolated from roots, regardless of location, tillage treatment and season. Total Fusarium cfu per 100 root fragments in 1987 were about double those in 1986 at both locations in rootworm- and non-infested roots. Thus, tillage, season and rootworms affect the number of roots infected with F. graminearum.

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DIFFERENTIAL EFFECTS OF VARIOUS PREPLANT SOIL TREATMENTS ON THE ROOT FUNGAL MICROFLORA, ROOT GROWTH AND YIELD OF STRAWBERRY. G. Y. Yuen, M. N. Schroth, J. G. Hancock, and A. R. Weinhold. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Preplant soil fumigation with methyl bromide-chloropicrin (MB-C) (33% MB:67% C; 364 kg/ha) increased root growth, root health, and yield of strawberry, whereas soil treatments with metham (467 L/ha) or metalaxyl (1.9 kg/ha a.i.) had no effect. The differential action of MB-C was associated with a greater alteration in the population levels of various fungi on strawberry roots during the cropping period. Metalaxyl reduced root infection by Pythium spp. (mainly P. ultimum and P. irregulare) as compared to no treatment, but had no effect on other fungi. Both MB-C and metham treatments exhibited similar effects on Pythium numbers and also decreased population densities of Cylindrocarpon spp. and Fusarium spp. below the nontreated control. Numbers of Cylindrocarpon and Fusarium were significantly lower with MB-C than with metham treatment.

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FUNGISTASIS BY OILS OF BASIL AND OREGANO. R. L. Powell[1], B. D. Bruton[2], C. L. Patterson[1], and R. Reuveni[3]. Dept. of Plant Path., Wes Watkins Agricultural Research and Extension Center, Lane, OK, Oklahoma State University, Stillwater[1], SCARL, USDA-ARS[2], Ag. Res. Org., Newe Yaar Exp. Sta., Haifa Post 31-999, Israel[3].

Oils from 2 sweet basil (Ocimum basilicum) and 3 oregano (Origanum spp.) chemotypes were tested for fungistasis against isolates of: 3 Macrophomina phaseolina (MP), 2 Fusarium solani (FS), 2 Fusarium oxysporum (FO), 3 Rhizoctonia solani (RS), 2 Diaporthe melonis (DM), and 1 Sclerotium rolfsii (SR). The basil oils significantly decreased growth of DM (29-45%), RS (33-67%), and SR (76%) after 6 days. Two oregano oils significantly suppressed growth of all the fungi by 50-85% within 6 days. The third oregano oil suppressed growth of some fungi but was significantly less effective than the other oregano treatments.

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APHANOMYCES ROOT ROT RESISTANCE IN PEAS. J. M. Kraft, USDA-Agricultural Research Service, P. O. Box 30, Prosser, WA 99350.

Root rot caused by *Aphanomyces euteiches*, is a serious soilborne disease of peas (*Pisum sativum* L.) in North America. Until recently, resistance to *Aphanomyces* was diminished when genetically transferred into horticulturally acceptable types. Resistance to Aphanomyces root rot has been recovered in breeding lines with commercial type as evidenced by zoospore inoculation tests. When inoculated with zoospores for 1 hr and incubated for 4 days, more oospores formed in roots of susceptible 'Dark Skin Perfection' than in the resistant breeding lines. This resistances held up at high inoculum levels (300,000 zoospores/ml) and under field conditions where *Aphanomyces* is a problem in Minnesota, Wisconsin, and Idaho.

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THE EFFECT OF FOSETYL-AL AND PHOSPHORUS ACID ON PHYTOALEXIN PRODUCTION IN CITRUS. A. Sztejnberg and U. Afek, Dept. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100, Israel.

Citrus species resistant and susceptible to *Phytophthora citrophthora* were compared for production of the phytoalexin (scoparone) and lesion length in citrus bark treated and untreated with fosetyl-Al and phosphorus acid, after inoculation with *P. citrophthora*. Scoparone was induced in both citrus groups, but the concentration in the resistant species was higher reaching 440.0 µg/g fr. wt. as compared to 41.6 µg/g fr. wt. in the susceptible. In some cases, concentrations of scoparone were two to four-fold higher in inoculated bark treated with 300 ppm fosetyl-Al or 125 ppm phosphorus acid. Lesion length was inversely proportional to the increase in phytoalexin concentration caused by fosetyl-Al or phosphorus acid. Lesion length in untreated resistant and susceptable species were 2.5 and 12.0 mm respectively as compared to 0.5 and 2.0 mm when treated.

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CHARACTERIZATION OF PHYTOTOXIC COMPONENTS FROM SEPTORIA NODORUM SUITABLE FOR IN VITRO GERMPLASM SCREENING. S. Leath, USDA,ARS, Dept. of Plant Pathology, NC State Univ., Raleigh 27695-7616.

Mellein, a nonspecific phytotoxin from S. nodorum, illicits symptoms similiar to those of septoria leaf and glume blotch of wheat. Activity of mellein and a crude extract (CE) from two fungal isolates was assayed on roots and 3-wk-old wheat callus cultures. Filtrate from 3-wk-old fungal broth cultures was extracted with ethyl acetate to obtain CE which was purified by flash chromotography and TLC to obtain mellein. CE, added at an equivalent weight mellein, and mellein were used in 2 ml aliquots and in amended agar in root growth and callus bioassays, respectively. Activity of mellein was not detectable below 200 ppm in root growth bioassays, however, CE caused clear inhibition at 100 ppm mellein. Similarly, mellein inhibited callus growth and development at 50 ppm whereas CE incited similar damage at just 10 ppm mellein and caused callus death in 24 hr at 50 ppm. Results show pure mellein does not exhibit phytotoxicity equal to CE with equal ppm mellein. Additional TLC fractions are being evaluated for phytotoxic activity.

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A CEREAL DISEASE SURVEILLANCE PROGRAM FOR MOROCCO. J. R. Burleigh and B. Ezzahir. Institut Agronomique et Veterinaire-Hassan II, Rabat, Morocco.

Cultivars and lines of barley and wheat, treated and nontreated for control of foliar pathogens were grown at 12 sites, that represented the environments for cereal culture. Disease severity was noted 4 times during eac- of 3 years. Yield, loss, and AUDPC were analyzed by ANOVA with years as replicates. Yields were significantly different among nontreated barleys. Pyrenophora teres severe on the cultivars. Cochliobolus sativus was severe on the lines. Significant grain loss was noted at 4 of 10 sites. Mycosphaerella graminicola was the dominant pathogen on bread wheat and caused significant grain loss at 2 of 10 sites. All cultivars/lines were susceptible to M. graminicola but only promising lines were resistant to Puccinia recondita. Yields of 1718 and Karim durums were greater than the standard, Kyperounda, but there was no grain loss from disease. Xanthomonas campestris pv. translucens and Pyrenophora tritici-repentis were most severe pathogens of durums.

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EVALUATION OF FOUR MALE STERILE FACILITATED RECURRENT SELECTION POPULATIONS OF BARLEY FOR IMPROVEMENT IN DISEASE RESISTANCE. M. E. Bjarko, M. Reinhold, and D. C. Sands, Plant Pathology Dept., Montana State University, Bozeman, MT 59717.

Four male sterile facilitated recurrent selection populations (MSFRSP's); RSP-5 Reg, RSP-5 Rph, RSP-5 Rpt, and RSP-5 Rrs, developed for resistance to Erysiphe graminis, Puccinia hordei, Pyrenophora teres, and Rhynchosporium secalis, respectively, were evaluated for changes in percent resistant plants with continued selection. In the MSFRSP's developed for resistance to E. graminis, P. hordei, and R. secalis the percentage of resistant plants increased with selection, while there was no increase in resistance in RSP-5 Rpt. However, the highest initial percentages of resistant plants were found in RSP-5 Rrs and RSP-5 Rpt. The greatest increase in the percentage of resistant plants over time was observed in RSP-5 Reg. The percentage of resistant plants increased most when the initial amount of resistance was low and selection pressure was high during each cycle of selection.

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LEAF RUST RESISTANCE IN SOUTH DAKOTA WHEATS. S. S. A. Rizvi, G. W. Buchenau, and F. A. Cholick, South Dakota State University, Plant Science Department, Brookings, SD 57007.

Several spring and winter wheats along with 27 lines near-isogenic for low reaction to Puccinia recondita var. tritici, were inoculated with 27 cultures of leaf rust, and infection types recorded. Cultivars were classified into eight distinct groups based on infection type analysis, known Lr gene content, and inspection of parentage. I. Butte, Len, Norak, Norseman, Olaf, Oslo and Wheaton with Lr 10; II. Erik/Marshall Lr 2a + Lr 10 and Butte 86 Lr 10 + Lr 24; III. A99AR, Alex, Apex 83 and Challenger Lr 1 + Lr 2a + Lr 10; IV. Guard and Shield Lr 2a + Lr 3 + Lr 10; V. Bob White and Pakistan 81 with at least Lr 26, Kohinoor 83 with at least Lr 1 and Pavon Lr 1; VI. Bennet, Brule, Lancer, Rita and Rose have Lr 3; VII. Dawn and Nell with Lr 3 and Lr 10, Centura and Sage Lr 3 + Lr 24; VIII. Siouxland Lr 3 + Lr 24 + Lr 26. Selected cultivars from these have been retained for confirmation through F_2 analysis.

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THE EFFECT OF ENVIRONMENT ON DURABLE RESISTANCE TO STRIPE RUST IN F7 POPULATIONS OF WINTER WHEAT. Tom Schultz and R. F. Line. Washington State University and USDA-ARS, respectively, Pullman, WA 99164-6430.

High-temperature, adult-plant (HTAP) resistance to stripe rust (Puccinia striiformis) has been durable for more than 25 years in the Pacific Northwest. F7 populations of six crosses using HTAP resistant cultivars Nugaines, Luke, Daws, and Stephens, plus a susceptible line, were evaluated at Mt. Vernon and Pullman, WA. Distribution of disease intensity for families of each cross were different between locations. Correlations between rust intensity at different plant growth stages and reduced kernel number, kernel weight, and spike weight were high when infection was early and rust was severe but not significant when infection was late and rust was moderate. The growth stages at which rust intensities were most highly correlated with yield components were different depending on the cross and location.

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DISEASE RESISTANCE OF TOBACCO SOMACLONES. M.E. Daub and A.E. Jenns. North Carolina State University, Raleigh, NC. 27695.

A total of 854 somaclones of two flue-cured tobacco cvs. were generated from protoplast cultures, and their progeny analyzed in greenhouse and field tests in 1986 and 1987. Under the culture conditions established in this study, approximately 55% of the somaclones were not self fertile, indicating that genetic variability was induced. Progeny of somaclones had normal phenotype and did not differ significantly from the parent cvs. in yield and leaf chemistry. Significant variation was found in resistance to black shank and bacterial wilt, two diseases for which the parental cultivars have low levels of resistance. Good correlations were obtained between greenhouse and field estimates of black shank resistance. Two lines have been selected which have moderately increased levels of resistance. No lines were isolated with resistance to tobacco mosaic virus or Meloidogyne incognita; the parent cvs. have no resistance to these pathogens. We conclude that stable variation in disease resistance occurs, but the changes are slight and depend on genotype of the parent cv.

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COMPARISON OF RESISTANCE TO *PHYTOPHTHORA CINNAMOMI* IN THREE AVOCADO ROOTSTOCKS. B. K. Gabor and M. D. Coffey. Dept. of Plant Pathology, University of California, Riverside, CA 92521

The resistance of three vegetatively-propagated rootstocks to Phytophthora root rot was compared quantitatively in greenhouse experiments. The susceptible rootstock Topa Topa (*Persea americana*) and resistant rootstocks Thomas (*P. americana*) and Martin Grande (*P. americana* x *P. schiedeana*) were inoculated with soil amended with *P. cinnamomi* at 35 propagules per gram (ppg) of soil. Twenty-four weeks after inoculation, the soil population for Martin Grande was 30 ppg, which was less than that for Thomas (53 ppg) and Topa Topa (56 ppg) ($P = 0.05$). The percent root infection of Thomas and Martin Grande was significantly less than Topa Topa as determined by percent recovery of *P. cinnamomi* from root segments and visual ratings of the entire root systems. The percent reduction in root dry weight for Topa Topa (80%) was greater than for Thomas (40%) and Martin Grande (55%) ($P = 0.05$).

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CONTROL OF VERTICILLIUM WILT OF PISTACHIO NUT TREES WITH A RESISTANT ROOTSTOCK AND THE COMPARATIVE SUSCEPTIBILITY OF PISTACIA SPECIES TO VERTICILLIUM DAHLIAE. W. C. Schnathorst. Department of Plant Pathology, University of California, Davis, CA 95616.

Large plantings of pistachio nut trees in California have been devastated by verticillium wilt. One approach to control is the use of a wilt-resistant rootstock. The following Pistacia spp. were inoculated with SS-4 and T-1 strains of Verticillium dahliae in a greenhouse: P. atlantica (the original rootstock), P. chinensis, P. integerrima, P. khinjuk, P. mutica, P. terebinthus and P. vera (the scion species). Only P. integerrima showed resistance to V. dahliae. In experimental plantings, hundreds of 18-year-old trees grafted to P. integerrima have been virtually free of symptoms. Over 900,000 trees on P. integerrima have been used in new plantings and to replace diseased trees on P. atlantica roots. This appears to be the first instance of control of a root-infecting vascular fungus in a perennial plant using a resistant rootstock.

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RUST DEVELOPMENT ON SLOW AND FAST-RUSTING ASPARAGUS CULTIVARS AFTER INOCULATION WITH BASIDIOSPORES AND AECIOSPORES OF PUCCINIA ASPARAGI. D. A. Johnson, Washington State University, P. O. Box 30, Prosser WA 99350.

Twelve asparagus cultivars that either rusted rapidly or slowly in the field were inoculated with urediniospores, aeciospores, or basidiospores. Number of aecia per shoot varied significantly ($P=0.05$) among cultivars after infection by basidiospores. Number of uredinia per linear cm of shoot and latent period varied ($P=0.05$) among cultivars after infection by either aeciospores or urediniospores. The cultivars that rusted slowly had longer latent periods and fewer uredinia after infection by either aeciospores or urediniospores than the cultivars that rusted rapidly. Number of aecia was not correlated to latent period or number of uredinia after either aeciospore or urediniospore infection. Latent period after aeciospore infection was correlated to latent period ($P=0.01$) and to number of uredinia ($P=0.05$) after urediniospore infection.

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MULTI-RACE RESISTANCE TO FROGEYE LEAFSPOT IN SOYBEAN, D. V. Phillips and H. R. Boerma, Departments of Plant Pathology and Agronomy, University of Georgia, Georgia Station, Griffin, GA 30223-1797

The gene Rcs_3 found in the soybean cultivar Davis conditions resistance to races 2 and 5 of Cercospora sojina. The cultivar Blackhawk is susceptible to all races of C. sojina. Plants in the F2 generation from Blackhawk X Davis crosses were selected for resistance to races 2 and 5. Resistant plants were kept vegetative and sequentially inoculated with from 2 to 5 other new races recently found in the southeastern U. S. There was no detectable effect of previous inoculations on the reaction of plants to subsequent inoculations. All plants which were resistant to races 2 and 5 were also resistant to the other races. Progeny from plants susceptible to races 2 and 5 were susceptible to all races. These results indicate that the single gene Rcs_3 is conditioning a multi-race resistance effective against the races of C. sojina currently present in the southeastern U. S.

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SYSTEMIC RESISTANCE TO PERONOSPORA TABACINA INDUCED BY TOBACCO MOSAIC VIRUS ON TOBACCO CV KY 14. X. S. Ye and J. Kuč, University of Kentucky, Lexington, KY 40546-0091.

Tobacco cv Ky 14 is resistant to tobacco mosaic virus (TMV). Localized necrotic lesions developed 2 days after inoculation with TMV and continued to enlarge for several days. Inoculation of 3 to 4 lower leaves of 2 to 3 month-old plants with TMV induced >90% protection of the upper leaves against blue mold caused by Peronospora tabacina. Disease was assessed by determining the percent of total leaf area covered by lesions, lesion diameter and spore production per cm^2 of leaf area. Induced resistance to blue mold was observed as early as 3 days after TMV inoculation and reached a maximum after 12 days. Removal of TMV-infected leaves at various times after inoculation with TMV revealed that resistance was induced by TMV 3 days after inoculation. However, induced resistance increased as the time period increased from 3-12 days between inoculation with TMV and leaf removal. TMV was not detected in protected leaves.

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RESISTANCE IN WILD BARLEY, Hordeum spontaneum, TO BARLEY LEAF RUST, Puccinia hordei, IDENTIFIED BY INFECTION TYPE AND LATENT PERIOD. L. M. Treeful and R. D. Wilcoxson. Dept. of Plant Pathology, University of Minnesota, St. aul, MN 55108.

Seedling and adult plants of 37 H. spontaneum accessions collected in Israel were inoculated with the most common Midwestern race and 2 other N. American races of P. hordei. Infection type (IT) of seedlings indicated 9 accessions with IT 0-2, and heterogeneity and temperature sensitivity of IT in the other accessions. Brown necrotic flecks without sporulation were observed on many seedlings and adult plants, indicating a high level of resistance. Five accessions were resistant in seedling and adult plants stages. Latent period of seedlings inoculated with each race was long in 2 accessions compared with the standard check cultivars at 18 and 25 C. Latent period of adult plants of 4 accessions was long compared with standard checks at 18 but not at 25 C. This study suggests H. spontaneum contains resistance genes against P. hordei that may be transferred to cultivated barley.

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EFFECT OF A SEVERE STRAIN OF PEANUT MOTTLE VIRUS ON ROOT AND SHOOT DEVELOPMENT OF PEANUT. H. A. Melouk, D. L. Ketring[1] and J. L. Sherwood. USDA-ARS, Dept. of Plant Pathology, and USDA-ARS, Agronomy Dept.[1], Oklahoma State University, Stillwater, OK 74078-0285

Germinated seed of four genotypes (cvs. Florunner, Pronto, Tamnut 74, and PI 109839) were planted in fritted clay growing medium in PVC tubes (10 cm dia and 76 cm long) fitted with rubber caps with drain (Crop Science 24:229-232, 1984). Four to five hundred ml of water was dispensed daily with an automated drip system. At two wks, seedlings were mechanically inoculated with a severe strain of peanut mottle virus obtained from infected Tamnut 74 plants exhibiting severe shoot dwarfing and malformation. Two weeks after inoculation plants showed virus symptoms. At 2, 4, and 6 wks after inoculation destructive sampling was made to determine root length, and shoot and root dry weight. Virus infection severely inhibited shoot and root development in all genotypes tested, however, cv. Florunner was the least affected.

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EFFECT OF PRUNUS NECROTIC RINGSPOT VIRUS AND PRUNE DWARF VIRUS ON SWEET CHERRY (PRUNUS AVIUM L.) POLLEN VIABILITY. W. E. Howell and G. I. Mink, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350

The effect of Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV) on the viability of pollen from sweet cherry (Prunus avium L. cv. Bing) was tested from 1984 to 1987. Pollen was collected just prior to full bloom from 39 trees previously inoculated with separate field collections containing PDV, PNRSV (including mild and severe isolates), or both. Pollen germination and elongation was observed in vitro, and ability of the pollen to initiate fruit set was tested on a caged sweet cherry tree of the cultivar Rainier. Healthy pollen germinated at an average rate of 75%, just slightly higher than pollen of the virus treatments (58 to 68%). Average germ tube elongation and fruit set were essentially equal for all treatments.

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CHERRY LEAFROLL VIRUS-WALNUT ISOLATE INFECTS BING SWEET CHERRY AND MAHALEB BUT NOT COLT CHERRY ROOTSTOCK. Adib Rowhani and S. M. Mircetich, Department of Plant Pathology, University of California, Davis, California 95616

Cherry leafroll virus - walnut (CLRV-W) is the causal agent of blackline disease of walnut. CLRV-W is serologically related but not identical to rhubarb, dogwood, golden elderberry, and cherry (Ch) strains of CLRV. CLRV-Ch did not infect walnuts (Phytopathology 88:in press). We inoculated CLRV-W to sweet cherry cv. Bing on two rootstocks, Mahaleb and Colt. CLRV-W infected eight of ten and five of five Bing scions on Colt and Mahaleb, respectively. However, we failed to detect the virus in the Colt rootstock of the infected Bing scions. Virus was detected in the Mahaleb rootstock. Similar number of trees per rootstock were inoculated with buffer and they remained ELISA negative during the experimental period. In conclusion, CLRV-W infected sweet cherry (Bing) tree as well as the Mahaleb rootstock, but failed to infect the Colt rootstock.

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GENETIC MULTIPLE VIRUS RESISTANT CAYENNE CAPSICUMS, B. Villalon, Texas Agricultural Experiment Station, 2415 E. Hwy 83, Weslaco, TX 78596.

Red, hot cayenne pepper, one of about 20 cultivated Capsicum annuum L. types, has for many years been associated with the hot sauce industry. The domestic market for hot sauce has grown rapidly during the past 15 years. Increased demand for the red, hot cayenne pepper has stimulated production in Texas and other areas throughout the world. All known commercial red, hot cayenne peppers are susceptible to virus diseases. Viruses are a limiting factor in most pepper production areas throughout the world. The Texas Agricultural Experiment Station at Weslaco has developed several hundred new red, pungent, multiple virus resistant, cayenne breeding lines. Genetic resistance to tobacco etch virus, potato virus Y, pepper mottle virus, and tobacco mosaic virus has been incorporated into high-yielding, large red thick cayenne types.

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EPIDEMIOLOGY OF BEAN POD MOTTLE VIRUS (BPMV) IN SOYBEAN IN KENTUCKY. S. A. Ghabrial, D. E. Hershman, and D. A. Johnson. Dept. of Plant Pathology, Univ. of KY, Lexington, KY 40546.

A 3-year survey of BPMV in soybean in Kentucky has indicated the virus was widespread throughout the soybean growing area in the state. Perennial weeds, seeds from infected plants and overwintering adult bean leaf beetles were examined as potential sources of primary virus inoculum. No BPMV was detected in any of the weeds examined. Serological analysis of seed from BPMV-infected plants showed BPMV antigen to be present in relatively high concentration in seed coats but not in embryos. The possibility that seedcoat borne virus may infect emerging seedlings via wounds could not be demonstrated. Overwintering adult beetles, collected from emergence traps were tested for virus content by ELISA using beetle regurgitants. Of 50 beetles tested positive by ELISA, only one transmitted BPMV to healthy plants. A similar decrease in specific infectivity has also been observed with viruliferous beetles maintained at 4C for one month.

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DETECTION OF POTATO LEAFROLL VIRUS BY DOT-BLOT HYBRIDIZATION. O. P. Smith, V. D. Damsteegt, USDA-ARS, Frederick, MD 21701 and A. D. Hewings, USDA-ARS, Urbana, IL 61801

A dot-blot hybridization assay has been used for the detection of potato leafroll virus (PLRV) in potato leaf extracts. Extracts were prepared by homogenizing leaf samples in phosphate buffer followed by (1) clarification via low speed centrifugation and (2) extraction with phenol/chloroform/isoamyl alcohol. Cloned cDNA to PLRV strain 4 (Phytopathology 77:1704-1705) was ^{32}P-labeled by nick-translation and used to hydridize to dot-blots of PLRV-infected samples submitted from Maine (F. E. Manzer, Cooperator), Washington (P. E. Thomas, Cooperator), and Wisconsin (S. A. Slack, Cooperator). Cloned cDNA to PLRV strain 4 hybridized to all samples and, therefore, has potential to be used for PLRV detection in disease diagnosis programs.

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VIRUSLIKE PARTICLES IN ROOTS INFECTED WITH SOIL-BORNE MAIZE WHITE LINE MOSAIC VIRUS AND IN AN ASSOCIATED FUNGUS. E. D. Ammar and R. Louie, Dept. of Plant Pathology, The Ohio State Univ., OARDC, and USDA-ARS, Wooster, OH 44691.

In a study to identify the vector of soil-borne maize white line mosaic virus (MWLMV) and its associated satellite-like virus (SLV), roots of infected and healthy control maize were assayed for MWLMV by ELISA, and then processed for and examined by light and electron microscopy. Aggregates of MWLMV- and SLV-like particles frequently were found in the cytoplasm and vacuoles of cortical cells of infected, ELISA- positive roots. Within these cells, hyphae of an unidentified fungus, sometimes containing two types of isometric viruslike particles similar in size to those of MWLMV and SLV, were observed. Neither viruslike particles nor fungal hyphae were observed in cells of healthy control maize roots. The possibility that this fungus is a vector of MWLMV and/or SLV is being investigated.

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A NEW VIRUS DISEASE OF IMPATIENS (IMPATIENS BALSAMINA L.) IN KENYA. M. E. Shaw, and D. E. Mayhew, California Dept. of Food and Agriculture, 1220 N St., Room 340, P. O. Box 942871, Sacramento, CA 94271-0001.

Several fields of impatiens (Impatiens balsamina L.) growing near Nairobi, Kenya, showed symptoms of vein yellowing, mottling, and chlorotic ring spots. The infection was widespread and caused substantial crop loss in infected fields. Double stranded RNA analysis, electron microscopy, and serology indicate that the causal agent is a tymovirus. The virus is serologically related to turnip yellow mosaic and clitoria yellow vein viruses. It is mechanically transmissible to a narrow host range.

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INFLUENCE ON PLANT GROWTH AND VIRUS TITRE OF THREE CUCURBIT POTYVIRUSES IN SINGLE AND MIXED INFECTIONS. S. J. Castle, J. A. Dodds and T. M. Perring. Depts. Entomology and Plant Pathology, Univ. of California, Riverside, CA 92521.

Virus activity and effects on plant growth were monitored in squash plants in a completely randomized experiment conducted in a glasshouse. Three potyviruses, zucchini yellow mosaic (ZYMV), watermelon mosaic 2 (WMV-2) and papaya ringspot (PRSV) were aphid-inoculated as single, double or triple infections into young plants assigned to one of four time blocks that were destructively sampled or a fifth treatment that was sampled throughout the experiment. Virus titre in plants represented as A_{405} values from ELISA varied with respect to the type of infection (single or mixed) and with time. Reductions in mean A_{405} values ($P<.01$, Anova) for ZYMV were recorded in time blocks 3 and 4 in the single-infected group and double-infected with WMV-2 group. Differences in various plant growth parameters were recorded dependent upon type of infection and time. WMV-2 had the least effect on plant growth relative to controls.

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POTATO LEAFROLL VIRUS TITER IN AND TRANSMISSION BY MYZUS PERSICAE ARE DIRECTLY RELATED TO VIRUS TITER IN SOURCE PLANTS. J.A.C. de Souza-Dias and S.A. Slack, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

Virus titer [PLRV] in the field resistant potato 'Katahdin' (K) is lower than in the susceptible potato 'Russet Burbank' (RB) and [PLRV] in M. persicae reflects host [PLRV]. Acquisition access period (AAP), latent period (LP), and inoculation access period (IAP) were compared at 50% infectivity (20C). AAP and LP were longer for K-aphids (24 h & 36 h) than for RB-aphids (16 h & 26 h), but IAP was similar for both sources (1.5-2.0 h). Fewer Physalis sp. were infected by aphids from K than RB: AAP_{50} from 3-35 h (<22%) and 3-83 h (<10%), LP_{50} from 6-72 h (<15%), and IAP_{50} from 10-50 min (<29%) and from 10 min to 48 h (<14%). With an AAP of 72 h, the relationship of source-[PLRV] x aphid-[PLRV] was confirmed (r=.70) with 13 potato cvs. Data show that [PLRV] in source plants directly affects PLRV transmission efficiency.

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IDENTIFICATION OF BARLEY YELLOW DWARF VIRUS (BYDV) STRAINS IN WEST CENTRAL MOROCCO. M. El-Yamani,[1] J. H. Hill,[2] and B. E. Lockhart.[3] INRA/MIAC, Settat, Morocco,[1] Dept. of Plant Pathology, Iowa State University, Ames, IA 50011,[2] and Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

In Morocco, BYDV disease of cereals has been studied only since 1980. During the 1985-86 through 1987-88 growing seasons, surveys were conducted on fields in the west central region of Morocco planted to durum and bread wheats, barley and oats. The virus strains were also investigated using both aphid transmission and ELISA. The disease incidence and severity were dependent upon the season and crop. Data on strain identification showed the relative abundance of the non-specific PAV-like strain of the virus. However, there was also evidence for the occurrence of some of the other strains.

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RELATIONSHIP OF POTATO LEAFROLL VIRUS IN POTATOES TO VIRUS SPREAD IN THE FIELD. J.A.C. de Souza-Dias & S.A. Slack, Dept. Pl. Path., University of Wisconsin-Madison, WI 53706.

In 1985 and 1986, field plots of healthy (H-) 'Katahdin' (K) and 'Russet Burbank' (RB) potatoes were planted with or without a central leafroll (PLRV)-infected source (SI) of K or RB (treatment=2 50-ft rows, 4 reps). PLRV titer [PLRV] was about 2x higher in PLRV-susceptible RB than in PLRV-field resistant K. Mean leaf-ELISA values of the 10 H nearest to the SI and the total spread of PLRV in treatments were regressed as dependent variables of [PLRV] in the SI. In 1985, r>.50 was noted for each SIxH treatment. In 1986, only K as SI showed r>.50. Lower PLRV spread was recorded with K than RB as SI for H-RB treatments (<16% in 1985; <22% in 1986, p<.05). No spread differences were noted for either K or RB SI in H-K treatments. Myzus persicae populations in 1986 ($\bar{x}$ peak=80/leaf) were high compared to 1985 ($\bar{x}$ peak=11/leaf). Data support the hypothesis that rate of spread of PLRV in potatoes is related to [PLRV] in the host.

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COMMELINA YELLOW MOTTLE VIRUS - A NON-ENVELOPED BACILLIFORM VIRUS CONTAINING DOUBLE-STRANDED DNA. B.E.L. Lockhart and Nezha Khaless. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Commelina yellow mottle virus (CoYMV), a non-enveloped, bacilliform virus with particles measuring 130 x 31 nm, was isolated from Commelina diffusa. Purified virions contain a 7.4 kbp DNA occurring as both circular and linear forms. The genomic DNA has a buoyant density of 1.567 in CsCl-ethidium bromide gradients, two S1 nuclease cleavage sites, major polypeptides of 37 kd and 39 kd and several minor polypeptides detectable by immunoblotting. CoYMV is serologically distantly related to rice tungro bacilliform virus, but not to bacilliform viruses occurring in banana, cacao, canna, Kalanchoe and yam.

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IDENTIFICATION AND SEPARATION OF TOMATO SPOTTED WILT VIRUS (TSWV) BIOLOGICAL STRAINS. J. J. Cho, and H. B. Bridgman. Dept of Plant Pathology, University of Hawaii, HITAHR Maui Research, POB 269, Kula, HI 96790.

Several TSWV isolates have been characterized for use in a tomato breeding program. In general, most isolates are

categorized in three major symptom groups based on symptom development on diagnostic plant hosts. Symptom groups included tipblight, necrotic, and ringspot types. Two new strain types have been identified using additional plant hosts. Monoclonal antibodies (MCA) were generated by the UH MCA laboratory to a mixture of TSWV strains. MCAs from two cell lines were found to differentiate between two TSWV strain groups. Antibody isotypes include IgG3 and IgM. These MCAs have been used in direct antibody sandwich ELISA to determine antigenic specificity among TSWV isolates.

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TRANSFORMATION OF CRYPHONECTRIA (ENDOTHIA) PARASITICA USING A VARIETY OF FUNGAL PROMOTERS. N.K. Van Alfen[1], A.C.L. Churchill[1], D.R. Hansen[1], L.M. Cuiffetti[2], and H.D. Van Etten[2]. Department of Biology, Utah State University, Logan, UT 84322-5305[1] and Department of Plant Pathology, Cornell University, Ithaca, NY 14853[2]

The filamentous fungus Cryphonectria parasitica, causal agent of chestnut blight, has been transformed using a variety of promoters. Two selectable markers were utilized in different plasmid constructs to transform sphaeroplasts of the fungus by polyethylene glycol treatment. The selectable markers used were the hygromycin B phosphotransferase gene (hygB) from E. coli, which confers resistance to the antibiotic hygromycin B, or a mutant beta-tubulin gene from Neurospora crassa conferring resistance to the fungicide benomyl. Plasmids containing the hygB gene and upstream promoters from Cochliobolus heterostrophus, Aspergillus nidulans, or Ustilago maydis each transformed C. parasitica, with the A. nidulans promoters being the most effective (20-30 transformants/μg DNA). A cosmid containing the beta-tubulin promoter and gene from a benomyl-resistant mutant of N. crassa also transformed C. parasitica. The foreign DNA in each case was integrated into the nuclear DNA of C. parasitica.

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CHARACTERIZATION OF FUNGAL GENES REGULATED BY dsRNA OF CRYPHONECTRIA PARASITICA. P. J. Kazmierczak, L. Zhang, and N.K. Van Alfen. Utah State University, UMC 5305, Logan, UT 84322.

Cryphonectria parasitica is the fungal pathogen that causes chestnut blight. Transmissible hypovirulence has been shown to be associated with a cytoplasmically transmissible double-stranded RNA. Hypovirulent strains of the fungus exhibit reduced virulence and sporulation. In previous studies we have shown that virulent strains accumulate specific poly(A) RNAs and proteins which are down-regulated in hypovirulent strains of the fungus. Two of the most abundant poly(A) RNAs found in virulent strains of the fungus are not found in hypovirulent strains. The genes encoding these two poly(A) RNAs are linked in a 4.2 kb region of the nuclear genome of the fungus. The two poly(A) RNAs are regulated together by both the virus and light, but during the growth cycle of the fungus, they appear to be separately regulated. To determine if they are mRNAs or structural in nature, we attempted to translate these poly(A) RNAs using a wheat germ system and hybrid arrest as a control. Two proteins of an abundance and size corresponding to that of the poly(A) RNAs were products of translation.

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COLONY IN SITU HYBRIDIZATION FOR THE DETECTION AND LOCALIZATION OF dsRNA IN CRYPHONECTRIA PARASITICA. R. Martin and N.K. Van Alfen, Department of Biology, Utah State University, Logan Ut 84322-5305.

The movement of dsRNA between different strains of fungus has been difficult to localize and quantitate. A method has now been developed for the visualization of dsRNA within colonies. Virulent and hypovirulent, dsRNA containing, strains of Cryphonectria (Endothia) parasitica were grown on agar plates overlaid with nylon membrane. The membranes were harvested and the mycelium were removed by enzyme digestion. The membranes were then probed with a biotin labeled cDNA clone of a dsRNA fragment. Visualization of the hybridization reaction was achieved by the addition of a streptavidin-alkaline phoshatase conjugate which turned the hybridized areas containing dsRNA a dark blue color. Using this method for detecting dsRNA we can visualize and quantitate the movement of dsRNA within a fungal colony and between different strains We hope to use modifications of this method for rapid detection of dsRNA in field isolates.

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OPTIMIZATION OF GENE TRANSFER IN THE PLANT WILT FUNGUS FUSARIUM OXYSPORUM F.SP. PISI. A. W. Barkdoll and H. C. Kistler, Dept. of Plant Pathology, University of Florida, Gainesville 32611.

The plasmid pHRC and the newly constructed cosmids pHRC-13 and pHRC-14 were used to transform Fusarium oxysporum f.sp. pisi race 2 and race 5 to hygromycin B resistance. Integration of the vectors into the genome appears stable and random but the frequency of transformation is low. To increase the rate of transformation, factors which affect protoplast formation (such as microconidial germination time, enzyme concentration, and $CaCl_2$ concentration) were examined. Protoplast viability and transformation frequency increased as $CaCl_2$ concentration increased from 5 mM to 50 mM. Osmoticum and buffer were 0.5 M sorbitol and 10 mM Tris, respectively. Optimal concentration of the cell wall degrading enzyme, Novozyme 234, and digestion time varied with race. One and 2% Novozym 234 and 15 and 12 hours germination were optimal for the two above races, respectively. Efforts are being made to increase the number of transformants above 1 /lg of vector DNA. The goal of these transformations is to utilize the random insertion of the vector to produce tagged mutants which are then screened for an alteration in their pathogenic reaction to a susceptible pea cultivar using a lesion assay.

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ISOLATION OF CLONED DNA FROM A BENOYML RESISTANT MUTANT OF ASPERGILLUS FLAVUS WITH HOMOLOGY TO THE benA GENE OF A. NIDULANS. E.R. Seip, C.P. Woloshuk, G.A. Payne and C.R. Adkins. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Aspergillus flavus produces the carcinogen aflatoxin. To study the genetics of A. flavus and aflatoxin biosynthesis, a genetic transformation system was developed utilizing a cosmid vector (pCW10) containing the pyr4 gene from Neurospora crassa. Recipient strains must be auxotrophic for uracil. In order to develop a vector with a more universal genetic marker, a cosmid library was constructed in pCW10 using genomic DNA from a benomyl (MBC) resistant mutant of A. flavus. The library was screened by colony hybridization and a clone was obtained that contains a DNA sequence which hybridizes strongly to the benA gene from A. nidulans. Analysis of the cosmid from this clone revealed a single Pst 1 fragment which hybridized to the benA gene of A. nidulans. This fragment was subcloned into the high copy vector pUC19. We are mapping this fragment and studying expression in A. flavus.

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FUSION OF ISOLATED NUCLEI WITH PROTOPLASTS OF TRICHODERMA HARZIANUM. A. Sivan, G. E. Harman and T. E. Stasz, Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY, 14456.

Nuclei were isolated from protoplasts of an auxotrophic mutant of Trichoderma harzianum and fused with protoplasts obtained from another auxotroph of the same strain. The fusion gave rise to stable prototrophic heterokaryons. Interstrain fusion was also done with nuclei from a prototroph of one strain and protoplasts from an auxotroph of a different strain. Slow-growing progeny strains were obtained. Although fusion frequencies in intrastrain and interstrain nuclear transfers were presumably similar, recovery of progeny strains was greatly reduced in interstrain transfers.

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TRANSFORMATION AS A NATURAL MECHANISM OF GENETIC EXCHANGE IN TRICHODERMA. T. E. Stasz, G. E. Harman, and N. F. Weeden. N.Y. Agricultural Experiment Station, Geneva, NY 14456

Protoplast fusion has been used to produce superior biocontrol strains and a variety of other nonparental progeny. However, neither interstrain heterokaryons nor diploids could be recovered, and extensive progeny analysis using isozyme and RFLP gene markers revealed no genetic recombination. These results have forced us to reject the hypothesis that nonparental progeny were products of classical parasexual processes (plasmogamy, heterokaryosis, karyogamy, and haploidization). To account for our results, we hypothesize that a natural transformation process occurs in Trichoderma following protoplast fusion. Our model predicts that, following fusion between dissimilar strains, nuclei of one parental type degenerate. DNA fragments are thus released in the cytoplasm, and these can eventually integrate into the genome of the other parental type, resulting in transformation.

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UNINUCLEATE PROTOPLASTS OF SCLEROTINIA SCLEROTIORUM FOR GENETIC MANIPULATION. N. K. Zidack, E. Ford, J. Henson, and D. C. Sands. Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717.

Sclerotinia sclerotiorum, a soilborne fungal pathogen, was tested as a biological control agent against the noxious weeds Centaurea maculosa (spotted knapweed) and Cirsium arvense (Canada thistle). Our goal of altering pathogenicity and the life cycle of S. sclerotiorum necessitates genetic manipulation. To facilitate genetic studies, a uninucleate form is desired. In an attempt to obtain uninucleate forms of the

fungus, protoplasts were prepared with NovoZym 234 and cellulase. Protoplasts were fixed and stained with 4,6-diamidino-2-phenylindole-hydrochloride (DAPI) and observed with a fluorescent microscope (UV range). Protoplasts containing from 0 to >10 nuclei were observed with ≈30% of the protoplasts appearing uninucleate. Methods of enriching the number of uninucleate protoplasts are being investigated, as well as different methods of genetic manipulation.

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LOSS AND REAPPEARANCE OF A dsRNA GENOME IN ENDOTHIA PARASITICA. C. Durbahn and D.W. Fulbright. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

The presence of transmissible cytoplasmic dsRNA is associated with hypovirulent strains of E. parasitica. These dsRNA genomes may vary in number, size and homology of dsRNA segments. To determine if dsRNA segments of various genomes can reassort to form a new genome, nonhomologous dsRNA from two hypovirulent isolates, GH2 and RC1, were transfered into a single E. parasitica isolate. Single conidia from this multiply infected isolate were cultured and assayed for dsRNA. No dsRNA segment reassortment was detected. However, one single-conidial isolate (ESS6) appeared to harbor only the RC1 genome, but upon single conidial isolation was shown to carry both the RC1 and GH2 genomes. Only the RC1 genome was detected in an isolate converted to hypovirulence by ESS6 indicating that the GH2 genome may not be a component of the cytoplasm. It appears that dsRNA can disappear and reappear from one conidial generation to another.

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MEIOTIC INSTABILITY OF CHROMOSOMAL SEGMENTS IN MAGNAPORTHE GRISEA. D.Z. Skinner, H. Leung, and S.A. Leong. Plant Pathology (DZS), USDA-ARS (SAL) Univ. Wisconsin, Madison 53706, and IRRI, Philippines (HL).

Magnaporthe grisea (anamorph = Pyricularia oryzae and P. grisea) causes rice blast. Transmission of random DNA segments through meiosis was studied with cloned probes. Most regions were inherited in Mendelian fashion. Some probes identified regions which were not inherited normally in random ascospores. These probes were used to analyze DNA from all eight progeny of one ascus. One probe hybridized to a fragment which segregated 4:2:2, where one class of two sister spores was different from both parents. This result indicated that one chromatid had undergone a nonreciprocal rearrangement during meiosis, suggesting a transposition event had occurred. A different fragment segregated 6:2, suggesting a gene conversion event. Thus, the stable transmission through meiosis of DNA fragments of this fungus is uncertain.

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Molecular Tagging of Fusarium sp. for the Study of Plant Disease. J.E. Partridge, M.B. Dickman, V. Hodgin, and C. Stryker. Dept. of Pl. Path., Univ. of Nebraska, Lincoln, NE.

Stalk rot of corn is a disease of uncertain etiology. Many causal agents have been proposed but Koch's Postulates have not yet been achieved. We have transformed two putative causal agents (Fusarium moniliforme and Fusarium graminearum) of this disease. Using an alkali salts procedure, Fusarium spores and mycelia were transformed using plasmid vectors containing separate antibiotic resistance genes. The transformed DNA was stably integrated into the chromosome. Plants were inoculated with both isolates and responded normally to the infection. Isolations were made at 2, 7, and 21 days onto appropriate antibiotic containing media. Serial inoculation and reisolation experiments easily distinguished the two species by their respective markers. Southern blot analysis indicated extensive deletions and/or rearrangements of the donor DNA. The strategy of using molecular tags should be of general use with complex multiple pathogen diseases.

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CLONING OF SPECIES-SPECIFIC DNA FROM PHYTOPHTHORA PARASITICA AND P. CITROPHTHORA. P. H. Goodwin, J. T. English, B. C. Kirkpatrick, and J. M. Duniway, Dept. of Plant Pathology, University of California, Davis, CA 95616.

Chromosomal DNA of Phytophthora parasitica was digested with Hind III and Eco RI, and cloned into E. coli. Three recombinant plasmids were identified which hybridized to P. parasitica DNA but not to DNA of other Phytophthora spp. or Pythium ultimum. A similar cloning procedure was used to identify a species-specific DNA probe to P. citrophthora. Southern blot analyses indicated that the DNA probes hybridized to repetitive DNA. When tomato leaf discs were used as bait on infested soil, P. parasitica was identified by hybridization of the DNA probe to blotted extracts of crushed leaf discs, and by dot-blots of DNA extracted from infected leaf discs. On soil dilution plates, P. parasitica was identified by hybridization of lysed fungal colonies grown on selective medium covered with a nylon membrane. P. parasitica colonies grown on agar medium were also identified by lysis and blotting of crushed agar plugs.

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MOLECULAR CHARACTERIZATION OF dsRNAs ASSOCIATED WITH HYPOVIRULENCE IN ENDOTHIA (CRYPHONECTRIA) PARASITICA AND INFLUENCE OF THESE GENETIC ELEMENTS ON LIGHT-REGULATED FUNCTIONS. B. I. Hillman, B. P. Rae, and D. L. Nuss. Roche Institute of Molecular Biology, Nutley, NJ, 07110.

At lower light intensities, pigmentation and conidiation were suppressed in the hypovirulent strain EP713 of *E. parasitica* compared to its isogenic virulent counterpart (EP155). The suppression was partially overcome in the presence of intense light, and conditions were achieved in which pigmentation and conidiation were equivalent in the two strains. No differences in protein pattern or dsRNA accumulation were observed. Phenotypic variation within hypovirulent strain EP713 was investigated by analyzing single conidial isolates (SCI's). The dsRNAs from SCI's with different phenotypes were analyzed by hybridization with probes from defined portions of the major 9.0 kbp dsRNA present in all EP713-derived SCI's. These studies compliment our current attempts at elucidating the structure and function of hypovirulence-associated dsRNAs by cDNA cloning, sequence analysis, and DNA-mediated transformation studies.

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CLONING THE PECTATE LYASE GENE FROM ASPERGILLUS NIDULANS, ITS EXPRESSION IN ESCHERICHIA COLI AND ITS ROLE IN PLANT DISEASE. R. A. Dean and W. E. Timberlake, Dept. of Genetics and Plant Pathology, Univ. of Georgia, Athens, GA 30602.

We have shown that A. nidulans has phytopathogenic potential and produces abundant cell wall degrading enzymes. The molecular genetic system of this fungus makes it amenable for the identification and cloning of genes associated with plant pathogenesis. The A. nidulans pectate lyase (PL) gene was cloned by purifying the protein from culture filtrates. PL antisera were produced and used to identify PL cDNA clones in a library made from polypectate-induced mRNA. All 13 PL cDNA clones identified immunologically also cross-hybridized at the DNA level. The longest cDNA was 1.2 kbp. This clone hybridized to a single genomic restriction fragment under low stringency hybridization conditions indicating that the gene is unique in the genome. The cDNA identified a single mRNA of 1.3 kb that was not present in cells grown in glucose. Cloning of the PL gene was confirmed in two ways: 1) several PL cDNA clones expressed high levels of PL activity in E. coli and 2) disruption of the A. nidulans PL gene resulted in complete loss of enzyme activity. Effects of PL gene expression on pathogenicity will be discussed.

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MONOCLONAL ANTIBODIES TO SURFACE ANTIGENS OF XANTHOMONAS CAMPESTRIS PV. ORYZAE. A.A. Benedict*, A.M. Alvarez**, and C.Y. Mizumoto*. Departments of Microbiology* and Plant Pathology**. University of Hawaii, Honolulu, HI 96822.

Three monoclonal antibodies (mAbs) specific for X c. pv. oryzae (Xco) separated 171 Xco strains from diverse geographical locations into 4 serological groups. MAb Xco-1 reacted with all Xco strains; Xco-2 with 88% of the Xco strains; Xco-5 reacted only with Xco strains isolated from the recent disease outbreak in Texas and Louisiana and weakly with some strains of X.c. pv. oryzicola. Xco-1 showed weak immunofluorescence and no binding in immunoelectron microscopy (IEM), whereas Xco-2 and Xco-5 showed intermediate and bright immunofluorescence, respectively, and similiar distributions with IEM. Heat stability, presence in phenol extracts, and results with immunoblots indicated that Xco-2 epitope is associated with LPS; the Xco-1 epitope is heat sensitive and of high mol.wt.

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CHEMOTAXIS OF PSEUDOMONAS SYRINGAE SUBSP. GLYCINEA. D. R. Hattermann and S. M. Ries. Department of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801.

Chemotaxis of P. s. glycinea (Psg) toward 10^{-2} M sodium citrate, as determined by Adler's capillary assay, is optimal in chemotaxis medium containing 10^{-2} M potassium phosphate buffer (pH 6.5), 10^{-5} M EDTA, 5 X 10^{-3} M $MgCl_2$, and 10^{-2} M glycerol at an assay temperature of 15-25 C (peak response at 20 C). Maximum assay sensitivity occurred when using a low cell concentration (4 X 10^5 cfu/ml) and a long incubation time (60 min). Psg is attracted to several organic acids and to a few amino acids, but to no sugars. The most powerful responses were exhibited toward malate, fumarate, cis-aconitate, succinate and tartrate. The best amino acid attractants were N-carbamyl-DL-aspartate, proline, and histidine. Psg is most strongly attracted to the organic acid fraction of soybean leaf extract and less so to the amino acid and neutral-basic fractions. None of the fractions were as attractive as unfractionated leaf extract.

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RATES OF INTRASPECIFIC SHIFTS IN PSEUDOMONAS SYRINGAE POPULATIONS UNDER FIELD CONDITIONS. S. S. Hirano, K. L. Carroll, J. D. Stock, and C. D. Upper*, Department of Plant Pathology and *ARS-USDA, University of Wisconsin, Madison, WI 53706.

To determine whether intraspecific shifts in P. syringae (Ps) populations occur when population sizes of the species undergo large changes, Ps cultures were isolated from bean leaflets harvested at various times during the 1985 growing season. The strains were tested for their ability to cause bacterial brown spot in a pod inoculation test. During each of 3 periods when population size of the species increased at least 10-fold following rain, the proportion of strains that were pathogenic also increased. For example, the proportion of pathogenic strains increased from 49% (of 71 strains) to 76% (of 98) during a period when Ps population size increased 64-fold. This corresponds to an increase in the pathogenic phenotype of about 1.08-fold per generation following rain. Thus, substantial intraspecific shifts in Ps populations can result from several iterations of a very small selection.

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GROWTH IN LEAVES OF XANTHOMONAS CAMPESTRIS OF DIFFERENT AGGRESSIVENESS TYPES ISOLATED FROM CITRUS BACTERIAL SPOT IN FLORIDA. D. S. Egel, J. H. Graham, and R. E. Stall, University of Florida, Department of Plant Pathology, Gainesville, FL 32611.

In greenhouse and field observations, different types of aggressiveness have been identified among strains of Xanthomonas campestris (Xc) isolated from citrus bacterial spot. It was predicted that strains of low aggressiveness would exhibit lower populations in citrus leaves than strains of high aggressiveness. Xc strains (ca. 10^3 cfu/ml) were infiltrated into leaves and quantified by dilution plating. Non-aggressive strains 12689 and 6200 reached lower numbers (3.16 X 10^7 and 9.17 X 10^6 cfu/cm^2, respectively) in Swingle citrumelo leaves, compared to the weakly aggressive 3401 and highly aggressive 3048 (3.13 X 10^8 and 9.55 X 10^8 cfu/cm^2, respectively). A highly aggressive strain of Xc pv. citri of the A type of citrus canker (9771) reached a maximum population less than non-aggressive 12689 (8.87 X 10^6 cfu/cm^2). However, in Duncan grapefruit 9771 grew as well as the highly aggressive 3048 (1.27 X 10^8 and 7.04 X 10^7 cfu/cm^2, respectively). These data confirm that the above strains of low aggressiveness exhibit low populations in leaves, and that populations might depend on the citrus host.

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CONDITIONS FOR ETHYLENE EVOLUTION BY XANTHOMONAS CAMPESTRIS PV. VESICATORIA IN CULTURE. R. E. Stall, and C. B. Hall. Departments of Plant Pathology and Vegetable Crops, University of Florida, Gainesville, 32611.

The production of ethylene in diseased tissue is important in development of some symptoms of the bacterial spot disease of tomato and pepper. Ethylene evolved (2.5 to 8.8 X 10^{-9} nl $cell^{-1}$ hr^{-1}) from cultures of Xanthomonas campestris pv. vesicatoria (Xcv) growing on slants of a mineral base medium containing methionine and glucose. Barely detectable amounts of ethylene were produced by cultures in a broth of the same medium. The maximum amount of ethylene was produced with 10^{-3} M methionine in the medium. No ethylene was produced in the mineral base medium containing glucose. However, the production of ethylene in the medium containing methionine was increased with the addition of glucose, which resulted in increased growth of the bacterium. Methionine was a poor source of carbon for Xcv.

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CORKY ROOT OF LETTUCE CAUSED BY A GRAM-NEGATIVE BACTERIUM. A.H.C. van Bruggen and K.N. Jochimsen, Department of Plant Pathology, University of California, Davis, CA 95616.

Corky root (CR) of lettuce is caused by an aerobic bacterium, ranging in morphology from small rods with one lateral flagellum to long filaments. Originally, the CR bacterium was thought to be Gram-positive (Waters, C.M., and Grogan, R.G. 1984. Phytopathology 74:857). According to the KOH stringiness test, the CR bacterium seemed Gram-positive, but with Hucker's Gram-stain stained Gram-negative. TEM sections of the cell wall were similar to those of Pseudomonas fluorescens. Lipopolysaccharides (LPS) were extracted from the CR bacterium and P. fluorescens. The KDO test on partially purified lyophilized LPS was positive for both species. Isoprenoid quinone analysis yielded Q10 ubiquinone and an unidentified quinone, but no menaquinones. Fatty acids were analyzed by thin-layer and gas-liquid chromatography/mass spectrometry. The fatty acid profile consisted of iso-methylated saturated and unsaturated fatty acids, and hydroxy fatty acids. All chemical analyses showed that the CR bacterium is truly Gram-negative.

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DETECTION OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS IN LATENT INFECTIONS OF TOMATO. R. D. Gitaitis and Curt Leben, Univ. of Georgia, Tifton, GA 31793 and The Ohio State Univ., Wooster, OH 44691.

Tomato seedlings were inoculated with Clavibacter michiganensis (CM) by clipping the terminal bud with a blade contaminated by cutting through a plant infected with CM. After 1-14 days, areas 1 cm below the inoculation point were surface-disinfested, dissected from the stem, and printed against the surface of selective media. CM was recovered 2 days after inoculation, whereas symptoms were not evident until after 17 days. After ten days, CM was 15 cm below the inoculation point. CM was detected at 3 days in 58% of plants exposed to 20 cfu/ml and in all plants inoculated with 2 x 10^7 cfu/ml. After 6 days, CM was in all plants at all inoculum levels. CM also was isolated from disinfested petiole-stem junctures of large greenhouse or field plants using the printing technique.

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PECTOLYTIC ACTIVITY IN AGROBACTERIUM TUMEFACIENS. A.L. Bishop[1], B.H. Katz[2], T.J. Burr[2], A. Kerr[3], and K. Ophels[3]. [1]Calif. Dept. Food & Agric., P.O. Box 942871, Sacramento, CA 94271-0001, [2]Dept. Plant Pathol., Cornell Univ., Geneva, NY 14456, and [3]Dept. Plant Pathol., Waite Agric. Res. Inst., Univ. of Adelaide, Glen Osmond, South Australia 5064.

All biovars of Agrobacterium tumefaciens digested sodium polypectate at pH 4.5 and 6.5 in an agarose-pectate gel assay (Ried and Collmer. 1985. Appl. Environ. Microbiol. 50:615-622), regardless of tumorigenicity. Biovar 3 strains produced significantly larger zones of pectolytic activity than other biovars at pH 4.5. Biovar 3 strains digested 8-10 mm zones in 3-4 hr when polygalacturonic acid (PGA) was substrate; PGA was not digested by biovars 1 and 2, at pH 4.5, though some strains attacked PGA at pH 6.5, and A. rubi digested PGA at pH 4.5 and 6.5. Strong pectolytic activity of AT3 at acid pH may be associated with its ability to decay grape seedling roots (Burr, et al. 1987. Phytopathology 77: 1424-1427).

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EXPRESSION OF PECTATE LYASE BY ERWINIA CAROTOVORA SUBSP. CAROTOVORA IN POTATO TUBER TISSUE. E.A. Maher and A. Kelman, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

To determine whether pectate lyase (PL) isozymes are expressed in potato tuber tissue infected by Erwinia carotovora subsp. carotovora (Ecc), PL was detected by different anti-PL monoclonal antibodies (MAbs) in extracts from decayed tissue transferred to nitrocellulose following electrophoresis and by tissue printing of soft rot lesions. Previous analyses suggested at least 6 different epitopes are recognized by MAbs prepared against PL purified from pectate-based Ecc cultures (Ecc strain SR319). PL was detected in blots of decayed tissue extracts or in tissue prints by several MAbs. However, the PL isozyme specifically recognized by MAb 2-3 was not detected in tissue. PL from pectate cultures but not from decayed potato extracts was retained by an immunoaffinity column prepared with MAb 2-3. At least one PL isozyme expressed by Ecc in culture media is not expressed in infected plant tissue.

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BIOLOGICAL CONTROL OF FROST INJURY TO POTATO WITH RECOMBINANT ICE$^-$ STRAINS OF PSEUDOMONAS SYRINGAE AND THEIR SURVIVAL AND DISPERSAL AT TEST SITES. S. E. Lindow, N. J. Panopoulos, C. Pierce, G. Andersen and G. Lim, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Ice$^-$ P. syringae strains comprised up to 10% of all bacteria on treated potato plants for up to 6 weeks after seed piece and foliar spray application. The population size of Ice$^+$ bacteria on treated plants was about 8-fold lower than on control plants during this period. Frost injury to young treated plants was reduced up to 80% compared to uninoculated plants during 3 natural frosts (min. air temp. = -2.5C to -5.0C). The number of aerosolized Ice$^-$ strains deposited around the sprayed plot decreased logarithmically with distance. No Ice$^-$ P. syringae strains were detected on vegetation outside of a 30 m bare soil buffer zone around the plot nor in soil, water, or insects nearby the plot.

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APPLICATION OF RESTRICTION FRAGMENT FINGERPRINT ANALYSIS TO PREDICTING COMPETITIVE INTERACTIONS BETWEEN INA+ AND INA- P. SYRINGAE ON STRAWBERRY. D. Misumi, T. V. Suslow. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA 94608.

Recently, the use of restriction fragment fingerprinting (RFF) and restriction fragment length polymorphisms (RFLP) has gained popularity in studying and perhaps redefining taxonomic relatedness among phytopathogenic bacteria. One anticipated benefit of determining such relationships is the facilitation of epidemiological studies, host range predictions and other ecological interactions. We have analyzed the RFF patterns of 56 random isolates of *P. syringae* obtained from healthy strawberry plants in several regions in California and one in Oregon. The RFFs define 16 groups by visual inspection. Two groups contain 55% of the total isolates. These preliminary groupings have been shown to be somewhat correlated to competitive interactions on strawberry blossoms in greenhouse frost protection tests (Lindemann and Suslow, 1987). Isolates were also examined by standard determinative tests, bacteriocin typing, LOPAT tests, nutrient utilization, and host range studies. Based on this limited sample size, some predictive correlations about competitive abilities can be applied for some groupings. This suggests an expansion to RFLP characterization may be justified.

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COMPARISON OF INDIRECT IMMUNOFLUORESCENCE (IIF) AND SPREAL PLATING TECHNIQUES FOR DETERMINATION OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA (XCV) POPULATIONS IN LESIONS ON A RANGE OF TOMATO GENOTYPES. G. Cameron Somodi, J. B. Jones, and J. W. Scott, IFAS, University of Florida GCREC, 5007 60th Street East, Bradenton, FL 34203

Hawaii 7998 (H7998), a tomato genotype resistant to XCV, was shown previously to have significantly lower populations in lesions than susceptible 'Walter' using the spread plate technique. IIF was used to verify these results. Approximately 12 days after inoculation with XCV, lesion populations from susceptible, resistant, tolerant and selected F_1's of these genotypes were sampled by both methods. For each IIF sample, cells were counted in 10 randomly selected fields. Both methods differentiated XCV populations in susceptible and resistant genotypes, with separation of F_1 genotypes from susceptible and resistant genotypes often possible. A positive correlation was found between the two methods. Both methods may be useful in a bacterial spot resistance screening program.

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PRESCENCE OF EXTRACHROMOSOMAL ELEMENTS IN THE SOFT-ROTTING BACTERIUM PSEUDOMONAS VIRIDIFLAVA. C. H. Liao. USDA, ARS,NAA, ERRC, Philadelphia, PA 19118

Pseudomonas viridiflava produces a single pectate lyase (Mr 42 KDa, pI 9.7) required for pathogenicity. This organism has been shown to lose virulence rapidly in culture. The present study was initiated to investigate the possible mechanism for genetic variability. The alkaline lysis procedure was used to screen for the presence of plasmid or viral DNA. An extrachromosomal (EC) element was detected in all eleven strains examined, including one strain that had naturally lost pathogenicity and mucoid phenotype. The EC-element migrated less slowly than the chromosomal DNA in agarose gel, and usually formed a diffusive band uncharacteristic of plasmid DNA. A yellow-pigmented strain (SJ074) was found to harbour, in addition to the EC-element, a large plasmid of 110 MDa. The role of the EC-element in mediating genetic exchanges in P. viridiflava is now under investigation.

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BIOSYNTHESIS OF FUSAROCHROMANONE AND ITS MONOACETYL DERIVATIVE BY Fusarium equiseti. Weiping Xie, Chester J. Mirocha, Robert J. Pawlosky, Yechun Wen and Xigen Xu, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA.

Fusarochromanone and its monoacetyl derivative were isolated and purified from a rice culture of Fusarium equiseti (Alaska 2-2). The identity of monoacetyl fusarochromanone was confirmed by analysis via mass spectrometry and nuclear magnetic resonance spectroscopy. Time course studies of biosynthesis of these two compounds on Czapek Dox media enriched with soybean peptone and autoclaved rice indicated that fusarochromanone was converted to its monoacetyl derivative in the cultures. The peptone concentration in the liquid medium is a critical factor affecting the synthesis of these two compounds. High concentrations of peptone in the medium may stimulate both processes of fusarochromanone synthesis and its conversion to the acetyl derivative.

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EVALUATION OF TRICHOTHECENE AND NONTRICHOTHECENE MYCOTOXINS PRODUCED BY FUSARIUM IN SOYBEAN. Hamed K. Abbas, U. Bosch, and C. J. Mirocha, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Two samples of soybean associated with toxicity in wild geese in 1986 in Minnesota were analyzed for the presence of Fusarium species and mycotoxins. The samples were negative for the known mycotoxins. Twelve isolates of Fusarium were identified as F. acuminatum (2), F. equiseti (1), F. graminearum (2) and F. moniliforme (7). All isolates when grown on rice caused a weight loss in rat feeding tests except one isolate of F. moniliforme. The same isolates when grown on autoclaved or surface sterilized soybeans caused no weight loss. Ten isolates grown on rice caused death in rat feeding tests whereas only one death occurred when the same isolates were grown on soybean media. The production of mycotoxins (zearalenone, DON, T-2, neosolanol, T-2 tetraol, wortmannin and moniliformin) was greater on rice than on soybean substrates. However, more HT-2 toxin was produced on soybeans than on rice.

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PRODUCTION OF ZEARALENONE, NIVALENOL, MONILIFORMIN AND WORTMANNIN FROM TOXIGENIC ISOLATES OF Fusarium OBTAINED FROM SOIL PASTURE SAMPLES COLLECTED IN NEW ZEALAND. Hamed K. Abbas and C. J. Mirocha, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Two cultures of F. avenaceum, one culture of F. oxysporum and 13 cultures of F. sambucinum were isolated from soil samples of pasture in New Zealand in 1987. All isolates, when grown on rice, and fed to rats caused a weight loss in rats as well as toxic signs including hemorrhaging and congestion, uterine enlargement and hematuria. Six out of 16 isolates caused death in rat feeding tests. F. oxysporum (MT6) killed rats (feeding test) within 5-12 hrs. Ten isolates produced zearalenone (19 to 8849 ppm), 8 isolates produced nivalenol (32 to 217 ppm), one isolate produced wortmannin (20 ppm) and iso-wortmannin and 5 isolates produced moniliformin (40 to 9000 ppm). We report for the first time the co-occurrence of zearalenone, nivalenol and moniliformin produced by F. sambucinum in culture. F. avenaceum and F. oxysporum cultures produced moniliformin alone.

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PRODUCTION OF MYCOTOXINS BY FUSARIUM CULTURES ON SUGAR BEET SUBSTRATE. Ursula Bosch, H. K. Abbas, and C. J. Mirocha, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Thirty-five Fusarium cultures were isolated from fungus infected sugar beet piles in Minnesota in 1987-1988. The cultures were grown on rice and fed to rats; 60% of the cultures were toxigenic and caused weight loss, uterine enlargement, intestinal hemorrhages, diarrhea, hematuria and death. The following mycotoxins were identified in the culture extracts: zearalenone, cytotoxic factor (HM-8), moniliformin, deoxynivalenol (DON), 15-acetyldeoxynivalenol(15-ADON), neosolaniol, acetyl-neosolaniol, T-2; HT-2, and minor trichothecenes. Fusarium cultures isolated from sugar beet piles (2 isolates) and from grains (4 species), produced mycotoxins when grown on sugar beet including (zearalenone, 6-44 ppm; DON, 3 ppm; 15-ADON, 5 ppm; acetylneosolaniol, 5 ppm; T-2, 41-49 ppm; HT-2, 17-69 ppm). This is the first report for the production of mycotoxins on sugar beet substrate.

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CONTROL OF SPORULATION OF PENICILLIUM DIGITATUM WITH IMAZALIL APPLIED TO ORANGES. Brown, G. E., Florida Department of Citrus, CREC, 700 Experiment Station Road, Lake Alfred 33850, and Craig, J. O., Florida Department of Agriculture, Winter Haven 33880.

An application of imazalil (1000 ug/ml for 15 sec) in a non-recovery spray (NRS) to fruit rotating on fungicide-saturated brushes was less effective for sporulation control than a similar treatment applied as a dip. Residues from a dip treatment were greater than residues from a NRS at a comparable concentration. Dips for 2, 7 or 15 sec exposure times produced comparable sporulation control. A NRS for 60 sec reduced sporulation more than one for 15 sec. Sporulation was controlled more effectively with a NRS at pH 4.5 than at 7.3. More of the residues at the lower pH were retained on the fruit surface. A NRS over PVC rollers controlled sporulation better than a similar concentration applied over brushes.

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POSTHARVEST CONTROL OF BOTRYTIS CINEREA ON GRAPES BY IODINE VAPORS. Joseph L. Smilanick. USDA-ARS, Fresno, CA 93727.

Decay of table grapes by Botrytis cinerea was reduced 90% or more for up to two months by iodine vapors. The dosage was controlled by the rate of iodine and the ratio of metallic iodine to iodide ion. Ethanol (80%; v/v) containing I_2 and KI in 1:1 ratio (w/w) was applied to 15 cm-dia paper disks. After drying 15 hr at 25 C, the disks were placed in plastic bags with 350 g clusters of Botrytis cinerea-inoculated grapes. Decay of Perlette grapes after 30 days at 1 C in bags with disks containing 0, 25, 50, 100 or 200 mg I_2 was 91.1, 17.7, 7.5, 0.6, and 8.2 percent, respectively. In a second test, the ratio of KI to I_2 was 1:2, 1:1, and 2:1 on disks with 100 mg I_2. Decay of Thompson grapes after two months at 1 C was 3.3, 1.7, and 1.0 percent, respectively, while the controls decayed 73.1 percent. Minor discoloration was observed on damaged grapes. Iodine residues were not determined. Iodine treatment may offer an alternative to sulfur dioxide for grape decay control.

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ANHYDROUS AMMONIA VAPOR AS A MICROBIAL INHIBITOR FOR THE AMBIENT AIR DRYING OF HIGH MOISTURE CORN. D. H. Gillman and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Twenty-eight hundred bu of freshly harvested corn of 25-26% moisture was loaded into a metal bin of 3000 bu capacity. It was equipped with full perforated floor and fans for ambient air drying. An average of 0.088 (range: 0.142-0.046) weight percent, dry basis, of ammonia vapor was applied during each of eight treatments to retard spoilage. In the ammoniated corn, surface disinfected kernels yielding fungi decreased from an initial 72% to 23%. In nonammoniated corn in control bins it increased from 23% to 72% in corn of 25-26% moisture, and from 51% to 72% in corn of 21% moisture. In the ammoniated corn, kernels yielding bacteria increased from an initial 5% to 55%, but did not increase after moisture fell below 18-20%. In the nonammoniated corn, kernels yielding bacteria ranged between 2-5% throughout the drying period.

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THE EFFECT OF CARBON DIOXIDE AND TEMPERATURE ON IN VITRO AND IN VIVO GROWTH OF SEVERAL POSTHARVEST FUNGAL PATHOGENS OF STRAWBERRY. C.K. Ragland[1], J.A. Bartz[2], J.K. Brecht[1], and S.A. Sargent[1]. [1]Vegetable Crops and [2]Plant Pathology Departments, University of Florida, Gainesville, 32611.

Growth of strawberry fungal pathogens [Botrytis cinerea (BC), Dendrophoma obscurans (DO), Colletotrichum acutatum (CA), C. fragarieae (CF), Glomerella cingulata (GC), and Alternaria tenuissima (AT)] was evaluated in vitro at 10 and 20 C in controlled atmospheres of: air, 10% CO_2, 20% CO_2, and 30% CO_2. The inhibitory effect of low temperature on growth of DO, CA, CF, and GC was equal or greater than that of high CO_2. The latter only had a significant effect on growth at 20C. In contrast, with AT, low temperature and high CO_2 inhibited the growth additively, whereas BC responded more to high CO_2 than to low temperature; growth inhibition was similar at both 10 and 20 C. At 20 C, the ranking of growth inhibition (most to least) caused by 20 or 30% CO_2 was: DO>BC>CA>GC>AT>CF. In strawberry fruit stored at 20 C, 20 or 30% CO_2 reduced disease severity most in fruit inoculated with GC and least with CA.

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EFFECT OF SUMMER APPLICATIONS OF CALCIUM CHLORIDE ON POSTHARVEST DECAY OF BOSC PEARS. David Sugar, Kate Powers, and S.R. Basile, Oregon State University, Medford 97502.

The potential of summer calcium chloride sprays to enhance resistance of Bosc pears to postharvest decay was evaluated by wound inoculation of treated fruit. Treated trees were given 3 spray applications of $CaCl_2$ at either 0, 1.2, 3.6, or 6.0 g Ca/L, and fruit were inoculated postharvest with spore suspensions of Phialophora malorum at either 0, 10, 10^2, 10^3, or 10^5 spores/ml. Lesion diameter was measured after 3 mo storage at 0 C. A significant interaction between rate of $CaCl_2$ and spore concentration was observed. At 6.0 g Ca/L the mean area of decay was reduced at spore concentrations 10^2/ml. At 3.6 g Ca/L lesion area reduction was significant at 10^2 and 10^5 spores/ml. At 1.2 g Ca/L, lesion area reduction was significant only at 10^2 spores/ml.

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SEED AND FUNGAL RESPIRATION IN CORN WITH 15-17% MOISTURE. D. B. Sauer, USDA-Agricultural Research Service, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502.

Corn in tightly closed containers was purged at intervals with humidified carbon dioxide-free air. The CO_2 was measured with an infrared gas analyzer and converted to percent loss in dry matter (DML). Sterile corn produced 10% as much CO_2 as corn with Aspergillus amstelodami at 15.5% moisture in 3 months at 25°C. Large differences were observed among treatments inoculated with different species of storage fungi; Aspergillus candidus produced the highest respiration rates under the conditions tested. Corn hybrids identified as relatively resistant or susceptible to storage fungi using other criteria were also different as measured by DML. Previously suggested guidelines of 0.5% DML as the maximum allowable without grade reduction appear to be too high for corn in this moisture range. Visible mold damage may be excessive with 0.2 to 0.3% DML.

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EVALUATION OF DISINFESTANT-FLOTATION SALT-SURFACTANT SOLUTION COMBINATIONS ON DECAY FUNGI OF PEAR IN A MODEL DUMP TANK. R.A. Spotts and L.A. Cervantes. Oreg. St. Univ., Mid-Columbia Agric. Research and Extension Center, Hood River, OR 97031.

Sixteen solutions were compared for effects on germination of spores of Mucor piriformis, Penicillium expansum, and Phialophora malorum and decay of pear caused by these fungi after exposure to a 7 hr dynamic circulation and spore addition phase, followed by a 16 hr static phase in a model dump tank. In flotation systems without soil added, chlorine (Cl) at 64 µg/ml inhibited germination from 90 to 100% in all salt solutions. Effectiveness of sodium ortho phenylphenate (SOPP) at 0.4% was highest in calcium or sodium lignin sulfonate (NaLGS) and lowest in sodium silicate (NaSi) solution. SOPP was less inhibitory to germination than Cl during the first 1 to 3 hr of the dynamic phase. In flotation systems with 6.25 mg soil/ml, Cl in sodium sulfate and SOPP in Na LGS inhibited germination of spores and reduced decay of fruit more than in NaSi. Inhibition of germination of the three fungi was greater at the end of the static than during the dynamic phase in 8 of 30 tests.

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DRY MATTER LOSSES CAUSED BY STORAGE FUNGI ON SOYBEAN SEEDS. F. A. Lazzari, and R. A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Soybean seed samples were stored with initial moisture contents of 14.0%, 17.0%, and 20.0% and maintained at temperatures of 15°C, and 25°C in growth chambers. Samples with initial 22% moisture content were stored at room temperature (22-32°C). These samples were periodically tested for moisture content and dry matter loss. With increasing time, invasion by storage fungi caused an increase in moisture content in all samples, accompanied by dry matter reduction. After a storage period of 180 days dry matter losses varied from 0.18% in the soybean kept at 14.0% moisture content and 15°C, to 36.61% in the soybean kept at 22.0% moisture content and at room temperature (22-32°C).

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THE RELATIONSHIP OF SITE FACTORS TO THE INCIDENCE OF CYTOSPORA AND SEPTORIA CANKERS AND POPLAR AND WILLOW BORER IN HYBRID POPLAR PLANTATIONS IN MICHIGAN. G. Abebe and J.H. Hart, Depts. of Forestry and Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824.

Two hybrid poplar plantations in Michigan were surveyed (1984-87) for the incidence of poplar and willow borer damage, and for Cytospora and Septoria cankers. Tree height, root starch level, leaf water potential, root development, disease incidence and clonal differences were measured. Soil samples were analyzed for physical and chemical properties. Using discriminant and stepwise regression analyses, equations accounting for more than 80% of the variations in the incidence of cankers and borer were developed. An interaction model relating pest incidence, and soil and stand factors was developed. The two plantations were different in most of the soil nutrient levels that were analyzed. Soil nutrients and tree height were the two factors which correlated most closely with the pest problems in both plantations.

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INTERSTERILITY GENOTYPES OF HETEROBASIDION ANNOSUM FROM NORTHERN CALIFORNIA AND SOUTHERN OREGON. T.E. Chase, W.J. Otrosina, F.W. Cobb, Jr., and J.W. Taylor. USDA Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA, and University of California, Berkeley, CA.

Collections of monobasidiospore isolates were established from sporophores of Heterobasidion annosum in northern California and southern Oregon. Intersterility genotypes of isolates were determined in mating experiments with tester strains from Finland and North America. The data suggest the existence of two mutually intersterile groups. However, members of both groups are interfertile with testers from eastern North America. Heterozygosity at the V3 and P intersterility loci was common. Collections from true fir (Abies) yielded only S group isolates (sensu Korhonen), whereas collections from pine stumps yielded both S and P group isolates (sensu Korhonen).

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SHORT-TERM SUSCEPTIBILITY TESTS OF EUROPEAN OAKS TO OAK WILT. J. Pinon, F. H. Tainter, and W. L. MacDonald. INRA, Champenoux, Seichamps, France; Dept. Forestry, Clemson Univ., Clemson, SC 29634-1003; and Dept. Plant Path. & Agr. Micro., West Virginia Univ., Morgantown, WV 26506-6057.

Trees of twelve provenances representing five species of various European oaks growing in arboreta in two locations in the United States were inoculated with Ceratocystis fagacearum and observed for symptom development. In West Virginia, virtually 100% of all trees from all provenances, including Quercus rubra and Q. alba controls, developed wilt symptoms. Symptom expression in West Virginia was dramatic and associated with much mortality by end of the season. In South Carolina, only 40.0% of Q. rubra and 18.5% of Q. alba developed wilt symptoms and symptom incidence for European species ranged from 62.9% for Q. pedunculata to 5.9% for Q. petrae. In South Carolina symptom expression was relatively mild with little mortality.

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HISTOLOGICAL EVIDENCE OF RESISTANCE TO ENDOCRONARTIUM HARKNESSII IN PINUS CONTORTA **Eric A. Allen**, **P.V. Blenis**, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, and **Y. Hiratsuka**, Northern Forestry Centre, Canadian Forestry Service, Edmonton, Alberta T6H 3S5.

Resistance to Endocronartium harknessii was observed in 3, 10, 20, and 33-month-old seedlings of lodgepole pine. In some seedlings, fungal infections were stopped in the cortex. Infected cells died, were isolated by necrophylactic periderm, and exfoliated with the bark. Other infections progressed to the cambium where infected cells and cambial initials were inactivated resulting in abnormal secondary xylem development. In a number of cases, cambial function was restored and infected lesions were overgrown. Live mycelium often escaped cortical lesions and reinvaded healthy cells. Such infections were usually blocked but occasionally progressed to reinfect the cambium. These latent-type infections resulted in the initiation of gall formation up to one year after initial resistance to infection.

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Impact of Peridermium filamentosum on the scenic beauty of ponderosa pine. F.A. Baker and D.R. Rabin. Department of Forest Resources, Utah State University, Logan, UT 84322-5215.

Peridermium filamentosum causes limb rust, an unusual disease, that progresses through crowns of Pinus ponderosa in stem and branch xylem and kills branches. Trees die when most of the branches are killed. Ponderosa pines infected with limb rust are prominent in the viewscapes near some national parks in Southern Utah, where the trees scenic value far exceeds their timber value. We photographed scenes with infected trees, and used computer graphics to alter the incidence and severity of limb rust while keeping all other variables constant. Varying amounts of crown were removed from 2 trees in each of 4 different scenes. For each scene, ten images ranging from severely infested to noninfested were generated onto 35 mm slides. Without mention of the disease, these scenes were shown to viewers who independently rated each image for scenic quality. Scenic beauty ratings decreased significantly with increasing incidence and severity of limb rust.

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ASH YELLOWS: GEOGRAPHIC RANGE AND ASSOCIATION WITH DECLINE OF WHITE ASH. W. A. Sinclair, R. J. Iuli, and A. T. Dyer, Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853; P. T. Marshall, Indiana Dept. Natural Resources, Vallonia, IN 47281; J. A. Matteoni, Agriculture Canada, Vineland Station, Ontario L0R 2E0; C. R. Hibben, Brooklyn Botanic Garden Research Center, Ossining, NY 10562; G. R. Stanosz, Pennsylvania Dept. Environmental Resources, Middletown, PA 17057; and B. S. Burns, Vermont Agency of Environmental Conservation, North Springfield, VT 05150.

Ash yellows (AshY) occurs primarily between 39° and 45° N from the Great Plains to the Atlantic coast. White ash (*Fraxinus americana*) on 72 sites in Indiana, New York, Pennsylvania, Vermont, and Ontario were examined for dieback and, by means of the DAPI fluorescence test, for evidence of infection by mycoplasmalike organisms (MLOs). MLO infection was found on 29 of 36 sites where dieback was scored as common and severe, and on 6 of 36 sites where dieback was scored as scarce or absent. These six sites were all located near areas of severe ash decline. AshY was more common and damaging where open and wooded lands are intermixed than where land is primarily forested. Unexplained slow growth of white ash was common and apparently independent of MLO infection on sites in both categories.

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SYMPTOMOLOGY, ETIOLOGY, AND ECOLOGY OF COTTONWOOD BUD PROLIFERATION, A NEW DISEASE FOUND IN ALASKA. D. Boyce and J. H. McBeath, Agricultural and Forestry Experiment Station, University of Alaska, Fairbanks, AK 99775-0080.

A new disease has been found on Populus balsamifera growing under alpine conditions(near the treeline), at Denali National Park and Donally Dome area. The disease can be readily recognized by the proliferation of terminal buds (up to 50), all fused together in a flattened fashion. Histological studies of diseased tissues revealed an excessive proliferation of secondary phloem. Leaves formed were small, malformed, and turned yellow-colored prematurely. Significant decreases of stem diameter, tree height, canopy size and root sucker production were found on diseased trees. Samples were harvested periodically from both diseased and healthy trees. Leaves, stems and roots were fixed and prepared for transmission electron microscopy. Mycoplasma-like organisms were observed in the sieve elements of the diseased but not in the healthy tissues.

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EFFECT OF ETHEPHON AND ECTOMYCORRHIZAL TREATMENTS ON *FUSARIUM* ROOT ROT OF RED PINE SEEDLINGS. Sandra F. Maynard and William H. Livingston, Dept. of Botany and Plant Path., Univ. Maine, Orono, ME 04469

Pinus resinosa seedlings (2 weeks old) were inoculated with an ectomycorrhizal fungus (unknown species) and treated with weekly drenches of 0, 25, or 75 ppm ethephon (an ethylene releasing agent) for 16 weeks (49 seedlings/treatment). Red pine seedlings infected by the ectomycorrhizal fungus exhibit increased numbers and swelling of short roots and increased resistance to *Fusarium* infection. Drenching with ethephon produced a preferred root structure with similarities to that of ectomycorrhizal seedlings, but such treatments did not increase resistance to *Fusarium* infection. To clarify how the ectomycorrhizal fungus and ethephon treatments affect conifer resistance to *Fusarium* infections, tissue studies were performed to compare morphological and histochemical characteristics of *Fusarium* infected, ectomycorrhizal inoculated, and ethephon treated roots.

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HOST SPECIALIZED BIOLOGICAL SPECIES OF PHELLINUS WEIRII. P. A. Angwin and E. M. Hansen, Dept. Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Phellinus weirii, cause of laminated root and butt rot, is heterothallic with a multiallelic bipolar system of mating compatibility. Single spore and homokaryon-heterokaryon crosses confirmed the near-complete genetic isolation of two biological species groups, the cedar-type and Douglas-fir type. Protein banding patterns obtained by SDS polyacrylamide gel electrophoresis show overall similarity between the groups, but differ at several locations. Douglas-fir-type isolates were found on Douglas-fir and six other species, including western red cedar. The cedar-type isolates were mostly confined to western red cedar, but were also identified on western hemlock and grand fir. The geographic ranges of the Douglas-fir and cedar-type Phellinus weirii overlap in much of the Douglas-fir region, indicating that the emerging biological species groups are likely the result of host specialization rather than geographic separation.

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GENETIC ARCHITECTURE OF WESTERN GALL RUST (PERIDERMIUM HARKNESSII) IN CALIFORNIA PINE FORESTS. D. R. Vogler, B. B. Kinloch, Jr., F. W. Cobb, Jr., W. J. Libby, Jr., and T. L. Popenuck, Department of Plant Pathology, University of California, Berkeley, CA, 94720.

Aeciospores were collected from single galls on seven native pine species from coastal and inland mountain forests in California. Sieved and air-dried spores were ground in glass tissue grinders or homogenized with a Mini-BeadBeater^{R}, and then subjected to starch gel electrophoresis in each of four gel-buffer systems. Resolution was obtained for 16 enzymes encoded by 18 putative loci. As reported previously (D. R. Vogler et al., Phytopathology 77:1242), all enzyme loci from coastal California isolates were monomorphic. Seven loci from Cascade and Sierra Nevada mountain isolates were polymorphic among sites, but monomorphic within sites; the remaining 11 loci were mostly monomorphic and similar to those in coastal isolates. These results suggest that P. harknessii propagates clonally and is very homogeneous genetically.

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DISTRIBUTION OF PINE WOOD NEMATODE IN SOUTHERN PINE BEETLE-KILLED LOBLOLLY PINES COLONIZED BY PINE SAWYERS. L. D. Dwinell. USDA For. Serv., Southeast. For. Expt. Sta., Athens, GA 30602.

The pine wood nematode (PWN) (Bursaphelenchus xylophilus) is transmitted during oviposition of Monochamus titillator into loblolly pine (Pinus taeda) killed by Dendroctonus frontalis (SPB). Six SPB-killed loblolly pines (mean height of 12 m) were felled, sawn into 30 cm long bolts, and the wood sampled for nematode assay. Nematodes were extracted from samples using the pie-tin technique, and the numbers of oviposition pits/m determined. Boles were divided into quarters for data analysis. The greatest concentration of nematodes (1225 PWN/g dry wood wt) was in the upper 25% of the bole. The nematode was fairly evenly distributed over the lower 3 quarters of the bole (100 PWN/g dry wood wt). Oviposition pits were linearly distributed (10 pits/m) and were not correlated with PWN concentrations.

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BIOTINYLATED CLONED DNA PROBE DETECTS ASH YELLOWS MYCOPLASMALIKE ORGANISM IN ASH. R. E. Davis, I. M. Lee, W. Sinclair, and S. M. Douglas. USDA, ARS, Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705; Plant Pathology Dept, Cornell University, Ithaca, NY 14853; and Connecticut Agricultural Experiment Station, New Haven, CT 06504.

Ash yellows (AshY), a disease responsible for decline and death of ash (Fraxinus spp.) in midwestern and northeastern states, is apparently caused by a mycoplasmalike organism (MLO). A biotinylated cloned DNA probe hybridized in dot blots with nucleic acid extracted from Ash Y-infected, but not healthy, white ash (F. americana), flowering ash (F. ornus), and periwinkle (Catharanthus roseus). The probe also detected other MLOs, but not the agent of elm yellows, in periwinkle. Although not specific for AshY MLO alone, the probe should aid research into the host range, geographical occurence, and tissue spread of these agents.

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METHODS FOR DESCRIBING THE EFFECT OF ARMILLARIA ROOT ROT ON GROWTH OF YOUNG PINUS TAEDA. J. E. Lundquist, North Dakota State University, Department of Horticulture and Forestry and Department of Plant Pathology, Fargo, ND 58105.

Five methods of calculating tree growth loss due to root disease and four of illustrating associated changes in stem growth patterns were examined using 8 year old Pinus taeda infected with Armillaria root rot in South Africa. Methods of calculating loss were able to show that disease caused an average of between 57% and 70% incremental growth loss for the final year of growth in trees that died and between 13% and 39% in trees that showed symptoms but were not yet dead. Loss estimates based on cumulative growth were less sensitive than those based on incremental growth. Graphical illustrations of growth patterns indicated a sharp reduction in ring width within the last two years at all heights of symptomatic trees, but no significant change in stem taper.

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INFECTION OF TISSUE-CULTURED ASPEN PLANTLETS WITH TOBACCO NECROSIS VIRUS. R. Belanger, J.D. Castello and P.D. Manion. State University of New York, College of Environmental Science and Forestry, Syracuse, NY 13210.

One leaf of each of 50 tissue-cultured plantlets of two trembling aspen (Populus tremuloides Michx.) clones (B-10 and B-70) were mechanically inoculated with a purified preparation of an aspen isolate of tobacco necrosis virus (TNV). Twenty plantlets of each clone were inoculated similarly with buffer as controls. At five day intervals five plantlets of each clone were harvested, frozen, and indexed for TNV by ELISA. Virus inoculated plantlets of clone B-10 began to show necrosis on day 15. On day 20 five plantlets died. Only slight necrosis and no mortality was observed in control or B-70 plantlets. Virus concentration in inoculated plantlets of both clones as measured by ELISA continued to rise from approximately 100 ng/ml on day 5 to over 1000 ng/ml on day 40. TNV is capable of replication and systemic spread in aspen. Clonal differences in response to TNV were observed.

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DISTRIBUTION AND TURNOVER OF ISOFLAVANOID CONJUGATES IN PMG INFECTED SOYBEAN TISSUES. T. L. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Accumulation of the phytoalexin glyceollin in response to Phytophthora megasperma var. glycinea (PMG) wall glucan is preceded by the de novo synthesis of the precursors, daidzein (DZ) and genistein (GT). We have found that soybean seed, root, hypocotyl and cotyledon tissues contain large constitutive pools of both DZ and GT in the form of complex conjugates. The identity and distribution of these conjugates varies markedly among tissues and their levels are often ten times that required for an ED50 glyceollin response. We are investigating the role of these preformed pools during discrete temporal events in the PMG and rhizobium infection processes. DZ and GT are very effectively released from their conjugates in PMG infected tissues, but not in response to purified PMG wall glucan. The mechanisms regulating DZ and GT release from these conjugates may thus play an important and previously undefined role in soybean responses to infection.

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EFFECT OF EXPRESSION OF BACTERIAL CHITINASE ON TOBACCO SUSCEPTIBILITY TO LEAF BROWN SPOT: ALTERNARIA LONGIPES. T. V. Suslow, D. Matsubara, J. Jones, R. Lee, P. Dunsmuir. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA 94608.

It has been proposed that overexpressing chitinase protein in plants may directly or indirectly increase disease resistance to certain fungal pathogens. To evaluate this model, a series of tobacco plants were transformed to contain *chi A*, which encodes a 58 kDa chitinase from *Serratia marcescens* shown to contribute to fungal inhibition in vitro and in vivo (Jones et al, 1986). Various populations of *chi A*-expressing plants were developed using mesophyll-specific or constitutive plant promoters fused to the bacterial gene. In these transformed plants bacterial chitinase protein approached 0.2% of the plant total soluble protein. At these levels the bacterial enzyme increased the endogenous chitinase activity by 30 - 40%. With the best comparable homozygous populations, *chi A*-expressing tobacco leaves had significantly reduced necrotic lesion development and reduced chlorosis when transformed control plants were at peak susceptibility. Continued maturation of *chi A*-transformed plants eliminated these differences. Points to consider in evaluating disease resistence of transgenic plants will be emphasized.

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INCREASE IN ACID PHOSPHATASE ACTIVITY ASSOCIATED WITH THE HYPERSENSITIVE RESPONSE IN POTATO-PHYTOPHTHORA INFESTANS INTERACTION. C. M. Jordan and J. E. DeVay, Department of Plant Pathology, University of California, Davis, California 95616.

Acid phosphatase activity was visualized cytochemically and ultrastructurally in healthy and diseased leaves of Solanum tuberosum cv. Kennebec. In hypersensitive reactions (HR), visible 2-5 days after inoculation with Race 0 of Phytophthora infestans, AP activity was observed in the cytoplasm of infected cells; cells in healthy leaves and leaves inoculated with the compatible Race 1.2.3.4 had less AP activity than in HR. Levels of hydroxyl free radical ($OH^{\cdot}$) in HR 48 h after inoculation were higher than in healthy controls and compatible interactions. Hyphae associated with HR were degenerated, and AP activity appeared to be associated with disrupted lysosomes. Two isozymes of AP were present in diseased and healthy leaves as indicated by cellulose acetate paper electrophoresis. Lysosome disruption associated with increases in $OH^{\cdot}$ appeared to contribute to cell death.

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THE LOSS OF VIRULENCE BY A CONSENSUS DELETION OF THE iaa OPERON IN PSEUDOMONAS SYRINGAE PV. SAVASTANOI. Scott Soby and Tsune Kosuge, Department of Plant Pathology, University of California, Davis, California 95616 USA.

A large (20kb) deletion which encompases the iaa operon of the 72kb plasmid pIAA2 of Pseudomonas syringae pv. savastanoi occurs at a high frequency (50%) of IAA- mutants. The deletion margins are conserved among a large number of independently isolated mutants and map from 12kb upstream to 8kb downstream of the IAA structural genes. Restriction enzyme fragment comparisons between the wild type and deletion mutants suggest that the insertion element IS51 resides in the region downstream of iaa and may be involved in mediating the deletion event. Two other native IS elements do not appear to be involved in the deletion. The presence of IS elements and other repetitive DNA flanking the IAA region may suggest a mechanism for transposition of the phytohormone genes.

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INDUCTION OF LINEAR FURANOCOUMARINS IN CELERY AND CELERIAC. S. Heath-Pagliuso, O. C. H. Kwok, and L. Rappaport., Dept. of Veg. Crops/Plant Growth Lab., Univ. of Calif., Davis, CA 95616

Linear furanocoumarins (LFCs), xanthotoxin, bergapten, psoralen, and isopimpinellin, are highly phototoxic compounds that are believed to be the causal agents of a contact dermatitis in celery handlers and processors. The recent invasion of a root pathogen, Fusarium oxysporum race 2 (FOA_2) into the prime celery growing regions of California prompted an investigation of the effects of this fungus on LFC production. The levels of LFCs typically found in petioles of mildly infected celery and celeriac plants were generally less than 10 ppm; levels as high as 60 ppm were measured in very diseased plant tissues. Celery cell suspension cultures are being used to create mutants in the pathway in order to study the regulation and function of these compounds.

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LIPOXYGENASE AND LIPID PEROXIDATION IN TOMATO TREATED WITH SPECIFIC AND NON-SPECIFIC ELICITORS FROM CLADOSPORIUM FULVUM T.L. Peever and V.J. Higgins. Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

Glycoprotein non-specific elicitor (NSE) and a specific elicitor preparation made from intercellular fluids (SE) of tomato infected with race 2.4.5 of Cladosporium fulvum were injected into cv. Sonatine (resistant to race 2.4.5) to compare lipoxygenase activity and lipid peroxidation induced in response to these elicitors. Increased lipoxygenase activity was detected 6 hours after injection with either elicitor. Activity peaked at 12 hours with both elicitors and declined to low levels by 24 hours, when visible necrosis was detected. A 6-8 fold increase in lipoxygenase over the controls was observed with NSE at 12 hours compared to 2-3 fold with SE. Lipid peroxidation in elicitor-treated tissue was also assayed at 6, 12 and 24 hours after injection. Increased peroxidation was not detected until 12 hours with similar values obtained at 24 hours. No differences in amounts of peroxidation were detected between the two elicitors.

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EARLY ACCUMULATION OF CHITINASES, β-1,3-GLUCANASES AND OTHER b-PROTEINS IN TOBACCO IMMUNIZED AGAINST BLUE MOLD. S. Tuzun, M. N. Rao, U. Vogeli,* C. L. Schardl and J. Kuć. Department of Plant Pathology, *Department of Agronomy, Univ. of Kentucky, Lexington, KY 40546.

b-Proteins were extracted from leaves of Burley tobacco, Ky 14, stem-injected with water (control) or systemically protected against blue mold by stem injection with Peronospora tabacina (immunized). SDS-PAGE indicated the presence of b-proteins in immunized plants prior to challenge with P. tabacina; these were not detected in controls until 6 days after challenge. Western blot analyses and enzyme activity assays indicated the presence of low levels of chitinases, but not β-1,3-glucanases in controls prior to challenge. Both enzymes, as well as other b-proteins, were detected or increased earlier in challenged leaves of immunized plants. The increases in chitinases and β-1,3-glucanases and other b-proteins coincided with the onset of immunization in P. tabacina-injected plants.

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CYCLOHEXIMIDE DECREASE IN MAIZE PEROXIDASE MAY INVOLVE LECTINS. M. Akhtar and M. O. Garraway, Dept. Pl. Path., OARDC and Ohio State University, Columbus, OH 43210.

Detached leaves of maize (Zea mays L.) were exposed to cycloheximide (40 ppm) in the dark at 28 C for 24 h. Compared to distilled water control, cycloheximide decreased the activity of buffer extractable peroxidase (BEP) but not its cathodic isozymes separated by starch gel electrophoresis. This suggests that neither synthesis nor degradation is involved in the decrease in BEP in response to cycloheximide. BEP or commercial peroxidase when mixed with pellets from homogenized maize leaves showed a decrease in enzyme activity. Similarly, peroxidase activity decreased when either BEP or commercial peroxidase were mixed with concanavalin A. Thus cycloheximide decrease in peroxidase activity in maize may involve lectins. These findings may facilitate further research which has as its goal the evaluation of the role of peroxidase in regulating the response of maize to fungal infection.

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ROLE OF PROTEOLYTIC ACTIVITY IN ELECTROLYTE LEAKAGE FROM MAIZE LEAVES EXPOSED TO HIGH TEMPERATURE STRESS (HTS) PRIOR TO INFECTION BY BIPOLARIS MAYDIS. M. O. Garraway and M. Akhtar, Dept. Pl. Path, OARDC and Ohio State Univ., Columbus, OH 43210.

Detached leaves of susceptible or resistant isolines of the maize inbred W64A, were incubated for 6 h in the dark at 42 C (HTS), inoculated with Bipolaris maydis race T (BMT), then further incubated for 24 h in the dark at 28 C. Controls were inoculated but not exposed to HTS. Electrolyte leakage (EL) from infected susceptible leaves was more than from infected resistant leaves and increased when leaves were exposed to HTS prior to inoculation. Protease in cell free leaf extracts degraded protein more rapidly at 42 C than at 28 C indicating parallel trends in proteolytic activity and EL in response to HTS. Leaves infiltrated with protease leaked more electrolytes than controls. Moreover, they were more prone to leak electrolytes following inoculation with BMT, or exposure to HTS or both. Proteolytic activity may mediate in part EL seen in response to HTS.

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AEROBACTIN PRODUCTION BY A STRAIN OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA. C. A. Ishimaru and J. E. Loper, USDA, ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330

Aerobactin is a hydroxamate siderophore produced by certain members of the Enterobacteriaceae and is one of several virulence factors in Escherichia coli. Pathogenic strains of Erwinia carotovora were screened for aerobactin production by bioassay and by homology to aerobactin biosynthesis genes of E. coli. Of 23 strains tested, E. carotovora subsp. carotovora CC105 provided iron to an E. coli mutant (LG1522) that grows under iron-limiting conditions only when given exogenous sources of aerobactin. Southern analysis, using labeled aerobactin biosynthesis genes of E. coli as a probe, identified in the genomic DNA of strain CC105 a single homologous 16 kb EcoRl fragment, which appeared chromosomal in origin. No homology was observed with the other strains tested. Aerobactin production by soft rot erwiniae is probably not common nor is it a requirement for pathogenicity, since only one of the strains tested produced aerobactin.

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CUTINASE FROM *VENTURIA INAEQUALIS*. Wolfram Köller, Dept. of Plant Pathol., Cornell University, Geneva, NY 14456.

Venturia inaequalis was grown on purified apple cutin as the sole carbon source. An enzyme with esterase activity was isolated from the culture fluid after eight weeks of growth and purified to apparent homogeneity. The purified esterase hydrolyzed tritiated cutin, and the hydrolysis products were identical to the fatty acids present in polymeric cutin. This indicated the cutinase nature of the enzyme. The enzyme is a glycoprotein (5.4 % carbohydrate) with a molecular weight of 22,000. Structural features are two disulfide bridges, a high content of glycine, a high content of nonpolar amino acids and a high degree of hydrophobicity. Cutin hydrolysis was optimal at pH 6 and different from the alkaline pH-optimum reported for other cutinases. The enzyme activity was inhibited by diisopropyl fluorophosphate. The phosphorylation of one active serine residue was sufficient for complete inhibition.

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ISOLATION OF TOXINS FROM *SEPTORIA APIICOLA*. O. C. H. Kwok and L. Rappaport, Dept. of Vegetable Crops/Plant Growth Laboratory, University of California, Davis, CA 95616.

Two necrosis-inducing toxins were isolated from the culture filtrates of *Septoria apiicola* grown in modified Fries medium. The toxins were detected using a leaf-prick bioassay. Partial purification was accomplished by methanol precipitation and Sephadex LH-20 column chromatography using 60% isopropanol as the developing solvent. A single active peak was obtained, which was resolved into two active peaks on reversed phase HPLC developed isocratically with 45% methanol, with retention times of 10 (toxin I) and 50 (toxin II) minutes, respectively. Toxin I was highly water-soluble, but toxin II was only slightly soluble. The toxins were not host-selective, as they both caused necrotic spots on leaves of celery, corn, cowpea and pea.

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TOXICITY OF TERPENOID PHYTOALEXINS FROM COTTON TO DEFOLIATING AND NONDEFOLIATING ISOLATES OF VERTICILLIUM DAHLIAE. Mace, M. E. and R. D. Stipanovic. USDA, ARS, Cotton Pathology Research Unit, P. O. Drawer JF, College Station, TX 77841.

The terpenoid phytoalexins hemigossypol (HG), methoxyhemigossypol (MHG), desoxyhemigossypol (dHG), and desoxymethoxyhemigossypol (dMHG) from Verticillium dahliae-infected, wilt-resistant Seabrook Sea Island cotton were tested at pH 6.3-7.5 in liquid nutrient media for toxicity to two defoliating and two nondefoliating isolates of V. dahliae. Terpenoid concentrations of 35-40, 90-95, 15-20, and 45-50 g/ml of HG, MHG, dHG, and dMHG, respectively were required to kill mycelia, as measured by subsequent growth, of all four isolates of V. dahliae. Thus, differential sensitivity to terpenoid phytoalexins appears not to be a factor in the differences in virulence of the defoliating and nondefoliating isolates of V. dahliae on cotton.

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AUTOFLUORESCENCE, SILICON, AND HYPERSENSITIVE CELL DEATH IN BARLEY EPIDERMIS - POWDERY MILDEW INTERACTION. H. Koga, R. J. Zeyen, and W. R. Bushnell, Department of Plant Pathology, University of Minnesota and USDA/ARS Cereal Rust Laboratory, St. Paul, MN 55108.

Viability or death of epidermal cells containing the Mla resistance gene was determined by uptake of neutral red and cell plasmolysis. A low percentage of cell death first occurred at 15h post-inoculation, reaching a maximum, 72%, at 24 h. Autofluorescence was weak in dying or recently dead cells but gained intensity with time after death, suggesting release of phenolic compounds from cell vacuoles after membranes lost semipermeability. Insoluble silicon (Si), determined by ED X-ray microanalysis with SEM, did not correspond to initial appearance of autofluorescence but occurred gradually after cell death, suggesting that Si reacts with phenolics released at cell death and forms insoluble Si-phenolic complexes when monosilicic acid bathes dead cells during passive transpiration transport.

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PURIFICATION AND CHARACTERIZATION OF A POLYGALACTURONASE PRODUCED BY *COLLETOTRICHUM GLOEOSPORIOIDES* PATHOGENIC ON AVOCADO. D. Prusky and N. T. Keen, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

A highly purified endopolygalacturonase preparation was obtained from culture filtrates of *Colletotrichum gloeosporioides* by a two step purification process involving hydrophobic interaction chromatography on phenyl sepharose CL4B and isoelectric focusing. The purified preparations were resolved into two bands on SDS-gel electrophoresis with estimated molecular weights of 62 and 64 kDa. However, both bands possessed endo-PG activity and had identical N-terminal amino acid sequences, suggesting that they were isoforms due to glycosylation or other differences. The isoelectric point of both forms was approximately 4.95 and the optimum pH for activity was 5.1. The purified enzyme efficiently macerated avocado fruit slices, raising the possibility of pathogenic involvement.

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SCREENING *ASPERGILLUS* AND *PENICILLIUM* SPP. FOR POTENTIAL BIOLOGICAL CONTROL OF PHYTOPHTHORA GUMMOSIS OF CITRUS. A. Sztejnberg, H. D. Ohr, and P. H. Tsao. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Fourteen antagonistic fungal isolates (in 5 *Aspergillus* and 7 *Penicillium* spp.) were evaluated as potential agents for biocontrol of Phytophthora gummosis of citrus. Wounded bark of stems (8-15 mm diam) of sweet orange seedlings was coated with conidia (in 0.3% agar) of each antagonist immediately before inoculation with a mycelium disc of *Phytophthora citrophthora* or *P. parasitica*. Bark lesion size at 2-3 weeks was greatly reduced (up to 97%) by *Aspergillus flavipes, A. ochraceus, A. wentii, and Penicillium funiculosum* in repeated tests. *A. niger, A. versicolor, P. chrysogenum, P. citrinum, P. decumbens, P. frequentans, P. janthinellum,* and *P. ochrochloron* were less effective or ineffective in reducing *Phytophthora* lesion size. In 3 field experiments, lesion size of both pathogens on trunks of mature lemon trees also was reduced (77-84%) by some antagonists, especially *P. funiculosum* and *A. wentii*.

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USE OF BACTERIZED SAND AS A DELIVERY METHOD FOR RHIZOSPHERE-COLONIZING BACTERIA. M. Elliott Juhnke. University of Florida, Fort Lauderdale, Fl. 33314

Six bacterial isolates, used previously as seed inoculants for wheat, were screened for their ability to colonize established bermudagrass roots. The antibiotic-resistant isolates were applied individually to sand which was then top-dressed onto bermudagrass. One month after the bacterized sand application, the Streptomyces griseus isolate was not detected on roots. The Pseudomonas fluorescens, Xanthomonas maltophilia and coryneform isolates were present but at minimal levels. The Bacillus pumilus and B. subtilis isolates were colonizing at $\log_{10}$ 2.1 and 1.4 CFU per mg root (dry weight), respectively. Three months after application, B. pumilus and B. subtilis root-colonizing populations remained the same. However, the populations dropped substantially two months later.

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Biological Control of Black rot of cabbage using an avirulent Xanthomonas campestris strain
M.D.Kawalek, and N.W.Schaad, Plant Science Dept., University of Idaho, Moscow, Id. 83843.

A naturally occurring avirulent strain of Xanthomonas campestris (B122) was tested for biological control of black rot in greenhouse and growth chamber experiments. Cabbage seedlings (Brassica oleracea L.) producing guttation droplets in a dew chamber were spray-inoculated with 10^4-10^5 CFU/ml of Xanthomonas campestris pv. campestris strain B24 simulating natural infection of hydathodes. Plants sprayed with 10^6 CFU/ml of strain B122 during or prior to inoculation of the pathogen had 45-65% fewer lesions than unprotected controls, but lesion size was not effected. Using genetically marked isolates to quantify bacterial population size of leaf tissue, reduced lesion size was correlated with a delay in the growth of the pathogen when coinoculated with strain B122.

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INDIRECT EVIDENCE FOR OOMYCIN A EXPRESSION IN SITU: EFFECT OF SOIL TEMPERATURE, MOISTURE, AND TEXTURE. W. Howie, M. Correll, N. Gutterson, and T. Suslow. Advanced Genetic Sciences, 6701 San Pablo Ave. Oakland, CA 94608.

The effect of soil temperature, moisture, and texture on expression in situ of oomycin A was determined indirectly by using a lux-transcriptional fusion in the afuE operon of Pseudomonas fluorescens strain Hv37aR2 (pJZ71::lux4) denoted as strain WH137. Cotton seeds of Acala SJ-2 had 5 x 10^5 cfu/seed of WH137 at planting. A Hesperia Fine Sandy Loam (HFSL) and a Brentwood Clay (BC) were adjusted to 16, 20, or 24 C and to -.05, -.3, -1.0, or -4.5 bars. Seed populations of WH137 and bioluminescence (single photon scintillation counts) were determined at 24 and 48 hr after planting. Maximum expression occurred at 20 C and at -.3 and -1.0 bars for the HFSL and BC respectively. Both seed populations and expression were higher by 0.5 to 1 log units in the BC than in the HFSL. Bioluminescence, seed colonization by WH137, and incidence of infection of cotton-seed coats by Pythium ultimum was determined from 0 to 48 hr in the HFSL at 20 C and at -.3 bars. Seed colonization began by 3 hr, whereas bioluminescence did not occur until 10-12 hr after planting. P. ultimum infection was detected 6 hr after planting. Seed colonization and bioluminescence peaked by 18-20 hr and incidence of infection peaked by 14-16 hr.

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EFFECT OF THE SATELLITE OF TOBACCO RINGSPOT VIRUS-RNA ON CHERRY LEAFROLL VIRUS-WALNUT ISOLATE IN WALNUT TREES. Adib Rowhani, F. Ponz, S. M. Mircetich, and G. Bruening, Department of Plant Pathology, University of California, Davis, CA 95616.

Blackline disease of English walnut/Northern California black (NCB) or Paradox rootstock is caused by cherry leafroll virus (CLRV-W). Recent in vitro studies showed that the satellite RNA of tobacco ringspot virus (STobRV RNA) inhibits the translation of CLRV-W-RNA. We investigated the effects of STobRV-RNA on replication of CLRV-W in vivo by inoculating English walnut trees on NCB rootstock with CLRV-W and STobRV RNA. Five trees each were inoculated with a mixture of CLRV-W and STobRV RNA (1:1), CLRV-W or buffer. These trees were tested 3 months later by ELISA. Virus accumulation was prevented or reduced substantially in four of five CLRV-W and STobRV RNA inoculated trees (Avg. OD_{450} = 0.168) as compared to five trees inoculated with CLRV-W alone (OD_{450} = 1.649) and five trees inoculated with buffer only (OD_{450} = 0.094). Therefore, STobRV RNA could be used to prevent the replication of CLRV-W and possibly used to control blackline disease.

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A STRAIN OF *MYROTHECIUM RORIDUM* AS A POTENTIAL BIOCONTROL AGENT AGAINST *PHYTOPHTHORA CINNAMOMI*. R. Gees and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

To test for potential antagonists of *Phytophthora cinnamomi*, over 60 fungi were isolated from the rhizosphere of avocado roots, growing in a suppressive soil where *Phytophthora* had been present for over 40 yrs. Strain TW of *Myrothecium roridum* was the most active in controlling *P. cinnamomi* in repeated greenhouse-pot tests using highly susceptible seedlings of *Persea indica* inoculated with *Phytophthora*. *M. roridum* was grown on a wheat-bran medium and introduced into a peat-perlite mixture at 2.5% (w/v), 2 weeks prior to inoculation with *Phytophthora*. In a UC-mixture inoculated with *P. cinnamomi* zoospores, *M. roridum* reduced root infection of *P. indica* by 50-94% compared to the controls (P = 0.05). In three naturally-infested field soils, root infection ranged from 12-54% in the presence of *Myrothecium*, compared to 58-93% for the controls over the same 4 wk period.

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EVALUATION OF BINUCLEATE RHIZOCTONIA-LIKE FUNGI FOR PROTECTION OF CUCUMBER SEEDLINGS FROM RHIZOCTONIA SOLANI. M. A. Cubeta and E. Echandi. Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695.

Twenty binucleate Rhizoctonia-like fungi isolates from soil and several monocot and dicot hosts were tested in the greenhouse and field for protection of cucumber seedlings from damping-off caused by R. solani. Isolates were screened for pathogenicity on cucumber by the method of Cardoso and Echandi (Plant Disease 67:167-170). Fifteen isolates that were avirulent or weakly virulent on cucumber were then evaluated for their ability to protect cucumber seedlings from R. solani. Fourteen of the isolates significantly (P=0.05) reduced preemergence damping-off by 11-21% and postemergence damping-off by 68-91%. Eight isolates significantly (P=0.05) reduced disease incidence and severity.

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CROSS PROTECTING FUSARIUM STRAINS INCREASE YIELDS FROM FUSARIUM CROWN AND ROOT ROT INFECTED TOMATO PLANTS GROWN IN SAWDUST CULTURE. R.J. Copeman, B.E. Mauza and G.W. Eaton. Plant Science Department, University of British Columbia, Vancouver, British Columbia V6T 2A2 and British Columbia Ministry of Agriculture and Fisheries, Abbotsford, British Columbia V2S 1K2.

A mixture of three cross protecting Fusarium strains (CPS) was applied to 9-day-old tomato ('Dombito') seedlings. Some of these seedlings were inoculated with Fusarium oxysporum f. sp. radicis-lycopersici (FORL) 26 days after seeding. Protected, pathogen-inoculated plants had significantly increased mean yields (35%) over a 20-week harvest period compared to pathogen-inoculated but unprotected controls. The observed increase was caused by a significant increase in the mean fruit number and to a selective increase in the percentage of fruit grading into the extra large category. A second CPS treatment at 58 days did not further increase yield. CPS-treated plants not inoculated with FORL had mean yields comparable to the unprotected, healthy controls.

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PROTECTION OF TOMATO FROM SEVERE CUCUMBER MOSAIC VIRUS DISEASE USING PREVENTIVE INOCULATION WITH AN ATTENUATED VIRUS-SATELLITE COMBINATION. M.S. Montasser and J.M. Kaper. Botany Dept., Univ. of Maryland, College Park and Plant Sciences Inst., ARS, USDA, Beltsville, Maryland 20705.

Cucumber mosaic virus strain S (CMV-S), carrying the nonnecrogenic satellite S-CARNA 5 (=CMV-Associated RNA 5), was used for preventive inoculation of tomato (Lycopersicon esculentum cv. UC82B) plants to block their necrotic response against challenge infection by CMV-D, which contains the necrogenic D-CARNA 5, or against challenge by satellite-free CMV-16, which normally causes severe stunting in tomato. The above studies have been used as the basis for a field test of satellite-mediated protection of tomato against CMV infection, in which it was attempted to use molecular probes for monitoring in planta the interrelationships and interactions of the CMV strains and CARNA 5 variants used for protective and challenge infection.

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Quantitative Assay for Parasitism of Criconemella xenoplax by the Nematophagous Fungus Hirsutella rhossiliensis. B. A. Jaffee, J. T. Gaspard, H. Ferris, and A. E. Muldoon. Department of Nematology, University of California, Davis 95616.

An assay was developed to quantify numbers of Criconemella xenoplax (Cx) parasitized by Hirsutella rhossiliensis (Hr) in peach orchard soil. Cx were extracted from soil, surface disinfested, and aliquots spread onto water agar containing 200 ppm streptomycin. After 5 days at 22 C, plates were examined at 20-70x, and Cx with or without sporulating Hr were counted. The assay was used to measure the rate at which parasitized Cx degrade. Time required for 50% of the population to degrade in natural field soil was 61, 26, or 16 days at 10, 15, or 20 C, respectively. Parasitized juveniles degraded much faster than parasitized adults. Rate of infection can be estimated if numbers of parasitized nematodes and their rate of degradation are known.

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EVALUATION OF ENDOPHYTIC *PSEUDOMONAS* SPP. ISOLATED FROM LIVE OAK TREES FOR POTENTIAL BIOLOGICAL CONTROL OF OAK WILT. D. S. Brooks, C. F. Gonzalez, and D. N. Appel, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843; T. H. Filer, USDA-Forest Service, Stoneville, MS 38776.

Experiments were conducted to evaluate the ability of oak-inhabiting pseudomonads to inhibit oak wilt development in artificially-inoculated, containerized live oaks (*Quercus fusiformis*). Trees were inoculated with bacteria (10^8 CFU) and at day-7 challenged with the oak wilt fungus, *Ceratocystis fagacearum* (10^8 spores). After 8 weeks, an average crown loss of 41.6% per tree was observed in 9 of the 10 trees challenged with *C. fagacearum* without previous bacterial treatment. Only 4 of the 10 trees preinoculated with *Pseudomonas denitrificans* strain 1-15 exhibited oak wilt symptoms, with an average crown loss of 25% per tree. The *P. denitrificans* pretreatment significantly (P = .05) reduced disease symptoms over the control treatment. Pretreatment with *P. putida* strain 5-48 failed to inhibit symptom development. Other endophytic bacteria are currently being evaluated as agents for biological control of oak wilt.

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ANTAGONISM OF ARMILLARIA OSTOYAE AND PHELLINUS WEIRII BY TRICHODERMA SPP. IN CULTURE. J. L. Reaves, USDA Forest Service, Forestry Sciences Laboratory, Corvallis, OR 97331.

The antagonism of Armillaria ostoyae and Phellinus weirii by four species (11 isolates) of Trichoderma is reported. Culture filtrates from all isolates of Trichoderma reduced or prevented growth of A. ostoyae or P. weirii. When A. ostoyae was grown in dual culture with Trichoderma isolates, a large portion of the submerged A. ostoyae rhizomorph tips were nonviable. In contrast, submerged A. ostoyae rhizomorph tips from 35 day-old axenic culture remained viable. Growth rates of eight of the 11 Trichoderma isolates were increased significantly over those of controls when the medium was amemded with filtrates obtained from P. weirii. These results support the use of antagonistic fungi such as Trichoderma spp. as potential biological control agents for A. ostoyae and P. weirii.

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HISTOPATHOLOGICAL STUDY ON THE EFFECTS OF LIGHT INTENSITY AND TIME OF APPLICATION ON THE INFECTION PROCESS OF ALTERNARIA CASSIAE ON SICKLEPOD. R. A. Hudson, J. C. White, and J. S. Bannon, Mycogen Corp., 3303 McDonald Ave., Ruston, La.

The effects of light intensity and application time were determined on the infection of sicklepod seedlings (Cassia obtusifolia L.) by Alternaria cassiae Jurair and Khan. Each experiment was replicated six times. Sicklepod percent kill and percent biomass reduction were directly proportional to light intensity in plants not predisposed to reductions in intensity prior to inoculation. Predisposition of plants to various light intensities prior to inoculation did not significantly alter percent sicklepod kill or biomass reduction. Time-of-day applications were conducted at two-hour intervals from 0600 hours to 2200 hours, inclusive. The only significant reduction in the efficacy of A. cassiae on sicklepod was at 2200 hours. Histopathological studies revealed differences in response to light intensity.

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INTERACTION OF TURNIP YELLOW MOSAIC VIRUS (TYMV) WITH ARABIDOPSIS THALIANA. S. Ramachandra, S.A. Lommel and F.F. White, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A single TYMV isolate differentially infects five Arabidopsis thaliana accessions. Landsberg erecta (L.e.), Nd-0, Col-0 and Bur-0 were inoculated with 100, 10 and 1 ug/ml of TYMV under various temperature regimes (greenhouse conditions, 18°C & 28°C) and assayed for local and/or systemic infections using ELISA. TYMV was detected only in the inoculated leaves of L.e. and Nd-0 at 18°C at the lowest inoculum level. Systemic infections occured at higher inoculum levels and higher temperatures in L.e. and Nd-0. TYMV systemically infected both Col-0 and Bur-0 at all three inoculum levels and temperature regimes. Accession Fi-3 was inoculated with TYMV under the same temperature regimes and was assayed by ELISA. No virus was detected either in the inoculated or uninoculated leaves at any temperature.

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A MONOCLONAL ANTIBODY WHICH DISCRIMINATES ISOLATES OF CITRUS TRISTEZA VIRUS. T. A. Permar, S. M. Garnsey, U.S. Department of Agriculture, 2120 Camden Rd., Orlando, FL, 32803, D. J. Gumpf, Univ. of California, Riverside, CA 92521, and R. F. Lee, Univ. of Florida, Lake Alfred, FL 33850.

A monoclonal antibody produced to the Florida citrus tristeza virus (CTV) isolate T36 reacted to decline, seedling yellows, and stem pitting isolates of CTV from Florida, California, and Spain. It did not react to mild CTV isolates from these same areas which produce symptoms only in Mexican lime. All CTV antigen sources used reacted strongly to polyclonal antibodies in double sandwich ELISA and to a CTV-polyspecific Spanish monoclonal (3DF1) in comparable double sandwich indirect ELISA assays. Similar levels of discrimination to CTV isolates were observed in indirect ELISA tests with plate-trapped antigen and in double sandwich indirect ELISA tests with antigen trapped on polyclonal antibody-coated plates. The monoclonal, coded CTV-MCA13, is a IgG2a immunoglobulin and did not react to extracts of healthy citrus or citrus infected with other viruses.

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EVIDENCE FOR A DOUBLE-STRANDED DNA GENOME IN SMALL NON-ENVELOPED BACILLIFORM PLANT VIRUSES. B.E.L. Lockhart, M. Bouhida, and N. Olszewski. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Evidence based on sensitivity to nuclease treatment, electrophoretic and buoyant density properties, electron microscopy, cloning and hybridization experiments suggests that small non-enveloped bacilliform plant viruses, including banana streak and commelina yellow mottle viruses contain a circular double-stranded DNA genome 7.3-7.5 kbp in size. Viruses of similar morphology occur in cacao, canna, Kalanchoe, rice, sugarcane, yam and yucca, and have also been found in several invertebrates. Further investigation will determine whether all of these bacilliform viruses contain DNA.

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CLONING OF THE DSRNAS OF AN ILAR-LIKE VIRUS FROM RASPBERRY AND NUCLEOTIDE SEQUENCE ANALYSIS OF RNA-3. W. Jelkmann and R.R. Martin. Agriculture Canada Research Station, Vancouver, B. C., Canada V6T 1X2.

Agarose gel electrophoresis of dsRNA extracted from commercial raspberry plantings in the Pacific Northwest showing leaf spot symptoms yielded three major dsRNA bands (1, 2 and 3) with sizes of 3.7, 2.6 and 1.8 kbp, respectively. This virus is aphid-borne but is being referred to as an ilar-like virus because of its dsRNA pattern. Gel purified dsRNAs were reverse transcribed into cDNA using as little as 500 ng of template and cloned into pUC9. Plasmid DNA extracted from colonies that showed strong signals in filter hybridization tests contained inserts ranging from 0.5 to 1.6 kbp. Northern hybridization analyses indicated that cDNA clones of all three dsRNA components were obtained. Some cloned fragments hybridized to more than one dsRNA component suggesting homologies between different segments. Inserts specific to RNA-3 were subcloned and analysed by dsDNA sequencing.

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AN RNA OLIGOMER CORRESPONDING TO THE VIROID CENTRAL CONSERVED REGION RETAINS THE NOVEL TERTIARY STRUCTURE FOUND IN INTACT VIROID. C.P. Paul, B.J. Benenfeld, A.D. Branch, and H.D. Robertson. The Rockefeller University, New York, NY 10021

Viroids are small infectious RNAs. The interactions between viroid RNA and host cell components are undefined. The central conserved region (CCR) of the viroid RNA is thought to be important in viroid replication. UV crosslinking studies of intact PSTV RNA and viroid transcripts show that the CCR has an unusual tertiary structure. This novel structure resembles a regulatory binding site found in 5S RNA, suggesting an approach for studying viroid-host interaction. A synthetic 54 base DNA template has been constructed corresponding to the viroid CCR. Transcripts of this oligomer were produced using T7 polymerase. UV crosslinking studies indicate that the CCR transcripts retain the tertiary structure of the complete viroid RNA. These results suggest that RNA oligomers will be useful tools in studying the functions of individual domains of the RNA genomes of plant pathogens.

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TRANSCAPSIDATION OF BARLEY YELLOW DWARF VIRUSES IN MIXED INFECTIONS. R. Creamer and B. W. Falk. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Interactions between luteoviruses in mixed infections have previously been detected by breakdowns of vector specificity and by serological neutralization of infectivity. We have directly trapped and analyzed virions from mixed infections of the barley yellow dwarf viruses, RPV AND MAV. Samples trapped with a monoclonal antibody (MAb) to RPV reacted with ^{32}P-labelled cDNAs to RPV and MAV, while samples trapped by MAV MAb, reacted only with MAV cDNAs, suggesting a one-way transcapsidation. Testing mixed RPV and PAV infections showed that PAV RNA could be trapped by RPV MAb, suggesting that structural interactions occur between these two viruses that are not detectable by changes in vector specificity.

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CHARACTERIZATION OF A SATELLITE ELEMENT ASSOCIATED WITH PEA ENATION MOSAIC VIRUS (PEMV). S. A. Demler and G. A. de Zoeten Department of Plant Pathology. University of Wisconsin-Madison, Wisconsin 53706.

In addition to the two genomic RNAs of PEMV, many strains also display a third RNA of mol. wt. 0.2-0.3x10^6 daltons, the significance of which has yet to be determined. The sudden emergence of an encapsidated RNA-3 in the Wisconsin strains of PEMV has led us to examine the role of this RNA in more detail. Using a 700bp cDNA clone generated from the RF of RNA-3, we have demonstrated through Northern and Southern blot analysis that RNA-3 lacks sequence homology to the genomic RNAs of PEMV as well as to total cellular RNA and DNA prepared from mock and uninoculated host pea plants. Infectivity analysis of electrophoretically purified genomic RNAs and of RNA-3 alone established that RNA-3 was neither mandatory for infection, nor was it infectious on its own. From these results we have concluded that RNA-3 fulfills the criteria as a satellite element for PEMV.

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USE OF cDNA PROBES IN STUDIES OF BARLEY YELLOW DWARF VIRUS. F.A. Fattouh, P.P. Ueng, D.J. Barbara, E.E. Kawata, B.A. Larkins and R.M. Lister. Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Reciprocal hybridization specificity of 32P-labelled cDNA clones from the RNA's of the MAV, P-PAV and RPV isolates of barley yellow dwarf virus (BYDV) was tested with virus preparations and extracts of leaves infected with the homologous or heterologous isolates. For both MAV and P-PAV, clones from the coat protein-coding region hybridized well only to the homologous isolate; those from elsewhere also hybridized heterologously. However, none of these clones hybridized significantly with the RPV isolate, and clones from this isolate hybridized only homologously. No clones tested hybridized with the RMV or SGV isolates of BYDV, or with isolates of beet western yellows or potato leaf roll viruses. Virus was readily detected in extracts of fresh, frozen or air dried leaves, as well as in aphids raised on infected plants.

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REPLICATION OF SOIL-BORNE WHEAT MOSAIC VIRUS IN ISOLATED BARLEY PROTOPLASTS. R. French. USDA, ARS, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

Soil-borne wheat mosaic virus (SBWMV) is a bipartite rod-shaped virus of cereals. Mechanical transmission of SBWMV is erratic and symptoms develop slowly making the study of SBWMV replication difficult. To circumvent these problems, protoplasts isolated from Larker barley seedlings were inoculated with SBWMV RNA by a polyethylene glycol procedure. Similar to whole plant experiments, productive infection was detected in protoplasts held at 12°, 15°, and 18°C, but not at 24°C. At 18°C, between 15 to 30 percent of the viable protoplasts were infected by 24 hours post-inoculation. SBWMV coat protein and genomic RNA 2 were readily detected in extracts of cells incubated with ^{35}S- methionine or ^{3}H-uridine, respectively. A virus-specific 90K protein could also be immunoprecipitated. This system should facilitate the study of early events in SBWMV replication.

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PROTEOLYSIS OF PLANT VIRUS CAPSIDS. R.N. Skopp, L.C. Lane, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE, 68583-0722.

Proteolysis is a convenient method to investigate protein structure. Highly structured regions generally resist proteolysis, but less structured ends or loops are often cleaved. Thermolysin, a metalloprotease, is an inexpensive probe of virus structure. It is stable and active to about 80°C, but it is inactivated by dithiothreitol during virion dissociation for SDS gel electrophoresis. The latter property insures proteolysis of virions and not denatured protein. Thermolysin cleavage generates characteristic protein fragments and is a useful aid to virus identification. Some virus capsids (for example white clover mosaic) are highly resistant to proteolysis, while others, notably wheat streak mosaic are highly sensitive. In all cases, virion structural integrity survives proteolysis under physiological conditions. Enzyme/virus ratios of 1/500 to 1/10 give useful digestion patterns at 40°C.

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COMPLEMENTARY DNA CLONING OF THE GENOMIC RNA OF POTATO VIRUS S. J. Monis and G. A. de Zoeten, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Genomic RNA of the Andean strain of Potato Virus S (PVS-An) was extracted from virions. First strand complementary DNA (cDNA) was primed with oligo dT and reverse transcribed by the AMV reverse transcriptase. The second strand was produced by replacement synthesis using the following enzymes: DNA polymerase I, RNase H and E. coli DNA ligase. The cDNA produced (7.5 Kb) was either digested with restriction enzymes that produce sticky ends or attached to EcoR I linkers and cloned into E. coli plasmids pUC19 and pTZ18R. In order to determine the size of the inserts, the recombinant plasmids were screened by restriction endonuclease digests of small scale plasmid preparations. The size of the overlapping clones range from 0.9-6.3 Kb. Sequence specificity of the inserted DNA was confirmed by Southern blot hybridization using PVS-An single stranded cDNA as a probe. The physical map of the PVS-An clones will be presented.

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CHARACTERIZATION OF LETTUCE INFECTIOUS YELLOWS VIRUS. R. C. Larsen[1], H -Y. Liu[2], B. W. Falk[1], and J. E. Duffus[2]. [1] Dept. of Plant Pathology, Univ. of California, Davis, CA 95616; and [2] USDA-ARS, Salinas, CA 93901.

Virions of lettuce infectious yellows virus (LIYV), a whitefly-transmitted clostero-like virus, were purified from infected N. clevelandii and characterized. A single major protein of 32,000 MW was detected by SDS-PAGE. This protein specifically reacted with antiserum to LIYV nucleoproteins in immunoblots. Immunoblot analysis of total protein extracts of LIYV-infected plants also gave reactions only to a 32,000 MW protein. Nucleic acid extracted from purified LIYV virions revealed a single major species of ss-RNA of ca. 7000 bases. Ds-RNA analysis of LIYV-infected plants showed multiple bands with a major band of ca. 5 million molecular weight.

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CORN AS A DIFFERENTIAL HOST FOR STRAINS OF WHEAT STREAK MOSAIC VIRUS. C.L. Niblett[1], M.A. Newman[2] and J.R. Young[3]. [1]Plant Pathology Department, University of Florida, Gainesville 32611; [2]University of Tennessee, Jackson, TN 38301; and [3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66502.

Wheat streak mosaic virus (WSMV) is a serious pathogen of wheat throughout the world. In Kansas alone it caused yield reductions of 5.5, 10.9 and 8.2 x 10^5 metric tons (20, 40 and 30 x 10^6 bushels) in 1949, 1959 and 1974, respectively. To distinguish possible strains of WSMV 32 virus isolates were collected from 9 states (CO, IA, IL, KS, NE, OH, OK, SD, TX). These were inoculated to "Seneca Chief", B37Ht, H84, H93, N28Ht and Oh28 corn, "Eagle" wheat and the agrotricums CI 15092 and 15322 in greenhouse and field experiments. All isolates infected "Eagle" wheat. None infected the agrotricums or H84 and H93 corn. But there was consistent differential infection of the other corn genotypes. On this basis, four strains of WSMV were distinguished.

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CHARACTERIZATION OF THE COAT PROTEIN GENE OF WHEAT STREAK MOSAIC VIRUS. C.L. Niblett[1], L.A. Calvert[2], D.M. Stark[1], S.A. Lommel[2] and R.N. Beachy[1]. [1]Department of Biology, Washington University, St. Louis, MO 63130; [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66502.

Wheat streak mosaic virus (WSMV), a flexuous rod-shaped (ca. 710 X 15 nm) virus transmitted by mites, is generally classified as a member of the potyvirus group. To examine this relationship complementary DNA clones were prepared to the 3′ portion of the WSMV genomic RNA. Transcription and translation of cloned DNA produced a protein which reacted with antiserum to WSMV. DNA sequence analysis revealed an open reading frame of 1292 nucleotides, five potential sites for hydrolysis by a viral-coded protease, a relatively short (147 nucleotides) 3′ untranslated region and a polyadenylated (A=16) 3′ terminus. Thus as with potyviruses, the coat protein gene of WSMV is proximal to the 3′ terminus of its genomic RNA. However, computer-based comparisons (Chou-Fasman prediction) of the structure of WSMV coat protein with those of known potyviruses showed only limited homology.

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CHEMICAL AND BIOLOGICAL PROPERTIES OF RH-7592. H. E. Carley, W. S. Hurt and S. Shaber. Rohm and Haas Company, Independence Mall West, Philadelphia, PA 19105 and Spring House, PA 19477.

RH-7592 is a new broad spectrum fungicide discovered and being developed by Rohm and Haas Company. Laboratory and field evaluations have shown RH-7592 to possess strong protectant, curative and eradicant properties against a wide range of crop diseases. RH-7592 is most effective when applied as a protectant or presymptomatic infection treatment. The balanced biological and chemical attributes of RH-7592 provide new opportunities for the effective control of selective treefruit, vine, vegetable, field and ornamental crop diseases. An overview of the chemical physical, toxicological and fungicidal properties of RH-7592 will be presented.

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ACTIVITY OF RH-7592 IN GREENHOUSE AND IN-VITRO STUDIES. J.A. Quinn, A.R. Egan, W.J. Wilson, C.L. Kohls, R. Ross and S.H. Shaber, Rohm and Haas Co., Spring House PA 19477.

RH-7592 is 2-cyano-2-phenyl-2-(beta-p-chlorophenethyl)-ethyl-1H-1,2,4-triazole. C-14 demethylase activity was inhibited 50% by 0.2 µM RH-7592 in enzyme extracts from Saccharomyces cerevisiae. The compound was active in agar or broth, with 75% control of mycelial growth observed below 0.2 µm/ml for fungi in the genera Cercospora, Septoria, Venturia, Guignardia, Monilinia, Pseudocercosporella, Piricularia, Rhizoctonia and Ustilago; and Cochliobolus, Pyrenophora, Alternaria, and Botrytis below 5 µg/ml. In greenhouse tests, control was equal or superior to standards for wheat powdery mildew, stem and leaf rusts, and glume blotch, barley spot blotch, tomato Septoria leaf spot, peanut early leaf spot, sugar beet Cercospora leaf spot and rice blast. It suppressed tomato early blight and gray mold. Relative to other EBI fungicides it had: less water solubility, volatile activity, activity against established infections, systemicity and PGR activity; maintained drought tolerance and activity against presymptomatic infections; more residual activity.

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PERFORMANCE OF RH-7592, A NEW FUNGICIDE FOR CONTROL OF FOLIAR DISEASES OF WHEAT. W. J. Wilson and H. E. Carley, Rohm and Haas Co., Spring House, PA 19477 and Independence Mall West, Philadelphia, PA 19105.

RH-7592, 2-cyano-2-phenyl-2-(beta-p-chlorophenethyl)-ethyl-1H-1,2,4-triazole, is a new experimental fungicide that has been evaluated for disease control in wheat. Field efficacy studies have been conducted at dosages ranging from 0.045-0.18 lb ai/a. It is effective against Puccinia recondita, Puccinia graminis-tritici, Puccinia striiformis, Septoria tritici, Septoria nodorum and Pyrenophora trichostoma. RH-7592 is most effective when used as a protectant fungicide. Consistently, throughout the testing program, the Feekes GS-9 to GS-10.2 timing provided the best disease control and yield response.

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PARTIAL CONTROL OF LEAF RUST, TANSPOT AND SEPTORIA AVENAE LEAF BLOTCH OF WHEAT WITH CHLORIDE FERTILIZATION. G. W. Buchenau, P. E. Fixen, F. A. Cholick and S. S. A. Rizvi, Plant Science Department, South Dakota State University, Brookings, SD 57007.

Reduction in leaf diseases due to Pyrenophora tritici-repentis (PTR) and Septoria avenae var triticea (SAT) and/or leaf rust was closely associated with yield increase in chloride treated plots in several field studies from 1984 - 87. Significantly fewer leaves from chloride treatments developed PTR conidia or SAT pycnidia in moist chambers when compared with untreated leaves. Reductions in leaf rust pustule size and number were observed. For example in one study, KCl and $CaCl_2$ reduced leaf rust severity on Marshall spring wheat from 65% in untreated or KNO_3 treated plots to 25% in KCl and $CaCl_2$ treatments. Similarly, pustule type was 'MR' in 'no chloride' and 'R' on leaves from chloride treatments. Leaf spot was reduced from 90% to 30% by chloride in the same test. We have observed cultivar by chloride interactions in both yield and foliage disease.

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EFFECTS OF CHLORPYRIFOS ON PLANT GROWTH, YIELD, AND STALK LODGING OF MAIZE. W. L. Pedersen, H. W. Kirby, and T. A. Melton. Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801.

Soil insecticides are often applied to control insects when maize is grown in monoculture. The soil insecticide, chlorpyrifos, has been shown to be fungicidal against several soil-borne pathogens. Three maize hybrids were treated with chlorpyrifos (Lorsban 15G at 187 g a.i./h) at planting to determine if plant growth, yield, or stalk lodging were affected. All plots were planted in fields previously cropped to soybeans. Significant increases in seedling weight, leaf area (measured at three growth stages), and yield were obtained for at least two hybrids in 1986 and one hybrid in 1987. Significant reductions in stalk lodging were observed for two hybrids in 1986, no stalk lodging was observed in 1987. No insect damage was detected in treated or untreated plots.

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APPLICATION OF ETHYLTRIANOL VIA CHEMIGATION AND GROUND SPRAYS FOR PEANUT DISEASE CONTROL. T. B. Brenneman and D. R. Sumner, University of Georgia, Coastal Plain Station, Tifton 31793.

Ethyltrianol (0.25 kg/ha) was applied seven times to Florunner peanuts via ground sprays or center-pivot

irrigation (chemigation). Ethyltrianol applied by chemigation was diluted in water or nonemulsifiable oil and applied in 25.4 kl water/ha. Chemigated plots were either trafficked or not trafficked with a tractor. Nontreated plots were defoliated by late leafspot (Cercosporidium personatum) and had significant limb rot (Rhizoctonia solani AG-4). Ground sprays and chemigation using the oil diluent gave the best leafspot control. All fungicide treatments reduced limb rot and nontrafficked plots had the least disease. All treatments increased yields; greatest increases were in chemigated plots. Treatments did not affect soil populations of Rhizoctonia-like fungi, R. solani AG-4, or Pythium spp., although total post harvest populations of saprophytic soil fungi were reduced by the fungicide.

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EFFECT OF METALAXYL PLUS PCNB OR TOLCLOFOS-METHYL ON PEANUT POD ROT. A. B. Filonow[1], K. E. Jackson[1], and P. M. Inskeep[2]. Departments of Plant Pathology, Oklahoma State University, Stillwater[1] and Montana State University, Bozeman[2].

Metalaxyl plus PCNB or tolclofos-methyl were field tested in 1985-1987 in Oklahoma for their effect on pod rot of 'Florunner' and 'Spanco' peanut caused by Pythium spp. and Rhizoctonia solani. Fungicides reduced pod rot severity on both genotypes at 2-8 wks prior to harvest. At harvest, however, no significant (P=0.05) differences were observed. Yields were increased by ca. 220-620 kg/ha; but few increases were significant. Fungicides generally reduced Pythium spp. populations in soil at several sampling dates, but few of the reductions were significant. In 1986 and 1987 Pythium populations in soil peaked at 60 and 75 days, respectively, then declined. At these peaks treated soils generally had less (P=0.05) Pythium spp. than nontreated soils. Populations of R. solani were variable and responded less to treatments.

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ACIDIFIED CUPRIC ACETATE SEED TREATMENT CONTROLS BARLEY STRIPE. **R.L. Forster and J. Olson. Division of Plant Pathology, University of Idaho Res. and Ext. Center, Kimberly, ID 83341**

While evaluating spring barley (Hordeum vulgare) plants grown in the field from untreated seed and seed treated with acidified cupric acetate (ACA), the incidence of culms with barley stripe symptoms caused by Pyrenophora graminea in six replications was 10.1 and 0.3 per 4.9 m in the untreated and ACA-treated plots, respectively. In a greenhouse study, 'Bracken' barley seed naturally infected with P. graminea was soaked in 0.5% ACA at 45 C for 20 min, rinsed twice with water, and air dried. Treated and untreated seeds of the same lot were sown in a peat:sand:vermiculite (1:1:1) mix in 15 cm pots (4 seeds/pot; 20 pots/replicate; 3 reps of ACA-treated and 1 rep of the check) and incubated in a growth chamber at 10 C with a 12 hr photoperiod for 30 days after which they were moved to a greenhouse bench for an additional 41 days. Evaluations of signs and symptoms of barley stripe revealed that 0.4% (1 of 235 plants) and 36% (22 of 61 plants) were diseased in the ACA-treated and check treatments, respectively.

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SOIL FUMIGATION WITH TELONE II AND TELONE C-17 FOR CONTROL OF SUGARBEET ROOT ROT DISEASES. C. M. Rush, Texas A&M University, Texas Agricultural Experiment Station, Bushland, Texas 79012.

The predominant root rot pathogens of sugarbeets grown in the Texas Panhandle include Rhizoctonia solani, Fusarium oxysporum, Aphanomyces cochlioides, and Pythium spp. Often, all four pathogens can be found in individual fields and therefore, cultural practices and resistant varieties do not provide sufficient disease control. In 1987, test plots four rows wide and 30 m long were fumigated with Telone II or Telone C-17 at rates of 93 and 187 ℓ/ha. Plant stand and seedling disease were not affected by either treatment but root rot was significantly reduced, and yield in kg/ha and percent sugar were both significantly increased over nonfumigated control plots. At equivalent rates, Telone II outperformed Telone C-17. Telone C-17 at 93 ℓ/ha did not reduce root rot or increase yield when compared with the control but did significantly improve sugar content.

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THE TREE ROW VOLUME CONCEPT AND THE EFFECT OF TREE CANOPY DENSITY ON SPRAY DEPOSITION IN APPLE TREES. G. G. Clarke, K. D. Hickey and J. W. Travis, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

The Tree Row Volume (TRV) Concept was developed in the early 1970s in an attempt to better define trees as spray targets. In its current form the TRV formula calculates spray solution rates based on the volume and density of the tree canopy. The TRV concept has been incorporated into the spray recommendations of several eastern, fruit-growing states. Experiments conducted in 1986 and 1987 evaluated the effect of canopy density on deposits of chelated micronutrients when spraying one or two sides of target trees pruned to different canopy densities. Results indicate that canopy density has a relatively small impact on spray deposition. Distance from sprayer to target area was shown to greatly affect spray deposition. Deposits on leaves were 0.35 μg/cm and 0.10 μg/cm^2 at 1.5 and 6.0 m from the sprayer, respectively.

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MANAGEMENT OF GRAPE GRAY MOLD CONSIDERING FUNGICIDE RESISTANCE. M.L. Gullino and A.Garibaldi, Istituto di Patologia vegetale, Via Giuria 15, 10126 Torino, Italy.

In 1986 and 1987, dicarboximides had 20 to 70% reduced efficacy for control of gray mold (Botrytis cinerea Pers.) on "Moscato" cultivar of grape in north Italy. This was correlated with the presence of dicarboximide resistant populations. Most of the strains of B.cinerea were already resistant to benzimidazoles, thus the double resistance complicated gray mold management. A simple, rapid technique for monitoring fungicide resistance is used by the extension service in the Piedmont viticultural areas to help design spray schedules in consideration of the pathogen population for that region. In the presence of Botrytis strains resistant to benzimidazoles and/or double resistant to benzimidazoles and dicarboximides, diethofencarb, with negative cross resistance to benzimidazoles, is active even in the presence of severe disease pressure. Diethofencarb has been used as a mixture with carbendazim in one treatment/season, alternated with a dicarboximide.

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FIELD EVALUATION OF SYSTEMIC FUNGICIDES FOR CONTROL OF PHYTOPHTHORA ROOT ROT, STEM CANKER OF PEACH. C. H. Graves, L. N. Wilson, J. F. Killebrew, and R. A. Haygood, Dept. of Plant Pathology and Weed Science and Extension Plant Pathology, Miss. State, MS 39762.

Metalaxyl and fosetyl Al effectively controlled stem canker following "wound-plug" inoculation of container-grown peach trees with Phytophthora cinnamomi, P. cactorum and P. nicotianae var. parasitica. Treatments of an inoculated planting of small Lovell rootstock peach trees confirmed the potential of these chemicals to control the disease in the field. Mixed inoculum of the three species was applied with wounding at the base of the trees. Constant moisture was maintained by drip irrigation. Two rates of metalaxyl (0.46 and 0.92gm ai/m^2) applied three times at 3-mo intervals as a soil drench, and two rates of fosetyl Al (9 and 18gm ai/gal) applied four times at 2-mo intervals as a foliar spray significantly reduced canker, root rot development. Reisolations from controls suggested that P. cinnamomi is the most aggressive pathogen.

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PHOSPHONATE LEVELS IN AVOCADO SEEDLINGS AND SOIL FOLLOWING TREATMENT WITH FOSETYL-AL OR POTASSIUM PHOSPHONATE. D. G.

Ouimette and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

The levels of ethyl phosphonate and phosphonate in avocado seedlings and soil were determined up to 8 wk following foliar or soil applications of the fungicides fosetyl-Al (Aliette®) or potassium phosphonate. With fosetyl-Al, low levels of ethyl phosphonate were present in soil, roots and stems 1 wk after soil application while no residues were detected 1 wk following foliar application. Soil treatment with either fungicide resulted in much higher phosphonate levels in all tissues compared to foliar treatment. Following both soil and foliar applications of the two fungicides, high phosphonate levels were maintained in avocado tissues for 8 wk. The phosphonate levels found in roots after soil or foliar application of either fungicide are sufficiently high to control avocado root rot caused by *Phytophthora cinnamomi*.

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FUSARIUM WILT OF CHICKPEA IN CALIFORNIA. I. W. Buddenhagen and F. Workneh, Dept. Agronomy & Range Science, University of California, Davis, CA 95616.

Traditional chickpea cultivation in the Central Coast has declined markedly due mainly to Fusarium wilt and the lack of wilt-resistant, high yielding varieties. Isolations from commercial cultivars and from the international chickpea differential lines planted in soils from eight different sites in three counties yielded Fusarium oxysporum f.sp. ciceri which proved highly pathogenic in greenhouse tests. Colonies were white on PDA. Three of the differential lines wilted rapidly in most of the soils. Five others wilted later while two remained healthy. Challenge of the differentials with different isolates indicated the presence of at least two pathogenicity groups. However, breeding lines derived from Sonora (R) x UC5 (S) remained resistant in all soils and to challenge by the isolates recovered. Two of them were released as new wilt-resistant, high yielding 'Kabuli' chickpea varieties (UC15 and UC27).

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THE ROLE OF FUSARIUM SOLANI IN SUDDEN DEATH SYNDROME OF SOYBEANS. B. S. Lucas, T. D. Wyllie, and E. W. Palm Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Isolates of Fusarium solani characterized by slow growth and blue-purple pigmentation on acid PDA, were recovered from soybeans with symptoms of sudden death syndrome (SDS). The fungus was also isolated from asymptomatic plants collected from a field where SDS subsequently developed, but not from plants collected from fields where no SDS occurred. The slow growing, blue-purple cultures of F. solani were isolated from lateral roots and taproots, but never from the cotyledonary nodes or higher. Healthy soybean plants (Williams 82 and Lee 74) in stage V2 were inoculated with mycelium and macroconidia placed against the taproot, just below the soil surface. Inoculated plants either developed the typical SDS foliar symptoms plus extensive root rot, or root rot only. These observations suggest that SDS is a root rot type disease caused by F. solani with possible toxin involvement.

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FUSARIUM SPECIES ASSOCIATED WITH DISEASED SUGARBEET. E. G. Ruppel, USDA-ARS Crops Research Lab, 1701 Center Avenue, Fort Collins, CO 80526.

Sugarbeet roots showing Fusarium yellows symptoms were collected from six western states. Isolations on Komada's *Fusarium*-selective medium yielded 48 isolates of seven *Fusarium* spp. Besides *F. oxysporum* f. sp. *betae*, the reported cause of Fusarium yellows, an isolate of *F. acuminatum* from Colorado, two Texas isolates of *F. avenaceum*, an Oregon isolate of *F. sambucinum*, and a nonsporulating "Roseum" type were pathogenic on sugarbeet seedlings, all causing foliar yellowing, wilt, root necrosis, and eventual death. When roots of 3-mo-old plants were wound-inoculated with the seedling pathogens, only *F. o. betae* and *F. acuminatum* induced typical yellows symptoms. All isolates caused some necrosis of secondary roots and apparently-arrested necrotic lesions on the tap root, but no plant death. In both pathogenicity tests, all pathogenic and nonpathogenic isolates were reisolated from surface-disinfested roots 2 mo after inoculation.

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CHRONOLOGY OF GAS EXCHANGE EFFECTS IN VERTICILLIUM WILT OF POTATO. R. L. Bowden and D. I. Rouse, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Axenically propagated plants of potato cv. Russet Burbank were grown in a growth chamber (550 μE PAR m^{-2} sec^{-1}; 25C day, 15C night; 50% R.H.) for approximately 100 days. The potting medium was infested with various levels of Verticillium dahliae microsclerotia. CO_2 and water vapor gas exchange rates (GER) of individual leaves were measured with a Li-Cor 6200 system. $^{14}CO_2$ autoradiography was used to visualize within-leaf patterns of photosynthesis. Disease development could be divided into 3 phases. Phase I: The fungus was present in stems but no symptoms or GER effects were detectable. Phase II: There was an acropetal procession of wilting, chlorosis, or senescence of leaves or parts of leaves. Visible symptoms were typically preceded by a period of reduced GER. Young leaves had normal GER and no visible symptoms. Phase III: Acropetal defoliation continued. Young leaves had reduced GER and were often stunted. GER effects were uniform over the whole leaf.

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DETECTION OF SYNCHYTRIUM ENDOBIOTICUM RESTING SPORES IN WESTERN MARYLAND. M.L. Putnam and M.C. Hampson. MD Dep. Agric., Annapolis, 21401 and Agriculture Canada, St. John's West, Nfld A1E 3Y3.

Potato wart, caused by S. endobioticum, was first detected in the U.S. in Pennsylvania in 1918. In the following two years it was discovered in West Virginia and in home gardens in Allegany Co., Maryland; all three states had declared the infested areas quarantined by 1921. Eradication efforts over the years appeared to have eliminated the disease, and in 1974 the FAO declared the United States free of potato wart. Maryland however did not rescind its quarantine until 1986. In response to requests from a country desiring to import potatoes, the Maryland Department of Agriculture re-surveyed the originally infested area. A soil sample from one suspect home garden was analyzed by Agriculture Canada and yielded resting spores of S. endobioticum. This is the first time the causal agent of potato wart has been detected in Maryland since 1951 and is the only current report of the pathogen in the U.S.

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PREFERENTIAL HOST SELECTION OF ISOLATES OF MACROPHOMINA PHASEOLINA. G. L. Cloud and J. C. Rupe, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Host specialization of single sclerotial isolates of M. phaseolina from sorghum and soybean was evaluated in the greenhouse. On a differentiating chlorate medium, growth of sorghum isolates was dense and growth of soybean isolates was restricted. Treatments were: 1) sorghum grown in soil infested with sorghum isolate, 2) soybeans grown in soil infested with soybean isolate, 3) sorghum and soybeans grown in soil infested with both isolates, and 4) soybeans grown in sterile soil. Roots from each treatment were assayed periodically for M. phaseolina and the chlorate phenotypes determined. After 11 wk, 82 and 49% of isolates from sorghum in treatments 1 and 3 were of the dense type and 90 and 88% of isolates from soybean in treatments 2 and 3 were of the restricted type. Soybean roots in treatment 4 remained free of M. phaseolina. Preferential isolation from the original source host suggests that host specialization occurs in M. phaseolina.

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RELATIONSHIP BETWEEN STEM CANKER INCIDENCE AND AGRONOMIC PERFORMANCE OF SOYBEAN AT DIFFERENT DATES OF PLANTING. Medani A. Omer, V. T. Sapra, and R. P. Pacumbaba. Dept. Plant and Soil Science. Alabama A&M Univ. Normal, AL 35762

Four soybean cultivars obtained from four sources and planted at four dates of planting showed highly significant differences ($p=0.01$) on disease incidence, yield and their agronomic performance. Among all planting dates, early planting (May 5 and May 20) had the highest disease rating and the highest yield. At these dates environmental conditions were optimum for both plant establishment and disease development. However, late planting (June 24) showed the greater escape of the disease. Source of seed had no significant effect on most of the parameters measured. Soybean stem canker (SSC) appeared to have more effect on seed quality than on the quantity. This was shown by the negative correlation between disease rating and seed vigor index in all four planting dates. Path analysis showed that SSC had a significant effect on soybean yield components.

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STRUCTURAL COMPARISON OF C_4 BUNDLE SHEATH CELLS WITH AND WITHOUT A VIRUS. L. L. Hoefert, G. L. Fail, and R. L. Pinto, USDA-ARS, Salinas, CA 93905.

A plant often used for virus host-range studies is Gomphrena globosa L., a member of the family Amaranthaceae. It is a dicotyledonous member of the C_4 group of plants and exhibits the typical "Kranz" anatomy of bundle sheath cells surrounding the vascular bundles of the leaf. Structural comparisons by light and electron microscopy were made of the bundle sheath cells from healthy G. globosa leaves and from those infected with beet distortion mosaic virus, a recently described sugarbeet virus mechanically transmitted to G. globosa. Associations between the virus and chloroplasts of the bundle sheath cells were observed. The significance of the virus presence in cells specialized for nutrient transfer will be discussed.

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MANIPULATING POLYCLONAL ANTISERA AGAINST FUSARIUM SPECIES TO VARY CROSS-REACTIVITY. Elie Gendloff. Agrigenetics Advanced Science Company, 5649 E. Buckeye Road, Madison, WI 53711.

A polyclonal antisera was made against a mixture of extracts from Fusarium graminearum (Fg) and F. moniliforme (Fm). This antiserum recognized many heterologous fungi when these fungi were tested in an indirect ELISA. When this antiserum was used in indirect competitive ELISAs by coating with either antigen, there was cross-reactivity to several Fusarium species but not to fungi that were not Fusarium. An indirect competitive ELISA for Colletotrichum graminicola (Cg) (a fungus recognized by the antiserum) was developed using the anti-Fusarium antisera. This ELISA did not cross-react to any fungus tested, including the Fusarium antigens that was used to make the antiserum. The Cg-specific ELISA detected Cg isolated from corn stalks that was naturally infected with Anthracnose stalk rot.

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FIRST REPORT OF HEAD SMUT OF CORN IN COLORADO. W. M. Brown, Jr. and E. A. Milus. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Head smut of corn (Zea mays), caused by Sphacelotheca reiliana, was confirmed July 1987 in Adams County northeast of Denver. Infected plants were variously stunted and had typical smut sori with coarse strands of host vascular tissue in ears and most tassels. Many tassels exhibited proliferated leaf-like growth. Disease incidence was highest at row ends. Teliospores were dark reddish brown to black, globose, spiny, and 8-12 µm in diameter. Subsequent disease surveys in Colorado found head smut in Larimer, Weld, Logan, Sedgwick, Phillips, Morgan, and Yuma counties in the northeast and Otero, Bent, and Prowers counties in the southeast. Disease incidence within commercial fields was less than 0.1%, and yield losses were slight. Sphacelotheca reiliana probably was introduced on contaminated seed and has probably been present for several years in Colorado.

413 Withdrawn

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VARIATION IN VIRUS ACCUMULATION IN SORGHUM GENOTYPES DIFFERING IN 'RESISTANCE' TO MDMV-A. L. M. Giorda and R. W. Toler, Dept. of Plant Path. and Microbiol., and F. R. Miller, Dept. of Soil and Crop Sciences, Texas A&M University, College Station 77843.

Significant differences were observed within and among cultivars (CVS) in the amount of MDMV-A which accumulated over time. Differences among cultivar means were not uniform over time. Thus, area under the curve (virus accumulation over time) was used as a parameter for identifying cultivar resistance. In the 36 CVS assessed, virus accumulation increased for 32 days after inoculation (DAI) and decreased thereafter except for 4 CVS with intermediate or long maturity. In these CVS, the virus titer remained high up to 64 DAI. The older the plant at inoculation, the lower the virus accumulation at 24-30 C. Both resistant and susceptible sorghum genotypes produced the highest virus accumulation in the new, fully expanded leaf. Subliminal infections of MDMV-A were detected in both symptomless seedlings and older leaves of mature plants. The lowest virus accumulation was detected in the cultivar RTx430.

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INCIDENCE OF TOMATO SPOTTED WILT IN PEANUTS IN ALABAMA. R. I. Gudauskas, A. K. Hagan, J. R. Weeks, R. A. Shelby, W. S. Gazaway, and J. C. French. Departments of Plant Pathology and Entomology, Auburn University, AL 36849.

Systematic surveys for tomato spotted wilt virus (TSWV)-infected peanuts involved 54 and 65 randomly selected fields in 1986 and 1987, respectively. A total of 1000 row feet in each field was examined, and suspect diseased plants were tested for TSWV by enzyme-linked immunosorbent assay. TSWV-infected plants were found in 94% of the fields surveyed in 1986, and in 42% of the fields in 1987. Although average in-field incidence of virus-infected plants was low, heavy damage was seen in a few fields. Symptoms observed both years ranged from chlorotic ring-spotting, mottling of foliage, and internode shortening to severe stunting, reduced pod set, and seed coat mottling. A rapid decline in plant vigor characterized by yellowing and collapse of foliage, followed by premature plant death occasionally was associated with TSWV infection.

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CHARCOAL ROT OF SORGHUM IN THE BAY REGION OF SOMALIA, EAST AFRICA. F. A. Gray, J. D. Mihail and R. J. Lavigne. Wyo. Agric. Exp. Sta., Laramie, WY 82071, Ariz. Agric. Exp. Sta., Tucson, AZ 85721, and USAID, Somalia, East Africa.

A survey was conducted in the Bay Region of Somalia to determine the incidence of charcoal rot in sorghum incited by Macrophomina phaseolina and the soil population of M. phaseolina in sorghum fields and native vegetation. Thirty-four of 40 sorghum fields surveyed had charcoal rot present. Incidence (percent diseased hills) in the four regional districts was 21, 70, 20 and 35% for Baidoa, Burhakaba, Dinsoor and Quansadhere, respectively. Soil collected from 40 sorghum fields and 40 native vegetation areas all contained M. phaseolina. Mean soil populations for the 40 sorghum fields and 40 native vegetation sites were 25.2 and 2.5 microsclerotia/gram soil. Soil populations in sorghum fields ranged from 7-107 microsclerotia/gram of soil. Incidence of charcoal rot was highly correlated with soil populations of M. phaseolina (r=.53).

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SOFT ROT AND WILT OF CYCLAMEN SPP. CAUSED BY ERWINIA

CAROTOVORA SUBSP. CAROTOVORA. A. Dela Cruz, N. F. Schaad, and R. L. Forster, Plant Path. Div., Univ. Idaho, Moscow 83843 and Kimberly 83341

A soft rot and wilt disease of Cyclamen spp. was observed in southern Idaho in 1987. Symptoms were similar to those reported for a soft rot disease of Cyclamen in Greece in 1970 (Annals de L'Inst. Phytopath. Benaki 9:83-93) where Erwinia chrysanthemi was implicated. Cultures resembling Erwinia were isolated from the Idaho plants and two cloned strains produced soft rot and wilt of Cyclamen plants. These strains were reisolated and characterized by electron microscopy and biochemical tests. They were Gram-negative, motile by peritrichous flagella, approximately 0.8 µm x 1.5 µm, and facultatively anaerobic. Tests for phosphatase and gas production from glucose were negative while the potato soft rot test and sensitivity to erythromycin were positive. These results suggest that the Cyclamen soft rotting organism in Idaho is E. carotovora subsp. carotovora.

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INFLUENCE OF CULTURAL PRACTICES ON TOMATO PITH NECROSIS. N. B. Carroll, P. B. Shoemaker, and E. Echandi. Department of Plant Pathology. North Carolina State University, Raleigh, NC 27695-7616.

The influence of cultural practices on tomato pith necrosis caused by *Pseudomonas corrugata* was studied in western North Carolina (NC) and in northern Baja California, Mexico (MEX). In a 2 year field study in NC, disease severity in inoculated plants was significantly (P=0.05) higher at a nitrogen level above the recommended rate, in fumigated vs. nonfumigated soil, and in plants grown with black plastic mulch vs. bare ground. In MEX under high natural infection, disease incidence was significantly (P=0.05) higher in mature plants grown from seedlings receiving no antibiotic treatment vs. those treated with streptomycin sulfate (200 ppm) prior to transplanting, and in those that were handled by the stems vs. those that were handled by the leaves during transplanting. Also in MEX, disease incidence was positively correlated (P=0.05) with nitrogen and irrigation.

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REACTION OF LYCOPERSICON ACCESSIONS TO CLAVIBACTER MICHIGANENSE SUBSP. MICHIGANENSE. R.M. De Vries and C.I. Stephens. Department of Botany and Plant Pathology, Michigan State Univeristy, East Lansing, MI 48824-1312.

Tomato (Lycopersicon spp.) seedlings were inoculated either by cotyledon excision and/or apical stem excision using a scissors dipped in 10^6-10^8 colony forming units/ml of Clavibacter michiganense subsp. michiganense (CMM). Inoculum was prepared from either a highly virulent (DR63) or mildly virulent (CM103) isolate. The stem excision method proved to be more severe because only some reported resistant accessions exhibited survival (80-100%) after 8 weeks. In comparison, some susceptible accessions survived (<5%) to 8 weeks only when inoculated by the less severe cotyledon excision method. We will use these inoculation procedures to evaluate mutated tomato germplasm for resistance to CMM.

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Occurrence and pathogenicity on bean (Phaseolus vulgaris) of Pseudomonas syringae pv. syringae (Pss) recovered from weeds in New York. D. E. Legard, and J. E. Hunter. Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station. Geneva NY, 14456.

Epiphytic Pss strains were recovered on KB medium from weeds at several locations including sites near bean fields with a history of bacterial brown spot (BBS), to determine if weeds are a source of overwintering inocula in NY. A bean pod assay was used to determine pathogenicity of Pss strains to bean (BBS-type Pss). In 1986, 114 samples of 45 weed species were collected during May, June and July; Pss was recovered from 21 species (257 strains). Only 16 strains from 2 species (dogwood and chickweed) were of the BBS-type, possibly indicating overwintering on these weeds. In 1987, 134 samples of 48 weed species were similarily collected; and Pss was recovered from 17 species (177 strains). Only 5 strains from 2 samples (daisy fleabane and common buttercup) were BBS-type. Both samples were adjacent to beans that had emerged 3 weeks prior to sampling, therefore, the origin of BBS-type Pss was unclear. In western NY under conditions that favored BBS epidemics in 1986 and1987, the weeds sampled appear not to provide an important source of initial inocula for BBS.

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DRIPPY POD OF LUPINE CAUSED BY ERWINIA LUPINICOLA. K. M. Regner and D. C. Gross. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Production of lupine (Lupinus albus) for use as a forage crop in Eastern Washington has been hampered by drippy pod, a disease caused by an undescribed species of Erwinia. Profuse oozing and frothing of exudate from pod lesions characteristically occur along with less conspicuous watersoaked lesions on the stems. Contaminated seed appears to be the primary source of inoculum and secondary spread probably occurs by rain splash and insects which feed on lupine pods. Isolations from lupine field samples with drippy pod yielded a Gram-negative, facultative anaerobe, which was nonpectolytic and nonpigmented. The drippy pod pathogen was identified as a new species within the Amylovora group based on cultural, physiological, and biochemical characteristics and named Erwinia lupinicola.

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IN VITRO SCREENING OF GERANIUM SOMACLONES FOR RESISTANCE TO BACTERIAL BLIGHT. K. B. Dunbar and C. T. Stephens, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

A test tube assay was developed to detect resistance to Xanthomonas campestris pv. pelargonii in seed geranium (Pelargonium x hortorum) somaclones. Shoots from callus cultures were placed in culture tubes containing 15 ml of Hoagland's solution solidified with 0.7% agar. Somaclones were inoculated in vitro by rubbing the upper surface of individual leaves with a cotton swab dipped in a 10^7 CFU/ml suspension of X. c. pv. pelargonii cells. Three weeks after inoculation, seed geranium somaclones, regenerated from susceptible cultivars, showed a significantly lower survival rate than regal geranium (P. x domesticum) somaclones used as resistant controls. Bacterial blight symptoms did not develop when the inoculum was replaced with sterile water or a 10^7 CFU/ml suspension of X. c. pv. campestris cells. This assay holds promise as a means to rapidly identify resistant somaclones.

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SURVIVAL AND DISTRIBUTION OF EPIPHYTIC POPULATIONS OF CLAVIBACTER MICHIGANENSE SUBSP. MICHIGANENSE ON TOMATO FOLIAGE AND FRUIT. M. L. Gleason and E. J. Braun, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

In July, 1987, mutants of Clavibacter michiganense subsp. michiganense (Cmm), selected for resistance to rifampicin and for pathogenicity, were sprayed on tomato plants in the field. Symptoms (marginal scorch of leaflets and fruit spots) did not appear until the end of August. Epiphytic populations of Cmm were estimated at weekly intervals during July and August by shaking individual leaflets and fruit in an extraction buffer and plating dilutions on nutrient broth-yeast extract medium amended with rifampicin and cycloheximide. Mean populations (cfu) of Cmm per leaflet and per fruit were approximately 10^2 three days after inoculation; mean cfu/leaflet were approximately 10^7 in all subsequent samples, while mean cfu/fruit increased more gradually to approximately 10^6 by late August. Mean populations per leaflet or per fruit varied by a factor of 10^2 to 10^5 within a weekly sample set. After $\log_{10}$ transformation, no data sets met statistical criteria for normality, but were sufficiently close to normality to be acceptable for analysis of variance.

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PLASMID ANALYSIS OF CLAVIBACTER MICHIGANENSE SUBSP. MICHIGANENSE. J. Chamot and D. W. Fulbright. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Plasmids in the bacterial pathogen of tomato Clavibacter michiganense subsp. michiganense have been analysed in over 100 different isolates obtained from different locations since 1980. Some isolates lack detectible plasmids, but most contain 1 to three plasmids. All plasmids detected so far range in size between 30 to 60 X 10^6. Presence of plasmids was not correlated with pathogenicity or virulence of the pathogen. Some plasmids have been lost after storage of the bacterium at 5 C. Plasmid profile variation in agarose gels has been useful in differentiating isolates and in tracing the various sources of inoculum recovered from diseased plants in the field.

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ATTACHMENT OF PSEUDOMONAS SYRINGAE PATHOVARS TO LEAVES. C. A. Jasalavich and L. Sequeira, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Cucumber or soybean leaves sprayed with Pseudomonas syringae

pv. lachrymans (Psl) or pv. glycinea (Psg) were washed after treatments. Attachment was defined as the percent of bacteria on/in the washed leaf. After 2hr drying/24hr dew/2hr drying, Psl appeared to attach more to cucumber than to soybean leaves in 2 trails, and Psg more to soybean than to cucumber in 1 out of 2 trials. To determine if "attachment" included bacteria trapped inside the leaf with those attached to the surface, nonmotile mutants were selected. When injected into leaves, they were equally virulent to their motile parents in infectivity titrations, but when sprayed on leaves, were less infective, and so were used to estimate attachment to the leaf surface. Motile strains resisted washing more than nonmotile after the 2hr drying/24hr dew/2hr drying and apparently entered the leaf. Nonmotile mutants also attached to the leaf surface, but attachment was not host specific.

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SCREENING WHEAT GERMPLASM FOR CORYNEBACTERIUM MICHIGANESE SUBSP. TESSELARIUS. J. H. McBeath, M. Adelman and L. Jackson*, Agricultural and Forestry Experiment Station, University of Alaska, Fairbanks, AK 99775-0080 and *Department of Agronomy & Range Science, University of California, Davis, CA 95616.

A method has been developed to screen wheat germplasm for Corynebacterium muchiganese subsp. tessellarius. From each seed lot, 32 seeds were sampled. They were individually surfaced sterilized for 2 min. in 0.5% Chlorox, rinsed and imbibed in sterile distilled water at room temperature for 12 hours. The seeds were then cut longitudinally, and one of the halves was placed cut-faced down on CNS selective medium. Orange-colored colonies of C. michiganese subsp. tessellarius were observed around the seed halves after a 10-day incubation at 20 C. Presence of this bacterium has been detected in both common and durum wheat germplasm grown at Davis, California. Out of the 183 cultivars and breeding lines tested, 137 (75%) were found contaminated. This bacterium was detected in very high frequencies in 32 seed lots.

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LEAF PUBESCENCE CONFERS APPARENT RACE-NONSPECIFIC RUST RESISTANCE IN BEAN (PHASEOLUS VULGARIS). J.R. Steadman and M. Shaik. Dept. of Pl. Path., Univ. of Nebraska, Lincoln, NE, 68583-0722.

Leaf pubescence was studied in a number of bean cultivars that possess various types of resistance to rust (Uromyces appendiculatus). Densities of both hooked (0.1-0.3 mm long) and straight (1-2 mm long) hairs were quantified. Hair densities were greater on abaxial than on adaxial leaf surfaces. Leaves at higher positions on plants had greater hair densities than those at lower ones. Cultivar differences were, in general, significant regardless of leaf surface or leaf position. Rust races or isolates compatible on sparsely pubescent leaves showed significantly lower or absence of pustules compared to pubescent leaves of the same plant in field or greenhouse tests. Possible mechanisms by which leaf hairs could limit spore deposition on, and germ tube access to the leaf epidermis are discussed.

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EVALUATION OF CLOSELY RELATED CORN HYBRIDS FOR RESISTANCE AND TOLERANCE TO TWO VASCULAR WILT PATHOGENS AND A FOLIAR PATHOGEN. F. W. Philips and M. L. Carson, Plant Science Department, South Dakota State University, Brookings, SD 57007.

Field trials to evaluate tolerance and resistance of eleven closely related corn inbreds (Zea mays L.); crossed to two testers to Goss' Wilt (Clavibacter michiganese sp. nebraskense), Stewart's bacterial wilt (Erwinia stewartii), and northern corn leaf blight (Exserohilum turcicum), were set up in separate experiments in the summer of 1987 at the South Dakota State University agronomy farm near Brookings, SD. Experiments consisted of split plot arrangements of hybrids (whole plots) and inoculated vs. non-inoculated treatments (split plots). Disease progress and yield data were recorded. Yield losses caused by the three diseases as well as the relationship between resistance and tolerance to these diseases will be discussed.

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REACTION OF SELECTED SUGARBEET VARIETIES EXPOSED TO TWO PATHOGENIC PYTHIUM SPP. E. M. Baker and C. M. Rush, Texas A&M University, Texas Agricultural Experiment Station, Bushland, Texas 79012.

In the Texas Panhandle, two Pythium spp. designated P2 and P34 have been consistently isolated from diseased sugarbeet seedlings. Four cultivars, two experimental varieties, and a commercially primed cultivar were evaluated for their reaction to the two Pythium isolates. Seed were planted in artificially infested soil and incubated for two weeks at 25C in growth chambers. None of the varieties showed significant resistance to either isolate as determined by percent emergence and damping off, but there were significant differences between the primed and unprimed TX18. Primed seed had better emergence in both infested soils and more resistance to post-emergence damping off in P2 infested soil than nonprimed seed. There were also significant differences between isolates. P34 is much more virulent and appears to cause more preemergence damping off than P2.

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SCREENING CEREALS FOR RESISTANCE TO BARLEY YELLOW DWARF VIRUS (BYDV) IN MOROCCO. M. El-Yamani,[1] J. H. Hill,[2] and B. E. Lockhart.[3] INRA/MIAC, Settat, Morocco,[1] Dept. of Plant Pathology, Iowa State University, Ames, IA,[2] Dept. of Plant Pathology, University of Minnesota, St. Paul, MN.[3]

BYDV is present in Morocco and efforts are being put into the search for genetic resistance to this damaging virus. Local and imported plant material is challenged with the non-specific-PAV-like-strain using Rhopalosiphum padi as a vector in artificial inoculations. The material was then grown outdoors and monitored for disease development. Plant height, disease score and yield were taken. ELISA test was also used in parallel with visual scoring. Local material showed that barleys were the most susceptible followed by durum then bread wheats. Some wheats showed good tolerance. Most U.S. oats, tolerant to the virus, seemed to hold up well under our conditions. The imported material exhibited a wide variation in its reaction to the virus under local conditions. Therefore, chances of selecting some very tolerant material to the virus are high.

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HOST SUITABILITY OF BEAN GERMPLASM TO *Meloidogyne incognita* AND *M. hapla*. B. A. Mullin, G. S. Abawi, Dept. Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456, and J. Kornegay, CIAT, Cali, Colombia.

Bean (*Phaseolus vulgaris* L.) seeds were planted in 10-cm-d clay pots and inoculated

with 10,000 eggs of *M. incognita* (Mi) host-race 3 or *M. hapla* (Mh) and replicated at least 5 times. Mi populations from the U.S. and Colombia were used. Roots were evaluated after 6-7 wk for galling and egg masses on a 1-9 scale and for total egg production. Ratings of 1-3, 3.1-6, and 6.1-9 referred to resistant, intermediate, and susceptible responses respectively. NemaSnap, A211, G2587, G6278, G12727, BAT 477 and RIZ 30 were resistant to both Mi populations. Lines A252, A445, G4823 and BAT 1297 were resistant to the U.S. Mi but intermediate to the Mi population from Colombia. Eggs were produced on all plants; however, production on resistant lines was less than 7% of that on the susceptible Canario Divex, California Dark Red Kidney and lines A322, G3773, and G4450. A211, G2587 and NemaSnap exhibited no galling and averaged less than one egg mass per plant. Reaction of germplasm tested against Mh was similar to their response to the U.S. population of Mi.

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GROWTH RESPONSES OF FIELD CROPS FOLLOWING INOCULATION WITH MYCORRHIZAL FUNGI IN FUMIGATED AND UNFUMIGATED SOIL. U. Afek, J. A. Menge and E. L. V. Johnson, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Cotton and onion were planted and inoculated with *Glomus deserticola* and *G. interaradices* in fumigated or unfumigated soil field plots. Inoculum (167 g/m) placed 2-3 cm below seeds at planting gave the best growth response. Dry weight of cotton was 8.8 times greater than non-inoculated cotton in fumigated soil and 3.3 times greater than non-inoculated cotton in unfumigated soil. Dry weights of onion was 3 times greater than non-inoculated onion in fumigated soil. Mychorrizae did not significantly increase onion growth in unfumigated soil. For both cotton and onion, successful colonization with mycorrhizal fungi in fumigated soil was 70-77% greater than it was in unfumigated soil. This appears to be a result of *Pythium* spp. which interfere with mycorrhizal development during the first two weeks. A correlation was found between percent infection and dry weight of these crops.

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EFFECTS OF THE ECTOMYCORRHIZAL FUNGUS HEBELOMA ARENOSA ON THE PHOSPHORUS-RED PINE INTERACTION. MacFall, J.S., S. Slack, J. Iyer[1], S. Wehrli[2]. Pl. Path. Dept., [1]Soils Dept., [2]Chem. Dept., Univ. of WI, Madison, WI 53706.

Seeds of the red pines (Pinus resinosa Ait.) were planted in low P soil (Sparta loamy sand, P=11ppm) supplemented with 5 levels of P (0-133ppm) with and without Hebeloma arenosa inoculum. At harvest (19 wks), inoculated trees from low P soil had greater root (12x) and shoot (8x) dry wts compared to controls. Fungal colonization and growth enhancement was less at high levels of P. Shoot wts of inoculated and control trees were higher with higher fertility. With increased levels of P, root wts of inoculated trees were less and root wts of controls were greater. ^{31}P NMR showed polyphosphate accumulation by the fungus in mycorrhizal roots of trees grown in low and mid P soil, despite fertility conditions limiting shoot growth. These results suggest that P uptake is increased and strongly influenced by the fungus, but that it is not the only mechanism for growth promotion.

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INFLUENCE OF *GLOMUS ETUNICATUM* IN A TRIPARTITE PEANUT SYSTEM. J. S. Neck and R. A. Taber, Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

A study was undertaken to assess the effects of an endomycorrhizal fungus in a peanut (*Arachis hypogaea* L. cv Tamnut), mycorrhizae (*Glomus etunicatum* Becker and Gerd.[GE], *Bradyrhizobium*[BD] tripartite association. Treatments included GE and BD in all combinations with uninfested controls, at 0.05,0.25,1.25, and 6.25 ppm available phosphorus [P]. Plants were grown in a sand culture system using modified Hoagland's solution. At 80 days, the GE treatment minus BD had greater shoot biomass than uninfested controls at all P levels. The GE+BD treatment exhibited greater shoot biomass, number and wt of nodules than the non-mycorrhizal controls at 0.05-1.25 ppm P. Acetylene/ethylene reduction assays (nmol/mg/hr) were lower in the GE+BD treatment than in the non-mycorrhizal control at all P levels except 6.25 ppm P. Evidence indicates *G. etunicatum* can influence the vegetative growth and nodulation of peanut plants.

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INCREASE IN MYCORRHIZA FORMATION IN ONION DUE TO INOCULATION WITH ACTINOMYCETES. Robert N. Ames, USDA-ARS 800 Buchanan St., Albany, CA 94710.

Pots filled with a non-sterilized sand-soil mix containing Glomus macrocarpum and G. mosseae were inoculated with one of 12 actinomycetes (5 reps each), seeded with onion, and grown for 4 months. At harvest, plant dry weight, N and P content, mycorrhiza development, soil hyphal density, and soil bacterial and actinomycete populations were determined. Plant dry weight was larger in 10 of the 12 actinomycete treatments, however, only two were significantly (P = .01) greater than the control. Plant N and P content were not affected. None of the 12 actinomycete isolates significantly reduced plant growth or mycorrhiza development. Seven actinomycete isolates significantly (P = .001) increased the percent mycorrhizal root length while four significantly (P = .01) increased mycorrhizal fungus hyphal density in the soil. With the exception of one treatment, bacterial and actinomycete populations did not differ from the control.

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EFFECTS OF PHOSPHORUS, NITROGEN, AND SOIL TEMPERATURE ON THE INTERACTION OF VESICULAR-ARBUSCULAR MYCORRHIZAE (VAM) FUNGI AND MELOIDOGYNE INCOGNITA (Mi) ON PEACH. E. W. Dixon, R. W. Roncadori, and R. S. Hussey. University of GA, Athens, GA 30602.

Plant growth was increased by VAM, P fertilization, or use of NH_4-N as opposed to NO_3-N. Mi decreased plant growth in all treatments, but growth of Mi-infected plants was stimulated by VAM and P fertility. Nematode reproduction (eggs/g root) was significantly suppressed by VAM and increased with NH_4-N. VAM colonization was decreased by Mi and by NH_4-N, especially in high P soil. Decreasing soil temperatures from 22 to 10-14 C suppressed the activity of both VAM and Mi. Root penetration by Mi occurred more rapidly than VAM fungal penetration and colonization at all temperatures. Egg production by Mi was initiated by the time extensive VAM root colonization occurred.

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INTERACTIONS BETWEEN VA MYCORRHIZAL FUNGI AND FLUORESCENT PSEUDOMONADS IN THE RHIZOSPHERE. T. C. Paulitz and R. G. Linderman, USDA-ARS Hort. Crops Res. Lab., Corvallis, OR 97330

Cucumber seeds (Cucumis sativus L. 'Marketer Long') were treated with rifampicin-resistant derivatives of Pseudomonas putida (A12, N1R or R-20) or P. fluorescens (2-79 or Pf-5) and planted in soils with and without added inoculum of the VA mycorrhizal fungi, Glomus intraradices or G. etunicatum. Population densities of Pseudomonas spp. in the combined rhizosphere-rhizoplane soil were determined by dilution plating at 1, 2, 3, 6, and 9 wks. Rhizosphere population densities of all strains except R-20 were reduced significantly by G. intraradices but not by G. etunicatum, as compared to non-mycorrhizal controls. In other greenhouse tests on cucumber, Pseudomonas spp. had no effect on VAM inoculum potential or colonization. These results indicate that dual inoculation with VAM fungi and these biocontrol agents could be compatible, or that inoculation with bacteria alone would have no detrimental effect on colonization by endemic VAM fungi.

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EFFECT OF VA-MYCORRHIZAE ON ROOT ARCHITECTURE OF BIG BLUESTEM. B. A. Daniels Hetrick, J. F. Leslie, G. T. Wilson, and D. G. Kitt, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

In steamed soil mycorrhizal fungi significantly improved plant growth of Andropogon gerardii Vitm., and increased root length and the number and diameter of the primary, secondary, and tertiary branches. Differences between mycorrhizal and nonmycorrhizal plants diminished as phosphorus (P) levels increased. Topological analysis of root architecture revealed that mycorrhizal fungi reduce the relative amount of root branching. Roots of mycorrhizal plants develop in a more elongate, exploratory growth pattern, apparently allowing fungal hyphae to extract nutrients from a larger volume of soil. In contrast, roots of nonmycorrhizal plants develop a more highly branched root pattern, with roots themselves playing a more critical role in direct extraction of nutrients from soil. Differences in root topology were not directly associated with the level of exogenous P, but instead appear to be controlled by the mycorrhizal fungi themselves.

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PROPERTIES AND CYTOPATHOLOGY OF BEAN GOLDEN MOSAIC IN BRAZIL. R. L. Gilbertson[1], J. C. Faria[2], E. Hiebert[3], and D. P.

Maxwell[1]. [1]Dept. Plant Path., Univ. of Wisconsin-Madison, [2]EMBRAPA CNPAF, Caixa Postal 179, 74000 Goiania, Brazil, and [3]Dept. Plant Path., Univ. of Florida, Gainesville, FL.

Bean golden mosaic (BGM-BZ) has become a major constraint to bean production in Brazil. The causal agent is whitefly-transmitted and disease symptoms are similar to those caused by a mechanically transmissible geminivirus, bean golden mosaic virus (BGMV). Unlike BGMV from the Caribbean and Central America, however, the BGM-BZ virus has not been mechanically transmitted. To confirm that BGM-BZ is caused by a geminivirus, light and electron microscopic studies were completed. Infected plants had inclusion bodies consistent with a geminivirus. All attempts to mechanically transmit BGM-BZ virus using inoculum from field-collected and/or whitefly-inoculated greenhouse grown plants were unsuccessful. A virus mechanically transmitted from field-collected leaves was identified as bean rugose mosaic virus.

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MOLECULAR CHARACTERIZATION OF GEMINIVIRUSES CAUSING BEAN GOLDEN MOSAIC. R. L. Gilbertson [1], J. C. Faria [1], F. Morales [2], S. A. Leong [1], D. P. Maxwell [1], and P. G. Ahlquist [1]. [1]Dept. of Plant Path., Univ. of WI-Madison, and [2]CIAT Cali, Columbia.

Because recent evidence indicated that genetic variation might exist among geminiviruses causing bean golden mosaic (BGM), a molecular approach was taken to characterize BGM virus isolates from Brazil (BGMV-BZ) and Guatemala (BGMV-GA). DNA-DNA hybridization indicates that BGMV-BZ is surprisingly divergent from a previously characterized BGMV isolate from Puerto Rico (BGMV-PR), and that BGMV-GA contains sequences related to BZ and PR isolates. Double-stranded viral DNAs from infected plants were used to make full-length clones of DNAs A and B for BGMV-BZ and partial clones for BGMV-GA. Extensive DNA sequence analysis of BGMV-BZ clones showed sequence similarities of 60-85% with BGMV-PR. Limited comparisons with BGMV-GA clones showed 70 and 95% sequence similarity with BGMV-PR and BGMV-BZ, respectively. These results indicate that considerable differences exist among these BGMV isolates.

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SURVEY OF GRAPEVINE STEM PITTING IN NEW YORK AND ISOLATION OF DSRNA FROM A GRAPEVINE SELECTION INFECTED WITH STEM PITTING. O.I. Azzam and D. Gonsalves. Plant Pathology Dept., N.Y.S. Agr. Expt. Sta., Cornell University, Geneva, N.Y. 14456

Rupestris stem pitting (SP) is a virus-like disease widespread throughout New York. A survey showed that 170 out of 257 tested grapevines indexed positive for SP using graft inoculations to the woody indicator, Rupestris St. George. Infected St. George developed pitting on the woody cylinder, usually below the inoculum bud. Stem pitting was diagnosed in European, American-French hybrids, and American type cultivars. However, many of these SP-infected grapevines did not show pitting on the woody cylinder. Isolations of dsRNA were attempted from healthy grapevines and from grapevines of a selection that had indexed positive for SP but tested negative for grapevine leafroll virus (GLRV), corky bark (CB), and three nepoviruses. DsRNA was recovered from SP-infected but not from healthy plants. Extracts made from leaf and bark tissues from SP-infected plants yielded similar dsRNA patterns. DsRNA patterns associated with stem pitting differed from those associated with GLRV and CB.

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BIOCHEMICAL AND SEROLOGICAL CHARACTERIZATION OF CLOSTEROVIRUS-LIKE PARTICLES ASSOCIATED WITH GRAPEVINE LEAFROLL DISEASE. J. S. Hu and D. Gonsalves Dept. of Plant Pathology, Cornell Univ., NYSAES, Geneva, New York 14456.

The molecular weight of virus coat protein (NY-1 isolate) was *ca.* 43 x 10^3 daltons in SDS-PAGE analysis; the protein reacted with specific polyclonal and monoclonal antibodies in Western blotting tests. The possibility that the protein is a dimer has not been completely ruled out. A large dsRNA molecule (*ca.* 10 x 10^6 Mr), along with several low molecular weight dsRNAs, was consistently isolated from leafroll diseased grapevines. Polyclonal antisera to two European and to two US leafroll isolates were used to determine the serological relatedness of different isolates in a protein A-gold labelling immuno-electron microscopy. Results indicated that serologically distinct serotypes existed, and mixed infection of grapevines with different serotypes was common. High titer monoclonal antibodies to NY-1 isolate were produced and used in double diffusion, ELISA, ISEM, and Western blotting assays. A new antiserum to a California leafroll isolate was produced and used in ELISA for detection of virus from crude preparations.

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BIOLOGICAL AND SEROLOGICAL PROPERTIES OF FOUR STRAINS OF ZUCCHINI YELLOW MOSAIC VIRUS. H.L. Wang, D. Gonsalves, R. Provvidenti, and T.A. Zitter. Plant Pathology Dept., N.Y.S. Agr. Expt. Sta., Cornell University, Geneva, N.Y. 14456.

Four strains of zucchini yellow mosaic virus, ZYMV-CT (Connecticut), -FL (Florida), -FR (France), and -TW (Taiwan), were characterized and compared. All four strains could be distinguished by symptoms incited on melon, cucumber, zucchini squash, Black turtle #2 bean, Red Kidney bean, Ranger pea, and Chenopodium quinoa. ZYMV-CT, -FL, and -TW incited severe symptoms on melons, cucumbers and squash, whereas -FR caused only mild symptoms under similar conditions. ZYMV-CT, -FL, and -TW were transmitted by the green peach and cotton aphids with different efficiencies, but ZYMV-FR was not transmitted by either aphid species. Polyclonal antibodies produced to the four strains gave strong cross reactions with all strains. However, cross-absorption of antisera indicated the existence of different antigenic determinants among strains. Monoclonal antibodies (Mab) were produced to ZYMV-CT, -FL, and -FR. In indirect enzyme-linked immunosorbent assay, some Mab reacted only to ZYMV-FR, while others reacted to ZYMV-CT, -FL, -TW, but not to -FR.

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SCREENING OF CEREAL PROTOPLASTS FOR RESISTANCE TO BARLEY STRIPE MOSAIC VIRUS. Yu-Zhi Zheng and Michael C. Edwards, USDA-ARS Cereal Crops Research Unit and Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Protoplasts were isolated from barley and oats using a discontinuous gradient centrifugation procedure. Yields of up to 4 x 10^6 protoplasts per gram of tissue were achieved with a viability of up to 50% after 7 days of incubation. Protoplasts from both susceptible and resistant plants were inoculated with RNA purified from two BSMV strains, CV52 (ND18) and CV42 (ND159). Protoplasts from the susceptible cultivar Black Hulless were susceptible to both BSMV strains, as indicated by FITC staining and ELISA. Protoplasts isolated from barleys resistant to CV42, but not CV52, remained resistant to CV42. A small percentage of protoplasts from oats normally resistant to CV52, but not CV42, were susceptible to CV52. Percent infection of protoplasts varied and depended greatly upon the inoculation conditions. Up to 95% of the viable cells became infected under optimum conditions.

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DECLINE OF ORIENTAL PERSIMMON. S.W. Scott, Dept. Plant Pathology and Physiology, Clemson University, SC 29634 and Jerry A. Payne, USDA/ARS S.E. Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

A planting of 17 cultivars of Oriental persimmon (Diospyros kaki L.)intended to evaluate the suitability of the species as a fruit crop in the south-eastern United States has suffered extensive tree death (22 trees remain alive from an initial population of 238) during the 5 years of its existance at Byron, GA. Symptoms associated with the decline and preceeding the death of trees are: veinal necrosis in leaves, premature defoliation, bud death, and the death of individual scaffold branches. Crystals of isometric viruses were revealed by electron microscopy of fixed and embedded tissue from diseased leaves. Concentrated leaf dip preparations showed a few isometric particles. Sap-inoculation of herbaceous hosts using juvenile leaf tissue ground in 2% nicotine produced symptoms in Chenopodium quinoa, C. amaranticolor, and Prunus persica. This is the first report of a virus in persimmon in the U.S.A..

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EVALUATION OF CULTURE-INDEXING AND TWO IMMUNOASSAYS FOR DETECTION OF XANTHOMONAS CAMPESTRIS PV. PELARGONII IN GERANIUM. K. K. Rane and R. L. Wick, University of Massachusetts, 240 Beaver St., Waltham, MA 02154.

Culture-indexing (CI) was evaluated for sensitivity in detecting low levels of Xanthomonas campestris pv. pelargonii (Xcp) in the florist's geranium (Pelargonium x hortorum). Groups of 40 plants were inoculated with either sterile buffer or Xcp (approx. 200, 20 and 5 cfu/plant). Twenty-four hours later, 20 plants from each treatment were culture-indexed and the remaining 20 plants were observed for symptom development. False positive reactions, as indicated by turbidity, occurred in greater than 50% of plants inoculated with sterile buffer. For all Xcp treatments, the number of plants developing symptoms was greater than the number of verified Xcp positives obtained through CI. The experiment was repeated and similar results were obtained. Incorporation of ELISA and a dot-blot immunoassay with CI reduced false positive reactions and shortened the time needed to verify the presence of Xcp.

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COMPARISON OF SEROLOGICAL AND CULTURE PLATE METHODS FOR DETECTION OF PHYTOPHTHORA SPP. ON CONTAINER-GROWN PLANTS. J. D. MacDonald and J. Stites. Department of Plant Pathology, University of California, Davis, CA, 95616.

Monoclonal antibodies with genus-level specificity for Phytophthora were obtained in ELISA format from Agri-Diagnostics Associates, Cinnamonson, NJ. These were compared to traditional culture plate methods for detection of Phytophthora in roots of container-grown plants. In uncontrolled field tests at commercial nurseries, and in greenhouse experiments in which plants were inoculated with different numbers of zoospores and assayed over time to detect Phytophthora, the two methods exhibited very similar detection limits. Both methods reliably detected the pathogen only in roots with obvious symptoms of infection. If lesions were not evident, the detection limits of both methods, and agreement between them, declined. Antibody tests can detect Phytophthora much more rapidly than culture plate methods, and with a similar degree of confidence.

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PHYSICAL PROPERTIES OF PINE BARK MEDIA IN RELATION TO SEVERITY OF PHYTOPHTHORA ROOT ROT OF RHODODENDRON. B.H Ownley, D.M. Benson, and T.E. Bilderback. Dept. Plant Pathology, and Dept. Hortic. Sci., N. C. State University, Raleigh, NC 27695-7616.

Rhododendron were grown in 4 container media infested with Phytophthora cinnamomi. The media were pine bark, 3 pine bark: 1 sand (v/v), 3 pine bark:1 peat (v/v), and peat:sand: soil (v/v/v). After 20 wk, plants were evaluated for root rot symptoms and total porosity (TP), air space (AS), moisture holding capacities, and bulk density (Db) were determined for all media. The fresh shoot weight of plants in noninfested media was positively correlated with Db and water held in the 1.0 to 5.0 kPa matric tension range and negatively correlated with TP and AS of container media. Root rot severity was significantly greater for plants in infested peat:sand:soil, and intermediate for plants in infested pine bark:peat than for plants in infested pine bark and pine bark:sand. Root rot severity was negatively correlated with TP and AS and positively correlated with Db and water held in the 5.0 to 10.0 kPa matric tension range.

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CAUSE AND CONTROL OF BLACK LAYER IN TURFGRASS. W.L. Berndt, J.M. Vargas, Jr. and B. Melvin. Department of Botany and Plant Pathology, Michigan State University East Lansing, MI. 48824.

Research at Michigan State suggests that black layer is an accumulation of metal sulfides and is biologically produced. It suggests that metal sulfide formation is enhanced by organic matter and can be prevented by nitrate. Waterlogged soils incubated in vitro with sulfur readily developed black layer within 14 da while soils incubated with sulfur plus nitrate did not. When waterlogged soils were incubated with increasing amounts of organic matter, metal sulfide content increased provided S and iron were not limiting. Soils were also examined for the presence of sulfur reducing bacteria, which produce the sulfide. All soils examined contained these organisms.

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TOMATO SPOTTED WILT (TSWV), TOBACCO MOSAIC (TMV), AND OTHER UNIDENTIFIED VIRUSES ASSOCIATED WITH SEVERELY DISEASED IMPATIENS AND OTHER FLORAL CROPS IN OHIO. S. T. Nameth and C. C. Powell. The Ohio State University, Columbus, OH 43210.

In the winter and spring of 1987 and 1988 commercial greenhouses in the Toledo area of Ohio reported a variety of virus like symptoms associated with various floral crops. Hybrid New Guinea and regular bedding impatiens were most severely affected. Symptoms included mosaic, necrotic ring spots, and dieback. Plants were analyzed for virus infection using ELISA and viral-associated dsRNA analysis. ELISA tests indicated TSWV and TMV were present alone and in combination. In plants showing both necrotic ringspots and mosaic symptoms dual infections by TSWV and TMV were confirmed. Plants which tested negative for TSWV infection using ELISA were analyzed using dsRNA analysis, which indicated the presence of TMV and other viruses.

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ISOLATION AND CLONING OF PLASMIDS FROM THE WESTERN ASTER YELLOWS MLO. C. R. Kuske and B. C. Kirkpatrick. Depts. of Plant Pathology, Univ. of Calif., Davis, CA 95616.

Three to four supercoiled plasmids, ranging in size from approximately 6.5 kb to 1.5 kb, were isolated from celery infected with either the severe (SAY), dwarf (DAY), or Tule Lake (TLAY) strain of western aster yellows MLO (AY-MLO) by centrifugation in ethidium bromide/CsCl gradients. The apparent sizes of some of the plasmids varied between strains. ^{32}P-labeled SAY plasmid DNA was used to probe Southern blots of DNA from AY-infected and healthy plants and the leafhopper vector. Six to eight DNA species, probably supercoiled, linear and circular plasmid DNA, were identified in SAY, DAY and TLAY-infected celery, periwinkle, aster, and leafhoppers. No hybridization occurred with healthy plant or leafhopper DNA. Plasmids were also present in field isolates of AY-MLO but no plasmids were identified in plants infected with the BLTVA virescence agent. SAY plasmids were digested with EcoRI, ligated with pUC18, and cloned in E. coli for further characterization.

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PHENOTYPIC AND GENOTYPIC DIVERSITY OF CLAVIBACTER XYLI SUBSP. CYNODONTIS. J. Lampel and J.J. Anderson. Crop Genetics International, Hanover, MD 21076.

Clavibacter xyli subsp. cynodontis (Cxc) isolates from diverse geographical locations were tested for genotypic and phenotypic diversity. In order to assess the degree of genotypic and phenotypic drift, SDS-PAGE protein profiles of in vitro grown Cxc and DNA Restriction Fragment Length Polymorphisms (RFLP's) were compared. Both the protein patterns and RFLP analysis indicate a striking degree of uniformity among the isolates tested. The Cxc isolates were also characterized for their production of bacteriocins. Each of nine geographic isolates were used as the indicator host for either phage or bacteriocin production by the individual isolates. Temperate phage were not detected. All isolates produced bacteriocins. The pattern of bacteriocin production and resistance suggested that at least three types of bacteriocins are produced by Cxc.

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PSEUDOMONAS SYRINGAE HRP MUTATIONS BLOCK THE INCORPORATION OF EXOGENOUS METHIONINE INTO PROTEIN. I. Yucel, H.-C. Huang, A. Collmer and S.W. Hutcheson, Department of Botany, University of Maryland, College Park, MD 20742

Pretreatment of P. syringae pv syringae (Pss) with suspension-cultured tobacco cells abolishes the bacterial induction stage for elicitation of the hypersensitive response (HR). Apparent induction of the Pss genes necessary for HR induction is triggered by amino acid starvation rather than a factor derived from plant cells. Because some bacterial amino acid transport systems also respond to these conditions, we investigated incorporation of exogenous amino acids into Pss proteins. Induction of wild-type Pss strains stimulated the rate of ^{35}S-methionine incorporation into total protein. Pretreated Pss mutants carrying transposons in separate hrp complementation groups exhibited a near total inhibition of methionine incorporation into protein. These results suggest that some of the hrp genes necessary for bacterial parasitism of plants function in amino acid transport.

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MOLECULAR ANALYSIS OF A COMMON DNA SEQUENCE INVOLVED IN TABTOXIN PRODUCTION BY *PSEUDOMONAS SYRINGAE*. T.M. Barta, T.G. Kinscherf, R.H. Coleman, and D.K. Willis. USDA/ARS and Dept. of Plant Pathology, Univ. of Wisconsin, Madison.

An 11 kb region of the genome of *Pseudomonas syringae* isolate BR2, the causal agent of wildfire in bean, has been determined to be involved in the production of tabtoxin. Deletion of the 11 kb region or transposon insertions into this region abolished toxin production and pathogenicity of BR2. Southern hybridization experiments with the cloned region as a probe demonstrated that, while closely conserved within *P. syringae* pv. *tabaci*, the 11 kb region was not present in nomenspecies "*P. angulata.*" A relationship similar to that between *P. s.* pv. *tabaci* and "*P. angulata*" was investigated for two other closely-related bacteria, *P. s.* pv. *coronafaciens* and *P. s.* pv. *striafaciens* . Also included in this analysis was another tabtoxin producing pathogen, *P. s.* pv. *garcae.*

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TRANSCRIPTIONAL REGULATION OF A COPPER RESISTANCE OPERON IN *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. M. A. Mellano and D. A.

Cooksey. Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

We previously cloned and sequenced a cluster of four plasmid-borne genes that confer copper resistance in *Pseudomonas syringae* pv. *tomato*. The genes were closely spaced and all were oriented in the same direction. mRNA production from each of the four genes was coordinately induced with as little as 1 μM $CuSO_4$, and mRNA levels increased with increasing copper concentration. Induction of mRNA was detected within 30 min after the addition of copper and reached maximal levels at 3-4 h. The induction was apparently specific for copper, since subinhibitory levels of Hg^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} did not stimulate mRNA production. By cloning DNA fragments 5' to each of the four genes into the promoter probe vector pMP190, a single copper-inducible promoter was detected in front of the first gene of the cluster, suggesting that the four genes are transcribed as an operon.

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NOVEL ONCOGENES OF THE RI-PLASMID T-DNA DIRECT ROOT INITIATION AND GROWTH. Frank F. White and Farida Shaheen, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506

The individual rol genes of the Ri TL-DNA direct root formation in primary tumors as well as developmental abnormalties in transgenic plants. Selected genes have been transferred individually into Nicotiana tabaccum (tobacco) plants in order to determine the affects of each gene. Rol A, as shown previously, directs the severely wrinkled phenotype. Rol B causes increased adventitious root initiation, accelerated flowering and heterostyly. Rol C appears to promote autonomous root growth, adventitious shoot formation, and altered leaf morphology. The rol B gene has unusual regulatory features and over-expression of rol B is root-initiation inhibitory in some situations. Further analysis of rol B expression will be presented.

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EFFECTS OF STRIPE RUST ON POPULATION STRUCTURE, COMPOSITION AND YIELD OF WHEAT CULTIVAR MIXTURES. M. R. Finckh and C. C. Mundt. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

Five wheat cultivars possessing different race-specific genes for resistance to Puccinia striiformis were grown in the field in all possible combinations. Yield components and disease severity were measured on individual plants on a pre-cultivar basis. Ratios of the mixture components at harvest differed between diseased and non-diseased populations and also from the ratios that had been planted. Disease affected yield of a cultivar differently in mixtures than in pure stands. In contrast to predictions of theoretical models, disease reduction was greater in mixtures of resistant and susceptible cultivars than in mixtures of differentially susceptible cultivars. Two distinct factors affecting disease severity could be separated: an effect of host diversity on the rate of disease increase and an effect of selection for cultivars with greater competitive ability and/or resistance to stripe rust.

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ACTIVITY OF AEROSOLIZED ERWINIA CAROTOVORA CELLS AS CLOUD CONDENSATION NUCLEI. G. D. Franc and P. J. DeMott. Colorado State University, SLV Research Center, 0249 E. Rd. 9 N., Center, CO 81125 and Dept. of Atmospheric Science, Ft. Collins, CO 80523, respectively.

Aerosolized E. carotovora cells were tested to determine their relative activity as cloud condensation nuclei (CCN) in simulated cloud formations. CCN activities of aerosolized cells were compared with that of ammonium sulfate aerosols, which are highly efficient CCN. Aerosol concentrations and controllable pre-cloud conditions were standardized to facilitate relative comparisons. Results showed that E. carotovora cells were only slightly less efficient CCN than ammonium sulfate particles. Activation of cells appeared to occur at the same thermodynamic point as ammonium sulfate. Because bacterial CCN will form cloud droplets at low supersaturation of water vapor this may provide a mechanism for E. carotovora cells to survive atmospheric transport by protecting them from desiccation and radiation.

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USE OF ADAPTIVE SAMPLING FOR THE IDENTIFICATION OF NONRANDOM SPATIAL PATTERNS OF BACTERIAL BROWN SPOT. B. D. Hudelson, M. K. Clayton[1], K. P. Smith, D. I. Rouse, and C. D. Upper[2]. Departments of Plant Pathology and Statistics[1], and USDA-ARS[2], University of Wisconsin-Madison, Madison, WI 53706.

Information on the spatial patterns of bacterial brown spot incidence in 5 m snap bean (Phaseolus vulgaris L.) row segments was used to develop a 2,4-systematic sampling plan (rating disease on 2 adjacent plants, skipping 4 plants, rating 2 plants, etc.) for detecting additional spatial patterns in longer row segments. This plan was used to sample 45 m row segments from a 4 ha commercial snap bean field. The resulting sample was described using an ARIMA model which was then compared with a model predicted for this sample based on 5 m row segment data. Small scale spatial patterns in the 2,4-systematic sample were similar in type and magnitude to those predicted from 5 m row segment data. In addition, a previously undescribed negative correlation in disease on plants spaced approximately 18-23 plants apart was detected.

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ECONOMIC IMPORTANCE OF SECONDARY SPREAD OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS IN NORTHERN-GROWN PROCESSING TOMATOES. M.D.Ricker and R.M.Riedel. CIRT, Napoleon, OH 43545 and Dept. of Plant Pathology, OSU, Columbus, OH 43210.

Secondary spread of the tomato canker pathogen did not cause significant yield reductions of processing tomatoes in Ohio in 1986 or 1987. Greenhouse-grown plants of the determinate cv Easy Winner were transplanted into 24 rows of 75 plants each in June '86 and '87. One to 5 transplants in 20 rows were replaced with previously-infected ones. Check rows received no inoculated plants. The plots were sprayed weekly with a one-row sprayer moving in a set route. Leaf symptoms indicative of secondary spread of Cmm were wide-spread in both years. The bacterium was isolated on SCM medium from all leaflets sampled. Treated rows yielded as much fruit as the control rows. Fruit weight from each plant was plotted versus distance from the nearest inoculated plant in each of seven directions: north, south, east, west, up-row, within-a-row, and all. Plants adjacent to inoculated ones yielded 11% more than the mean of all non-inoculated plants, probably due to compensation.

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ALTERATION OF PHYSIOLOGICAL PROCESSES IN WHEAT FLAG LEAVES INFECTED BY PUCCINIA SPECIES. M. T. McGrath and S. P. Pennypacker, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Physiological measures of plant productivity were used to predict the impact of Puccinia graminis f. sp. tritici and P. recondita f. sp. tritici on flag leaves of field-grown winter wheat (Triticum aestivum 'Tyler') during the 1987 grain filling period. Nine percent rust was predicted by regression analysis to reduce apparent photosynthetic rate per unit leaf area (PRA) by 60%, chlorophyll content (CC) by 54%, chlorophyll a / chlorophyll b ratio by 12%, apparent photosynthetic rate per unit chlorophyll (PRC) by 35%, and transpiration rate by 18%, and to increase internal CO_2 concentration and stomatal resistance by 23% and 68%, respectively. Loss of photosynthetic tissue on an area basis could not account for the reduced PRA since the reduction greatly exceeded the leaf area covered by pustules. Decreased CC could not completely explain the reduced PRA because the relation between PRC and rust severity was significant.

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DISEASE DYNAMICS OF TUNGRO VIRUS IN TRANSPLANTED AND DIRECT-SEEDED RICE. E. R. Ferrer and P. S. Teng. Dept. of Plant Pathology, IRRI, P.O. Box 933 Manila, Philippines.

Rice Tungro Virus (RTV) epidemics in transplanted and direct-seeded rice, cultivar IR42, were studied at Los Banos during July-October, 1987 by quantifying their progression dynamics, spatial patterns, incidence-severity relationships, and the population of the green leafhopper (GLH) vector, Nephotettix virescens. The logistic model was found to adequately describe RTV progress curves. The spatial distribution pattern of RTV infected plants changed from random to clustered to regular with lateness of crop season. The "K-value" of the binomial distribution increased from mid to late season. Distribution pattern changes were relatively more obvious with transplated than direct-seeded rice. RTV severity was quantified using a 0-8 scale, and the incidence-severity relationship was found to be affected by GLH number and host development. GLH numbers increased markedly during the vegetative stage.

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EQUAL DISEASE RANKINGS ACROSS 5 SITES PREDICT HORIZONTAL RESISTANCE. K. F. Cardwell, R. A. Frederiksen, R. R. Duncan, P. R. Hepperly, and A. Ferreira da Silva (Texas Agricultural Experiment Station, College Station 77843; Georgia Agricultural Experiment Station, Griffin; Tropical Agricultural Research Station, Mayaguez, Puerto Rico; EMBRAPA, Sete Lagoas, Brazil).

At 5 sites (in Texas, Georgia, Puerto Rico, and 2 in Brazil), 40 sorghum lines were rated for reaction to *Colletotrichum graminicola*. At all sites, inoculum from previous season residue was introduced onto the end of each row. Twenty lines were a series of differential sorghum cultivars. They indicated that the anthracnose reactions of the pathogen population at the 5 sites differed with respect to virulence alleles. The other 20 lines were selected on the basis of green stem characteristics and intermediate reaction types in previous trials. Of these, 10 showed no significantly different rank across the 5 sites, which, by definition, is a predictor of horizontal resistance. Dilatory resistance, measured as epidemiological progress through time and reduced to a logAUDPC value, was low in only 5 of the 10 horizontally resistant lines. Dilatory (rate reducing) resistance may therefore be either vertical or horizontal.

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DISEASE GRADIENTS OF LEPTOSPHAERULINA LEAF SPOTS ON ALFALFA AND WHITE CLOVER. O. M. Olanya and C. L. Campbell, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Disease gradients of Leptosphaerulina briosiana from alfalfa and L. trifolii from white clover were studied in a field experiment with infected alfalfa or white clover plants surrounded by initially healthy alfalfa or white clover plants. Disease severity was assessed twice/wk on plants in radial arms around the disease focus. In spring 1987, disease gradients developed from diseased alfalfa to both alfalfa and white clover; little disease developed in either host around the clover disease foci. In fall 1987, disease gradients developed from alfalfa or clover foci to both hosts. Disease generally increased more rapidly on alfalfa and disease gradients were steeper on clover, regardless of disease focus plant or pathogen species. These studies confirm the cross-infectivity of Leptosphaerulina spp. and indicate that the inoculum-recipient host is more important in determining steepness and extent of disease gradients than is the host at the disease focus.

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SEVERITY AND INCIDENCE OF PASPALUM LEAF BLIGHT. T.V. Price and B.L. Williams, School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia.

The proportion of paspalum leaf area with symptoms of leaf blight (Ascochyta paspali) on three farms in the Northern Irrigated region of Victoria ranged from 0-49.5% (mean 21%) paspalum dry matter between 15 Nov 1983 and 29 Jan 1984. The percentage leaf area with symptoms was linearly related to the incidence of tillers or leaves with symptoms. Incidence of tillers with symptoms on 1 ha of a dairy farm at Undera, Vic. ranged from 8-20% between 20 Nov 1985 and 16 April 1986 when a W-sampling pattern was used. Between 3 Nov 1986 and 9 Feb 1987 incidence of tillers with symptoms on the same ha ranged between 1.75% and 27.3% when a stratified random sampling program 'Field Runner' was used in conjunction with an Epson portable microcomputer. 'Field Runner' allowed more samples to be taken and reduced bias. Using 'Field Runner' the incidence of tillers with symptoms on 12 farms between 12-14 Jan 1987 was found to range between 11.8% and 56.4%. Incidence levels were reduced following grazing.

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OPTIMUM NUMBERS OF SAMPLING UNITS FOR ESTIMATING INCIDENCE OF ALFALFA LEAFSPOT. J. A. Duthie and C. L. Campbell, Dept. of Plant Pathology, NC State Univ., Raleigh, NC 27695-7616.

Optimum numbers of replicate plots (P) and subplots (S) for estimating incidence of leafspot were evaluated for 2 periods of alfalfa regrowth. In each period, 5 treated plots (4m x 10m) were randomized in each of 5 replicate blocks. Six subplots (4 rows x 1 m) were established at the center of each plot. At 3-4 day intervals, incidence (proportion of leaflets with leafspots) was scored for each row in 2 randomly selected subplots per plot. Lagrange multipliers, based on a total cost of sampling constrained to 25 min per treatment, and estimates of costs and variances for plots and subplots were used to optimize P and S. Costs were 1.0 min/plot and 2.0 min/subplot. Plot variances increased and subplot variances decreased during both periods. In one period, P increased from 3, after 15 days of regrowth, to 8 after 30 days. In the other period, P increased from 5 to 9. In the 2 periods, S decreased from 4 to 1 and from 2 to 1.

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INFLUENCE OF TEMPERATURE AND WETNESS DURATION ON INFECTION OF MATURE AND IMMATURE STRAWBERRY FRUIT BY COLLETOTRICHUM ACUTATUM L.L. Wilson, M.A. Ellis, and L.V. Madden, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Strawberry fruits were inoculated with a conidial suspension and incubated under various temperature and wetness duration combinations. Incidence of fruit infection increased with increased wetness durations (0-48 hr) at temperatures between 6 and 30C. No infection occurred on immature fruit at 35C. At short wetness durations (<1 hr) at 35C, infection occurred on mature fruit and incidence of infection decreased with increased wetness durations. Optimum temperature for infection on both immature and mature fruit was between 25 and 30C, with >80% infection by 13 hr wetness. A regression model using the logit of disease incidence as the dependent variable accurately described infection as a function of wetness duration (W) and temperature (T). Terms in the model were W, WT, WT^2, and WT^3 and all estimated parameters were significant. Coefficients of determination for combined data from three replications were 0.71 and 0.83 for immature and mature fruit, respectively.

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MARYBLYT: A PREDICTIVE MODEL FOR EASTERN APPLE FIRE BLIGHT. Paul W. Steiner, Botany Department, The University of Maryland, College Park, MD 20742.

A comprehensive model for apple fire blight disease management is described. The model uses three time-temperature clocks, daily plus cumulative weather data and apple phenology to signal specific infection periods and predict symptom appearance. Four distinct types of fire blight are characterized: blossom blight (BB), canker blight (CB), shoot blight (SB) and trauma blight (TB, related to certain frost or hail events). A bud clock uses cumulative degree days (CDD) >4C to monitor the rate of apple bud development through the end of the bloom period. An inoculum clock runs on cumulative degree hours (CDH) >18C from first bloom (220 DD >4C from green tip) and, with adjustments for cool weather, monitors relative epiphytic populations of Erwinia amylovora available for BB and TB. A disease clock uses CDD >13C and predicts both the onset of overwintering canker activity and the appearance of symptoms following infections of all types.

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SUSCEPTIBILITY OF VITIS VINIFERA BERRIES TO BOTRYTIS CINEREA AS DETERMINED BY INOCULUM DENSITY-DISEASE INCIDENCE ANALYSES AND CLUSTER ARCHITECTURE. M.E. Vail and J.J. Marois. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Mature grape berries from seven varieties were inoculated with conidia of B. cinerea over a range of 100 to 10^6 conidia per ml. Disease incidence (DI) was determined after seven days by recording the number of berries with visible mycelium. Ten berries were used for each of six inoculum densities (ID) and the experiment was repeated twice. After 7 days the slopes of the ID-DI relationships ranged from 0.07 for Emperor to 0.23 for Muscat of Alexandria (MOA). Slopes for Cabernet Sauvignon (CASA) and MOA indicated greater susceptibility than generally observed in the field. When cluster compactness was quantified with a UC Firmness Tester, CASA and MOA were significantly less compact than the other varieties. Individual berry physiology, as determined by the slope of the ID-DI relationship, and cluster architecture, as determined by measurements of cluster compactness, are both important in disease development.

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DURATION OF LEAF WETTING AND DRYING PERIODS IN ORCHARDS. R. C. Seem, G. Rubin, and C. M. Becker, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Current weather simulators for disease models do not represent adequately periods of leaf wetting duration or drying between those periods. Based on hemp-string and electronic recordings, 1,674 leaf wetting periods in apple orchards exhibited a multimodal frequency distribution of hours of duration for wet and dry periods. The frequency distributions were highly skewed toward longer periods of wetting or drying with the primary mode at 1 hr, and increasingly smaller modes occurring at approximately 12-hr intervals. Periodicity was due to the likelihood of some periods being altered as a result of nightfall or daybreak. Duration of wetting was significantly correlated with rainfall, temperature (negatively), and time of initiation. A compound gamma distribution, where the scale parameter is also gamma-distributed, was used to describe the

distribution of wetting and drying duration. This distribution can be used to simulate wetting period duration.

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THE ROLE OF CHLAMYDOSPORES IN INFECTION OF TOMATO BY ALTERNARIA SOLANI. C. L. Patterson and R. L. Powell, Dept. of Plant Pathology, Wes Watkins Ag. Res. and Ext. Center, Okla. State Univ., Lane. OK 74555.

The mechanism for overwinter survival of Alternaria solani (cause of tomato early blight) has been reported as infected tomato debris in soil, alternate solanaceous weed hosts, and/or on seed. Production of chlamydospores by the fungus, and their role in infection, however, has not been reported. Chlamydospores of A. solani were produced on a defined medium (Phytopathology 63:765) at 27 C and 12 h diurnal light; conidia were not produced. Chlamydospores were dark brown, thick walled, single celled, 20-30 µm-dia., and occurred in clusters throughout the medium. The chlamydospores were extracted from the medium, suspended in distilled H_2O/0.1% tween-20, and inoculated on tomato plants. The plants were incubated 48 h in plastic bags in the greenhouse. Early blight lesions developed on the leaves within 14 days of inoculation and A. solani was re-isolated.

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VIABILITY AND PATHOGENICITY OF UNCINULA NECATOR ASCOSPORES IN CALIFORNIA. W. D. Gubler, D. G. Fogle, and D. O. Chellemi. Department of Plant Pathology, University of California, Davis, CA 95616.

The role of Uncinula necator ascospores as potential sources of primary inoculum for grape powdery mildew was investigated. Cleistothecia were collected periodically from bark tissues during spring of 1988, induced to release ascospores, and their viability determined. Cleistothecia from 4 unsprayed vineyards (var. Chardonnay) in Monterey and Napa counties produced ascospores which infected seedlings and caused disease. A dormant application of lime sulfur (0.89 l/ha) resulted in a reduction in ascospore release and germination. This is the first report of ascospore infection in California

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EFFECTS OF CULTURAL PRACTICES AND CAPTAFOL ON PEACH TREE FUNGAL GUMMOSIS. K. O. Britton, F. F. Hendrix, Dept. of Plant Pathology, Univ. of GA, Athens, GA 30602. P. L. Pusey, USDA-ARS, Byron, GA, and R. W. Miller, Dept. of Plant Pathology, Clemson Univ., Clemson, S.C.

'Summergold' peach trees were planted in 1984 in eight-tree plots with border trees. Six cultural trts were compared to evaluate the impact of tree stress and sanitation measures on peach tree fungal gummosis. Half of each plot was sprayed with captafol (2.14 l/h) in early Jan, after pruning, and after harvest. Disease was assessed in 1987 as cankers/m3 tree vol and gumming spots/cm trunk circ. These variables correlated (r = -.36 and -0.28, respectively) with tree growth rate. Irrigation with postharvest nitrogen decreased disease severity and increased yield in the third year after planting. Yield increases were due to irrigation rather than additional nitrogen. Flail mowing and shallow discing after pruning did not reduce disease. Three captafol applications reduced disease only 6%.

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PHYTOPHTHORA CROWN AND ROOT ROT ON NURSERY-GROWN MANGO TREES DELIVERED TO ARIZONA. M.E. Matheron and J.C. Matejka. Department of Plant Pathology, University of Arizona, Yuma Agricultural Center, Yuma, AZ 85364

Mango trees (Mangifera indica L.), grown for many years by home gardeners in southwestern Arizona, recently have attracted the attention of commercial growers. In 1987, a shipment of mango trees from a California nursery contained several plants that showed symptoms of crown and root rot prior to planting at the orchard site. All severely affected trees were maintained in a lath house in their original pots and eventually died. Phytophthora palmivora was recovered from decayed rootlets of declining trees. When zoospores of P. palmivora were added to pots containing 3-month-old mango seedlings, we observed rapid development of crown rot followed by plant death. The introduction of P. palmivora into Arizona, coupled with the wide host range of this pathogen, could endanger not only mango trees, but other commercial crops as well.

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PHYTOPHTHORA SPECIES ASSOCIATED WITH A CRANBERRY DECLINE SYNDROME IN WISCONSIN. S. N. Jeffers, Dept. Plant Pathology, University of Wisconsin, Madison 53706.

In 1987, Phytophthora spp. consistently were recovered from vines affected by a decline syndrome in Wisconsin cranberry beds. The syndrome was characterized by areas devoid of vines and/or by plants exhibiting stunted, unthrifty growth. Roots on affected plants were necrotic or lacking all together, and gray-black lesions occurred on underground runners. The syndrome typically occurred in poorly drained or low lying areas of beds. At least two species of Phytophthora were recovered from three cultivars ('Searles', 'Stevens', and 'Crowley') and native vines in nine beds at six locations. An unidentified species--which was heterothallic and had nonpapillate, internally proliferating sporangia--was isolated most often; P. megasperma was isolated less frequently. Pathogenicity of these species will be determined. This is the first report of Phytophthora spp. being recovered from cranberry vines in Wisconsin.

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GRAFT TRANSMISSION OF CITRUS BLIGHT. R. F. Lee, R. H. Brlansky, L. W. Timmer, D. P. H. Tucker, J. H. Graham, and K. S. Derrick, Univ. of Florida, IFAS, CREC, Lake Alfred 33850.

Citrus blight symptoms (including reduced water uptake, high levels of zinc in trunk xylem, and presence of amorphous plugs in xylem vessels) were transmitted to eight of eight 12-yr-old Hamlin trees on Carrizo stock 29 months after the roots were approach-grafted to roots of blighted Valencia donor trees on rough lemon stock. Nongrafted healthy trees equidistance from the donor trees remained healthy. In another test, Hamlin trees on Carrizo stock were grafted with root pieces collected from blighted Valencia trees on rough lemon stock. Four of five trees developed blight symptoms after 41 months. Six control trees remained healthy. Apparently the causal agent of blight is graft transmissible and not carried in soil adhering to the donor tree.

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STIMULATION OF LIGNIN IN PRUNING WOUNDS OF PEACH TREES BY VARIOUS EXOGENOUSLY APPLIED CHEMICALS. A.R. Biggs and C.A. Peterson. Agriculture Canada, Vineland Station, Ontario and University of Waterloo, Waterloo, Ontario, Canada.

One-year-old peach stems were wounded and treated alone or in combination with the following materials: fungal cell wall extract from the peach pathogen Leucostoma persoonii, plant cell wall extract from peach foliage, chitosan, calcium ions, gibberellic acid, auxin, cellobiose, abscisic acid, glutathione, ethrel, latex paint, or Lac Balsam. After 7 and 14 days, stems were harvested and assessed for lignin with thioglycolic acid. Pruning wounds also were analyzed histochemically and anatomically for total and suberin-related autofluorescence, and for the numbers of cells comprising tissues formed postwounding. Pruning wounds that received fungal cell wall extract and cellobiose had greater amounts of lignin relative to nontreated wounds or wounds treated with latex paint. Total tissue autofluorescence was correlated with the amount of lignin extracted. Suberin autofluorescence appeared diminished in wounds treated with Lac Balsam.

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DETERMINATION AND CONTROL OF THE OVERSEASONING STRUCTURE OF RAMULARIA TULASNEI IN CALIFORNIA. L.S. Lile, and W.D. Gubler. University of California, Davis, CA, 95616.

Ramularia tulasnei sclerotia, found on strawberry plants stored at -2 C, function as overseasoning structures. When sclerotia-colonized plant debris was buried in the field, R. tulasnei survived for seven months. Hot water and fungicide dips were examined as possible pre-plant control treatments. A hot water dip of 3 minutes at 52 C was effective against the sclerotia and had no detrimental effect on strawberry plants. However, treatments of five minutes or more at 52 C were lethal

to strawberry plants. The fungicides DPX-H6573, penconazole, captan, and chlorothalonil were investigated. Fungicide dip treatments had no phytotoxic effects, but were not as effective against the sclerotia as the best hot water treatment. Penconazole appeared to be the most promising pre-plant fungicide treatment. Soil fumigation with methyl bromide-chloropicrin or basamid provided complete control of buried sclerotia.

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THE FATE OF OVERWINTERING VENTURIA INAEQUALIS (CKE) WINT. CONIDIA ON APPLE LEAVES AND FRUITS IN NEW YORK. C. M. Becker, T. J. Burr, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456

The viability of Venturia inaequalis conidia, obtained from apple fruit and leaf lesions, was determined monthly from leaf fall (October) through the spring (May). In October 1986, V. inaequalis infected leaves were placed on the ground in an apple orchard, in a weather shelter, and in an apple tree, in Geneva, NY. In October 1987, infected leaves and fruit were placed only on the ground, but in two Geneva and one Ithaca NY orchards. Viability of conidia from leaves decreased over time, with no viable conidia observed after January in either year. Relative numbers of conidia from fruit lesions increased until January, then rapidly decreased. The percentage of viable conidia from fruit was > 8% through December, but declined to zero by March. These data indicate that conidia in overwintered leaf and fruit lesions are not important propagules for apple scab infections in Western New York.

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ENVIRONMENTAL EFFECTS ON CONIDIAL PRODUCTION BY UNCINULA NECATOR. D. O. Chellemi and J. J. Marois. Dept. of Plant Pathology. University of California, Davis, CA 95616.

Production of conidia by the grape powdery mildew fungus (U. necator) was quantified on Vitis vinifera (var Carignane and Chardonnay). Inoculations were performed by placing a single conidium onto designated areas of the three youngest leaves on each plant. Plants were incubated at various temperature and vapor pressure deficit combinations ranging from 19° to 31° C and 0.68 to 3.94, respectively. Sporulation on both varieties occurred within 7 days at all temperature-vapor pressure deficit combinations tested. Mean production of conidia after seven days ranged from 90 at 27° to 0.375 at 19°. Leaf age was inversely related to sporulation, with fewer than 10 conidia produced after 7 days on the oldest leaf inoculated. No effect of vapor pressure deficit or variety on production of conidia was observed.

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INFLUENCE OF TEMPERATURE AND MOISTURE ON SPORE GERMINATION OF PHYSALOSPORA OBTUSA. L. F. Arauz and T. B. Sutton, Dept. of Plant Pathology, NC State Univ., Raleigh, NC 27695-7616.

Maximum germination of Physalospora obtusa conidia occurred in free water and declined as relative humidity (RH) was reduced from 100 to 92%; no germination was observed at 88.5% RH. Germination reached 80% in 4 hr at 16-32 C and in 12 hr at 12 C in free water, but was only 23% at 8 C after 12 hr in free water. No germination was observed at 4 C after 12 hr. Higher temperatures were required for germination at 95 and 92% RH 16 and 28 C, respectively) as compared to 98, 99, and 100% RH (12 C). At 92% RH conidial germination was observed only after 12 hr. Differences in temperature and RH requirements for germination were observed among isolates. Requirements for ascospore and conidia germination were similar. Germ tube length was maximum in free water and decreased with RH. Ascospore germ tubes reached a mean length of 0.78 mm after 12 hr in free water at 28 C, whereas conidial germ tube length averaged 0.87 mm after 12 hr at 24 C. Germ tube length of both types of spore declined at 32 C.

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VIRULENCE OF PHYTOPHTHORA SPP. FROM VARIOUS SOURCES TO APPLE SEEDLINGS. G. T. Browne and S. M. Mircetich, USDA ARS, Dept. of Plant Pathology, University of California, Davis, CA 95616.

Apple seedlings were grown 3 mo in greenhouse soil infested with one of 49 isolates of P. cactorum, P. cambivora, P. megasperma, P. cryptogea, P. cinnamomi, P. citrophthora, P. citricola, P. drechsleri, and five unidentified Phytophthora spp. (PSPP) from: apple trees, plants other than apple, and surface sources of irrigation water (SSIW) in California. The average percent reductions in root fresh weight (RRFW) caused by the species (compared to controls) were directly proportional to severity of root and crown rot induced by the individual isolates. RRFW were as follows: P. cactorum, 26-73%; P. cambivora, 39-97%; P. megasperma, 0-52%; P. cryptogea 0-89%; P. drechsleri 7%; P. cinnamomi, 18-54%; P. citrophthora from SSIW, 28-40%; P. citricola from SSIW, 0-31%; and unidentified PSPP, 0-66%. Thus, 13 PSPP induced disease in apple. Virulence varied with species, source, and isolate, but virulence of most isolates from non-apple sources was similar to that of apple isolates of the same species.

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PHYTOPHTHORA SPP. CAUSING ROOT AND CROWN ROT OF KIWIFRUIT IN CALIFORNIA. K. E. Conn, W. D. Gubler, and S. M. Mircetich. Dept. of Plant Path., Univ. of Calif., Davis, CA 95616.

Phytophthora citrophthora, P. cryptogea, P. drechsleri, P. megasperma and three unidentified Phytophthora spp. have been isolated from kiwifruit vines (Actinidia deliciosa) affected with crown rot and root rot (CR & RR) in California commercial orchards. Phytophthora CR & RR-affected kiwifruit exhibited poor terminal growth, small chlorotic and drooping leaves and various degrees of dieback. The highest incidence of diseased kiwifruit usually occurred on sites subjected to prolonged and repeated soil saturation due to poor drainage, flooding or over irrigation. In greenhouse experiments, six month old seedlings of cv. Abbott developed various degrees of CR (0-100%) and/or RR (20-100%) within three months, depending on the Phytophthora sp. Thus, seven Phytophthora spp. are implicated in CR & RR of kiwifruit vines in California.

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RELATIVE VIRULENCE OF TEN PHYTOPHTHORA SPP. TO LOVELL AND NEMAGUARD PEACH ROOTSTOCKS. R. Felix-Gastelum and S. M. Mircetich, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

We studied relative virulence of 14 isolates of 10 Phytophthora spp. to Lovell (L) and Nemaguard (N) peach seedlings grown in infested soil in greenhouse. A peach isolate of P. cinnamomi caused 100% root rot (RR) in both rootstocks. Three peach isolates of P. megasperma caused 51-100% and 44-97% RR in L and N, respectively. P. citrophthora recovered from irrigation water (IW) caused 75% RR in N and 100% RR in L. P. citricola from IW and almond caused 77 and 85% RR in L and 97 and 80% RR in N, respectively. P. cactorum from almond induced 13% RR in L and 52% RR in N. P. cambivora almond isolate and IW isolate caused 52-71% RR and 53-78% RR in N and L, respectively. Two unidentified Phytophthora spp. recovered from IW caused 85 and 99% RR in L and 73 and 100% RR in N, respectively. P. cryptogea and P. drechsleri from peach caused 85 and 15% RR in N and 88 and 20% RR in L, respectively. Thus, these ten Phytophthora spp. can cause root rot in California's commercial peach orchards.

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INCIDENCE OF ILARVIRUSES IN YOUNG ORCHARD TREES OF ALMOND, CHERRY, PEACH, AND PRUNE IN CALIFORNIA. J. K. Uyemoto and C. F. Luhn, USDA-ARS, Department of Plant Pathology, Univ. of California, Davis, CA 95616.

Enzyme-linked immunosorbent assays (ELISA) were used to identiby prune dwarf (PDV) and Prunus necrotic ringspot (PNRSV) virus in infected extracts of succulent leaf tissues (extraction buffer was carbonate salts, pH 9.6, and 2% PVP-40, 0.2% egg albumin, and 0.45% sodium DIECA). Sampled trees were in their first- to fifth-leaf stages of growth; collections were made in March to May 1987 and 1988. ELISA results indicated infection percentages of 20 (73/360), 25 (128/513), and 25 (50/200), respectively, in almond (Prunus dulcis), peach (P. persica), and prune (P. domestica) trees. In sweet cherry (P. avium cv. Bing), the incidence was 4.4% (4/90). PNRSV was detected in all Prunus spp tested and PDV in all hosts except almond. Bioassays on Shiro-fugen flowering cherry (P. serrulata) trees of several peach trees were in good agreement with the ELISA results. These findings suggest that infected scion buds and/or rootstocks were used to propagate nursery trees.

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EFFECTS OF SOIL TEMPERATURE AND MOISTURE ON THE SURVIVAL OF COLLETOTRICHUM ACUTATUM. D. M. Eastburn and W. D. Gubler, Department of Plant Pathology, University of California, Davis, California 95616.

The survival of Colletotrichum acutatum on strawberry petioles buried in soil was evaluated at three temperatures and three soil moistures. Colonized petiole pieces were buried in field soil which had been air dried (Dry), maintained at 10% moisture (Moist), or fully saturated (Flooded). Soil temperatures were maintained at 10, 25, and 40 C with the use of water baths. Weekly samplings revealed that C. acutatum survived best in dry soil at all the temperatures tested. In moist and flooded soils survival was highest at 10 C and lowest at 40 C. At 10 and 25 C C. acutatum declined fastest in flooded soil, but at 40 C it declined most rapidly in moist soil.

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GENETIC DIVERSITY IN FIELD POPULATIONS OF RACES 2 AND 3 OF Bipolaris zeicola IN 1985. K. J. Leonard and S. Leath, USDA,ARS Dept. Plant Pathology, N. C. State Univ., Raleigh, NC 27695.

Polymorphisms for lesion type, mating type (MAT), pseudothecial production (P+), and sensitivity to cycloheximide (CyhS) and carboxin (CrbS) were identified in populations of B. zeicola collected in 8 North Carolina corn fields in 1985. Frequency of race 2 ranged from 54-96% in 6 fields in the western piedmont and from 68-97% in 2 fields in the coastal plain. The Shannon-Wiener Index of Diversity was greater for race 2 (2.35) than race 3 (2.07), but diversity in eastern vs. western NC fields did not differ significantly. Mean frequencies of P+ and CyhS were significantly greater ($P < .001$) in race 2 (.56 and .32) than in race 3 (.22 and .08). Mating type frequencies varied among fields, but their frequencies in races 2 and 3 in each field were significantly correlated ($r = .823$, $P < .05$). Mean frequencies of MAT1-1 in races 2 (.43) and 3 (.50) over all fields did not differ significantly. This suggests that mating type frequencies in races 2 and 3 do not vary independently even though the races are divergent in other gene frequencies.

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GENETIC VARIATION IN FIELD POPULATIONS OF RACES 0, 2 AND 3 OF BIPOLARIS ZEICOLA IN 1987. G. Welz and K. J. Leonard, Tropeninstitut, Univ. Giessen, 6300 Giessen, F.R. Germany and USDA-ARS, Dept. Plant Path., NC State Univ., Raleigh, NC 27695.

Virulence, mating type (MAT), pseudothecial production (P+) and sensitivity to cycloheximide (cy-), carboxin (cb-) and cadmium (cd-) were monitored in three sequential samples of populations of Bipolaris zeicola in two NC corn fields in 1987. An undescribed race, designated race 0, was found to be avirulent on corn genotypes that are susceptible to races 1, 2 and 3 of the pathogen. Race 0 isolates were less frequently sensitive to cd (<10%) than races 2 and 3 (99%) and also different in colony morphology on PDA. Frequency of race 0 declined in both fields from 53% and 3%, respectively, to very low levels. Race 3 produced larger lesions than race 2 but race 2 increased more rapidly than race 3. MAT frequencies did not change significantly within or between fields. Due to greater variation for P+, cy- and cb-, races 0 and 2 were more diverse than race 3.

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SUSCEPTIBILITY OF FLORIDA SUGARCANE VARIETIES TO CERATOCYSTIS PARADOXA . R. N. Raid, University of Florida, Everglades Research and Education Center - Belle Glade, FL 33430.

Pineapple disease, caused by Ceratocystis paradoxa , has become an important factor in the establishment of newly-planted sugarcane in southern Florida. Field observations suggest a relationship between the rising prominence of this disease and the increasing acreage of several new varieties. Greenhouse experiments were conducted to determine the susceptibility of 21 sugarcane varieties of Florida origin to C. paradoxa. Twenty-five seedpieces of each variety were artificially inoculated at one end and placed in flats with sterile soil. Colonization was measured following a four week incubation period at temperatures of 21-28 C. Percent colonization of inoculated seedpieces ranged from 18.9 for variety CP 78-2114 to 79.8 for CP 72-2005. Germination and vigor observations were also made. Susceptibility as indicated by these studies corrobates observations on stand establishment in commercial production fields.

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QUANTITATIVE RESPONSE TO INFECTION OF WHEAT SEEDLING ROOTS BY SINGLE CONIDIA OF COCHLIOBOLUS SATIVUS. D. L. Blunt and J. P. Hill. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Seed of winter wheat cultivars, 'Sandy' and 'CO 840186', that are field resistant and susceptible, respectively, to common root rot in Colorado, were surface disinfested with sequential treatments of detergent, Tween 20, ETOH, NaOCl and mercuric chloride under negative pressure and placed in petri dishes containing PDA. After 5 days, uncontaminated seedlings were transferred to test tubes containing water agar and a single germinated conidium of Cochliobolus sativus was placed on the largest root of half the plants. Four days later all seedlings were transplanted to pots containing steam treated soil and placed in a growth chamber under 18 C or 29 C. After 3 weeks there were no significant differences between cultivars in dry root and shoot wt, ht, total leaf length, and percent discoloration of roots and shoots. However, disease reaction was significantly greater under warmer temperature.

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AN IATROGENIC RELATIONSHIP BETWEEN THE SEED HERBICIDE ANTIDOTE CGA-92194 AND SORGHUM DOWNY MILDEW. J. B. Szerszen and R. A. Frederiksen, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

The seed herbicide antidote CGA-92194 significantly increased (up to 50%) the incidence of sorghum downy mildew incited in susceptible grain sorghum hybrids by oospores of *Peronosclerospora sorghi* pathotypes 1 and 3. This antidote retarded growth of sorghum roots and leaves up to 21 days after planting when used at 1.25 g a.i./kg seed. Increased dosages caused greater and longer lasting growth retardation and increased disease. SEM revealed that CGA-92194 affected development and growth of roots of sorghum seedlings, which were measured by estimation of root trichoblast elongation at the time and location of root hair papilla within trichoblasts. Natural seedling maturation was delayed and the pathogen-susceptible period lengthened at least 12%. Juvenile tissue was maintained making disease escape ineffective. CGA-92194 had no effect on Pythium root and seedling rot incited by *Pythium arrhenomanes*.

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THE EFFECT OF FOUR APHID SPECIES ON SUBSEQUENT LESION DEVELOPMENT ON WINTER WHEAT LEAVES AFTER INOCULATION WITH SINGLE CONIDIA OF COCHLIOBOLUS SATIVUS. V. Velasco, W. M. Brown, Jr., and J. P. Hill. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Five individual bird cherry (Rhopalosiphum padi), greenbug (Schizaphis graminis), Russian (Diuraphis noxia), or green peach (Myzus persicae) aphids were placed on two-week-old winter wheat cultivar 'Vona' seedlings (4 plants/treatment). After 8 days, aphids were removed, 4 leaves per plant were each inoculated with a single germinated conidium of Cochliobolus sativus and plants were placed in a dew chamber at 28 C for 16 hr. Inoculations were made either directly on, 1-2 cm from, or 5 cm from the aphid feeding site. The closer that the inoculation was made to the feeding site of all except the green peach aphid, the smaller the lesion. Lesions at least 5 cm from feeding sites and on plants colonized by green peach aphids were not significantly different from lesions on plants not colonized by aphids.

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QUANTITATIVE ADULT PLANT RESPONSE TO INFECTION OF WINTER WHEAT SEEDLING ROOTS BY SINGLE MACROCONIDIA OF FUSARIUM ACUMINATUM. M. Mergoum*, J. P. Hill**, and J. S. Quick*. Departments of Agronomy* and Plant Pathology and Weed Science**, Colorado State University, Fort Collins, CO 80523.

Winter wheat seeds of cultivars 'Sandy' and 'CO 840186' were surface disinfested and placed in petri dishes containing PDA. After 5 days, uncontaminated seedlings were transferred to test tubes containing water agar. The seedlings were vernalized by placing the test tubes in the dark at 1 C for 8 wk. After vernalization, a single, germinated macroconidium of Fusarium acuminatum was placed on the largest root of half the seedlings. Four days later, all seedlings were transplanted to pots containing steam treated soil and placed in the greenhouse. At maturity, within cultivars, inoculated plants were significantly shorter, had fewer tillers and lower fresh weight than the controls. There were no significant treatment differences in dry weight and relative water content of the flag leaf within cultivars.

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SOME SCLEROTIUM CEPIVORUM SURVIVES HOT WATER/F MALIN GARLIC SEED TREATMENT. F.J. Crowe and A.S.

Greathead, Oregon St. Univ. Central Oregon Exp. Sta., Redmond OR 97756 and Univ. Calif. Coop. Ext. Serv. Salinas CA 93901.

After 4 mo storage of white rot-diseased garlic bulbs, no Sclerotium cepivorum was isolated from symptomless cloves. Viability of sclerotia was determined (Phytopath. 70:64-69), as was viability and identity of mycelium by growth and sclerotia formation on a semi-selective medium (Phytopath. 62:545-549). After hot water clove treatment [37.8 C/30 min, followed by 48.9 C/20 min, then 16.7 C/10 min], only sclerotia within aggregates of mycelium and decayed garlic tissues remained viable (18%). After treatment in water at 48.9 C/20 min followed by 16.7 C/10 min, unaggregated and aggregated sclerotia were 18 and 20% viable, respectively. Viability of sclerotia was greater with reduced time/temperature treatments. Viability was 85-89% with water at 16.7 C/20 min. For sclerotia, addition of 1% formalin did not improve upon hot water treatment. Mycelium succumbed to all temperatures $\geq$ 37.8 C $\pm$ 1% formalin.

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FUSARIUM ROSEUM CULMORUM IN GARLIC CLOVES, AND ITS CONTROL. F.J. Crowe, S. Stahl, [1]P. Koepsell, and [2]A.S. Greathead, Oregon St. Univ. Central Or. Exp. Sta. Redmond OR 97756, [1]Oregon St. Univ. Dept. Bot. & Pl. Path. Corvallis OR 97331, and [2]Univ. Calif. Coop. Ext. Serv. Salinas CA 93901.

All Fusarium roseum culmorum isolates from diseased and symptomless garlic decayed dormant garlic cloves and young plants when mycelium was wound-inoculated into dormant cloves or young seedlings, but fifteen western U.S. cereal isolates were not effective. Garlic clove reinfection was followed through repeated production cycles, with and without disease occurrence. Diverse disease incidence was recorded for uniformly mixed and divided seedlots planted in various regions. Benlate 50W and Mertect 340F controlled seedborne disease when applied 2 kg a.i./100 kg seed, or 2.25 kg a.i./ha as in-furrow sprays at planting. Standard hot water/formalin clove treatment eliminated FRC spores but only half of internal clove infections.

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EXAMINATION OF GENETIC DIVERSITY IN POPULATIONS OF THE PITCH CANKER PATHOGEN, FUSARIUM SUBGLUTINANS. J. C. Correll, T. R. Gordon, A. H. McCain, and D. J. Jacobson. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Pine isolates of *Fusarium subglutinans* (FS) from California and the southeastern (SE) U. S. were examined for virulence, morphology, vegetative and sexual compatibility, isozymes, and mtDNA RFLPs. Pine isolates which produced microconidia in chains (on KCl medium) were avirulent on Monterey pine whereas all pine isolates which produced only false heads were virulent; non-pine isolates of FS were avirulent or weakly virulent on Monterey pine. One vegetative compatibility group (VCG) was predominant in the current known range of pitch canker in California; however, multiple VCGs were identified among isolates from two Monterey pine Christmas tree plantings in southern California. Examination of SE isolates revealed a great deal of VCG diversity within the population. We have been unable to cross either SE or California pine isolates with known fertile non-pine isolates of FS. No isozyme polymorphism was identified from seven enzymes resolved among all isolates of FS using starch gel electrophoresis. One enzyme, isocitrate dehydrogenase, was polymorphic among pine and non-pine isolates. mtDNA was analysed for RFLPs between pine and non-pine isolates and from isolates within several pitch canker pathogen populations.

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TEMPORAL AND SPATIAL DISTRIBUTION OF PHYTOPHTHORA PARASITICA IN CITRUS ORCHARDS IN FLORIDA. Timmer, L. W., S. E. Zitko, H. A. Sandler, and J. H. Graham, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850.

The temporal distribution of Phytophthora parasitica was determined at five sampling times per year for a 3-year period in four orchards. Season of the year, soil temperature, and soil moisture did not greatly affect measured propagule density. The horizontal spatial distribution was determined in eight orchards by using 49 quadrants, each consisting of a 3 x 3 tree plot. Populations best fit a negative binomial distribution and indexes of dispersion indicated varying degrees of aggregation of propagules. Aggregation was greatest in orchards where mean propagule densities were low. Sampling of citrus orchards to measure propagule densities can be conducted without regard to time of year or sample location, but repeated sampling may be needed to get a more accurate estimation of an orchard population.

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EFFECT OF TEMPERATURE ON EXPRESSION OF PATHOGENICITY IN STEM RUST TEST CULTURES ON SPRING WHEAT. J. D. Miller and N. D. Williams. USDA-ARS, North Dakota State Univ., Fargo, ND 58105

Seven test cultures of stem rust with different avirulence/virulence patterns were tested on cultivars, germplasm lines, and Sr gene lines at temperatures of 18C and 27C. Presence or absence of temperature sensitivity for virulence was determined by low (L) and high (H) infection types (ITs). Some cultures were insensitive at 18 and 27, but others were avirulent at 18 and virulent at 27 on some hosts. Cultures A-12 and A-21 were insensitive and produced LITs on 4 cultivars and 8 germplasm lines at 18 and 27. On some hosts, culture 72.00 was partially sensitive,and produced LITs at 18 and intermediate LITs at 27. Culture 46-2 was insensitive and produced HITs on 'Waldron' and LITs on 'Len', 'Stoa' and 4 germplasm lines; on 'Coteau' and 3 lines, it was sensitive and showed LITs at 18 and HITs at 27. Most cultures were insensitive on the single Sr lines except for Sr6, 10 and 11.

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NATURE OF INTERACTIONS BETWEEN FUSARIUM OXYSPORUM AND MELOIDOGYNE JAVANICA IN COWPEA. A. R. Harris and H. Ferris, Dept. of Nematology, Univ. of California, Davis, CA 95616.

Two cowpea cultivars were inoculated with 10^7 conidia of F. oxysporum f. sp. tracheiphilum (Fot) race 1 or 3. Three weeks earlier, half of the plants had been inoculated with 12,500 M. javanica (Mj) eggs. Cowpea cv. California Blackeye (CB) 3, but not cv. CB7977, had higher Fusarium wilt and vascular discoloration ratings in plants inoculated with both pathogens, than in plants inoculated with Fot alone. The extent and rate of systemic spread of Fot was also compared in Fusarium-susceptible (CB5), resistant (CB3) and Mj-predisposed cowpeas (CB3). Mj second-stage juveniles (1000/pot) and Fot race 3 chlamydospores (20,000 C.F.U./cm^3) were added to soil at sowing, and plants were sampled after 1, 2, 4, and 6 weeks by plating surface-sterilized root, stem and petiole sections onto PCNB agar. Fot rapidly invaded almost to the tops of CB5 plants, but seldom was found above the primary internode in CB3 with or without nematodes. The effect of Mj infection on Fusarium wilt of CB3 was also studied using split-root techniques.

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COLONIZATION OF MUSKMELON AND NON-HOST CROP PLANTS BY FUSARIUM OXYSPORUM F. SP. MELONIS AND OTHER FUSARIUM SPECIES. T.R. Gordon, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Muskmelon (Cucumis melo L.) honeydew, tomato, wheat, alfalfa, sugarbeet and cotton were grown, under greenhouse conditions, in field soil naturally infested with Fusarium oxysporum f. sp. melonis, race 2 (FOM), cause of Fusarium wilt of muskmelon. Colonization of roots by FOM, F. equiseti, F. solani, and non-pathogenic strains of F. oxysporum, was quantified both as the number of colonies per centimeter of root and the number of colony forming units per gram fresh weight of root tissue. FOM colonized roots, before and after shoot removal, and buried shoots, of all seven of the host species examined. Living roots of muskmelon and honeydew were the most heavily colonized while tomato roots consistently had the lowest levels of FOM. F. equiseti and F. solani were present at low levels on living roots of all plants; they became relatively more abundant following shoot removal. F. equiseti was the most abundant colonizer of buried shoots.

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DISTRIBUTION AND FREQUENCY OF PHYTOPHTHORA PARASITICA AND P. CITROPHTHORA ASSOCIATED WITH ROOT ROT OF CITRUS IN CALIFORNIA. J. A. Menge, E. L. V. Johnson, E. Pond, D. Ferrin, H. Liu, A. Lutz, M. Strother, D. Bartnicki, U. Afek, and J. Sjoerdsma. Dept. of Plant Pathology, University of California, Riverside, CA

Forty citrus groves were chosen to assess the distribution of *Phytophthora parasitica* (Pp) and *P. citrophthora* (Pc) on citrus in California. Ten trees/grove were sampled for populations of *Pp*, *Pc* and for root health. *Phytophthora* was found in all groves. Populations of *Pp* averaged 2.8 propagules/g rhizosphere soil (pgrs) in the winter and 7.0 pgrs in the summer. Populations of *Pc* averaged 2.7 pgrs in the winter. Only 5% of all trees had > 15 pgrs *Pp* in the winter and 12% in the summer. Only 5% of the trees had > 15 pgrs *Pc* in the winter. Sweet orange rootstock supported the largest average populations of both *Phytophthora* spp. but other rootstocks thought to be resistant also supported large populations. Clay content, K, and Na were positively correlated with populations of *Pp* or *Pc*.

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THE RELATIONSHIP BETWEEN INOCULUM DENSITY OF THIELAVIOPSIS BASICOLA AND SEVERITY OF BLACK ROOT ROT ON BURLEY TOBACCO AS INFLUENCED BY HOST RESISTANCE AND SOIL TYPE. Julie Meyer and H. D. Shew, Dept. of Plant Pathology, N. C. State University, Raleigh, NC 27695-7616.

The interactions among inoculum density of T. basicola, host resistance and pH-related soil factors and their effect on severity of black root rot were studied by generating inoculum density:disease severity (ID:DS) curves from fields with different soil types (low or high soil pH) and different cultivars (low or moderate resistance). Soil near 20 individual plants per field was assayed for initial inoculum density. The same plants were rated 6 weeks later for disease symptoms. A comparison of the ID:DS relationships for different host resistance and soil type combinations indicate how these variables interact to determine pathogen threshold levels under field conditions.

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INHIBITION OF GROWTH AND REPRODUCTION OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE BY ALUMINUM. T. H. DeLuca and H. D. Shew, Dept. Plant Pathology, N. C. State Univ., Raleigh, NC 27695-7616.

V-8 juice agar was amended with Al or Ca ions at 0, 2, 4, 6, or 10 meq/l of agar, and adjusted to pH 4.5, 5.0, or 6.0. Radial growth of P. p. var. nicotianae was determined after 12 days. All Al concentrations greater than 4 meq/l inhibited mycelial growth; greatest inhibition was at pH's 4.5 and 5.0. The effect of Al on sporangium production was determined by placing a blended mycelial suspension of P. p. var. nicotianae onto a nylon mesh in V-8 broth for 2 days. The mat that formed was transferred into distilled H_2O containing 0 or 0.33 meq Al/l. Sporangium production was inhibited 96% in the Al treatment. Zoospore motility was determined in distilled water containing 0, 0.056, 0.11, or 0.33 meq Al/l. After 30 min, compared to the control, only 15% of the zoospores were still motile at 0.056 meq Al; none were motile at higher concentrations.

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THE INFLUENCE OF *FUSARIUM SOLANI* ON ROOT ROT OF CITRUS CAUSED BY *PHYTOPHTHORA PARASITICA* AND *PHYTOPTHORA CITROPHTHORA*. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

The influence of *F. solani* (Fs) on root rot of citrus caused by *P. parasitica* (Pp) or *P. citrophthora* (Pc) was studied by transplanting citrus seedlings into non-infested soil or soil infested with Pp alone, Fs alone, or a combination of both Pp and Fs. Dry weight of roots was least for plants grown in soil infested with Pp plus Fs. Root length and % living roots were less for plants treated with Pp alone or in combination with Fs than control or Fs treated plants. In a second experiment, root weight, root lengths, but not % living roots, were least in plants co-inoculated with Fs and Pp or Pc. Plants inoculated with Pp or Pc had lower root weights, root lengths, and % living roots than the control. Fs alone did not decrease root weights, root lengths, or % living roots as compared to the controls. Studies also showed that fewer Pp zoospores were attached to roots pre-colonized by Fs than to non-colonized roots.

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EVALUATION OF HOST RESISTANCE AND PATHOGEN AGGRESSIVENESS ON SOYBEAN ROOTS INOCULATED WITH PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA. R.E. Wagner and H.T. Wilkinson. Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801-4709.

The rate of symptom development on taproots of soybean cultivars inoculated with different races of Phytophthora megasperma f. sp. glycinea (Pmg) was investigated at three temperatures (15, 20, and 25 C) using aeroponic culture. Differences among Pmg races 1,3,16,20, and 22 were detected on the susceptible cultivar Williams, for initial symptom expression, lesion size and rate of lesion expansion. The range in average lesion size 96 hr after inoculation was 1.1 to 5.5 cm at 15 C. Lesion size increased and time of symptom expression decreased as temperature levels were elevated. Initial symptoms were expressed and taproot elongation ceased within 24 hr at all temperatures on the resistant cultivars Williams 79 (Rpslc) and Williams 82 (Rpslk) inoculated with Pmg race 1.

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VERTICAL DISTRIBUTION AND GENERATION TIME OF BACTERIA IN THE RHIZOSPHERE OF MATURE SUGARBEETS. T. Tedla and M. E. Stanghellini. Dept. of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721 .

The vertical distribution and generation time of bacteria in rhizosphere sections of soil from the root-soil interface (RSI, [Phytopathology 73:1463-1466]) of 9- mo-old field grown sugarbeet tap roots were estimated by dilution plating. The bacterial populations at the 2-,6-,and 11-cm depths were 3.39, 5.12 and 3.26 X 10^7 cfu/g of RSI soil, respectively, prior to irrigation.Populations at the same depths 24 hr after irrigation were 10.8, 5.7, and 5.1 X 10^7 cfu/g RSI soil, respectively. Dry RSI sections were transported to the laboratory, irrigated, and incubated at 27 C. Soil samples were assayed as above at 0, 4, 8, 12, and 24 hr after irrigation. Bacterial populations doubled within 8 hr and then declined .

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Detection of Phytophthora in soybean soil by immunoassay analysis of infected bait. M. J. Klopmeyer, S. A. Miller, J. H. Rittenburg, F. P. Petersen, and G. D. Grothaus. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077.

A technique combining baiting and immunoassay was developed to detect Phytophthora megasperma f. sp. glycinea (Pmg) in soybean field soil. Naturally infested soils were dried, moistened, incubated for 3 days, then flooded and baited with soybean leaf discs. The discs were removed and individually macerated in buffer 1, 2 and 3 days after flooding. Extracts were analyzed in a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) containing antibodies prepared against Pmg. Pmg was detected in leaf discs from flooded, infested soil samples after 1 to 2 days, but not in leaf discs from flooded soil previously determined to be free of Phytophthora. The sensitivity of ELISA combined with amplification of the pathogen in leaf discs allows for rapid detection of Pmg in soil.

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ELISA DETECTION OF PHYTOPHTHORA FROM SOIL. A. F. Schmitthenner, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691 and Sally Miller, Agri-Diagnostics Associates, Cinnaminson, NJ 08077

An Agri-Diagnostics Phytophthora multiwell ELISA kit, developed for detection of Phytophthora in plant tissue, also readily detected Phytophthora in soil where soybeans were damaged by P. megasperma f. sp. glycinea (Pmg). Nonspecific background was eliminated by centrifuging the soil suspension. Only low levels of Phytophthora were detected in soil during winter or in soil stored at 3 C. Following cold storage, high levels of Phytophthora could be detected directly from soil, after Phytophthora damping-off of soybean seedlings was induced. Best Phytophthora detection was obtained from soybean leaf discs floated on water over infested soil for 24 hr. Pmg was the only Phytophthora isolated from such leaf discs using selective media. It was concluded that Phytophthora was detected best with an ELISA test of soil with actively rotting roots or from leaf disc baits with actively growing mycelium.

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COMPARATIVE USE OF CHLAMYDOSPORE-INFESTED SOIL TO SCREEN FOR RELATIVE SUSCEPTIBILITY IN CITRUS CULTIVARS TO PHYTOPHTHORA FOOT ROT. G. S. Smith, D. J. Hutchison, and C. T. Henderson. Texas A&I University, Weslaco, TX 78596 and USDA Horticultural Research Lab, Orlando, FL 32803.

Soil artificially infested with chlamydospores of Phytophthora parasitica was an effective inoculum source for screening citrus rootstocks for relative susceptibility to foot rot. Inoculum densities of 14 to 200 chlamydospores per plant resulted in mean percentage stem girdling, lesion area, and relative lesion area similar to results obtained with 1,000 zoospores per plant. Foot rot severity ratings, however, were significantly higher with inoculum of 0.5 cm diam mycelial agar disks compared with chlamydospores or zoospores. Commercial rootstocks considered moderately resistant or with field tolerance of foot rot were rated as susceptible when mycelial agar disks were used as an inoculum source. Use of quantifiable inoculum sources such as chlamydospores or zoospores may allow selection of rootstocks with intermediate levels of resistance to foot rot.

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BIOLOGICAL ACTIVITY OF MACROPHOMINA PHASEOLINA IN SOIL. D.J. Collins and T.D. Wyllie, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Glass slide technique was used to observe direct microsclerotial germination and behavior of Macrophomina phaseolina in soil and soybean rhizosphere. This method used glass microscope slides that were dipped into a water-agar suspension of microsclerotia of M. phaseolina. After the agar solidifed, slides were placed in soil at 45° angles with soil covering 7 cm of the slide. Soybean seeds were then planted 1 cm deep but directly on the agar-coated slide so that roots would grow down and across the agar film. After various incubation periods, slides were removed from the soil and examined microscopically; microsclerotial germination in sterile, nonsterile, and rhizosphere soils was 95%, 4%, and 35%, respectively. No apparent positive tropism of mycellium from germinating microsclerotia was seen except at the root tip. Competitive growth of other soil microorganisms around and on surfaces of microsclerotia may have contributed to reduced germination of microsclerotia in nonsterile soil.

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IMMUNOASSAY PROCEDURE FOR THE DETECTION OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE IN SOIL. Keith Jones and H. D. Shew, Dept. of Plant Pathology, N. C. State Univ., Raleigh, 27695-7616.

An immunoassay procedure (double antibody-ELISA) was developed for the quantitative detection of zoospores of Phytophthora parasitica var. nicotianae. A standard curve was generated for known numbers of zoospores added to extraction buffer and to soil solution. Zoospores were lysed prior to running a dilution series. As few as 2 zoospores per 100ul of either solution were detected, but the slopes of the standard curves were different for the two solutions. Zoospores released in naturally infested soils also could be detected by ELISA procedure. Finally, P. parasitica var. nicotianae was detected in naturally infested soil without the release of zoospores after freezing the soil with liquid nitrogen using the ELISA technique. The ELISA procedure detected <2.5 propagules of P. parasitica var. nicotianae per gram of soil based on paired samples run on selective agar medium.

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YIELD LOSS IN CORN CAUSED BY RHIZOCTONIA SOLANI AG-2 TYPE 2 AND NEMATODES. D. R. Sumner and N. A. Minton, University of Georgia and USDA/ARS, Coastal Plain Station, Tifton, 31793.

Corn was planted three successive years in a field infested with a high (1144 or 1430 kg/ha) or low (143 or 238 kg/ha) inoculum level of Rhizoctonia solani AG-2 type 2 grown on 3% cornmeal-sand (w/w), or noninfested. Each year soil was drenched with pencycuron or nontreated at planting. During the last two years, soil was treated with fenamiphos or nontreated before planting. Yield of grain averaged 6.9, 8.8, and 9.9 MT/ha in soil infested with high or low inoculum levels, or noninfested, respectively. Pencycuron reduced crown and brace root rot and prevented yield loss. Fenamiphos reduced Meloidogyne spp., and Pratylenchus spp. in soil, but interacted with R. solani AG-2 type 2 to increase severity of crown and brace root rot and reduce yield. Forty to 60% of the variation in yield was explained by crown and brace root rot severity and levels of nematodes in soil.

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EFFECT OF SUDAN GRASS AND WHEAT STRAW, NITROGEN, AND SPRINKLER IRRIGATION ON CONTROL OF VERTICILLIUM DAHLIAE. Gene D. Easton, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA 99350

Near Prosser, WA for two consecutive years, either sudan grass or wheat straw (5.4 T/ha) was rototilled into plots of soil fertilized at 112 and 448 kg N/ha. The field was plowed and planted to the Russet Burbank cultivar. Plots were sprinkler irrigated by an irrigation gradient system at very low (23-33 cm), low (37-54 cm), medium (57-75 cm) and high (70-103 cm) rates of water per season. Neither the straw or sudan grass had an effect on Verticillium wilt or potato production. Plots fertilized at 112 kg N/ha had more Verticillium wilt and less yield than plots fertilized at 448 kg N/ha. Rates of irrigation had no effect on Verticillium wilt. The very low rate had less % U.S. No. 1 grade tubers and less yield than any other rate. Evidently short-term applications of neither straw nor sudan grass amendments had any effect on Verticillium wilt or potato production.

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EVALUATION OF FUNGICIDES FOR THE MANAGEMENT OF CLADOSPORIUM VARIABILE ON SPINACH. G. Fuentes Davila and R. L. Gabrielson, Washington State University, Puyallup, WA 98371.

Complete control of C. variabile was achieved with benomyl at 4.8 g ai/l of water; chlorothalonil and DPX 965 at 2.4 g ai/l in greenhouse inoculated spinach. Fungicide mixtures that completely controlled the disease at dramatically lower rates and that were nonphytotoxic were benomyl+flusilazol at 0.07+-0.07 g ai/l; chlorothalonil+flusilazol, benomyl+DPX 965, and chlorothalonil+DPX 965 each at 0.15+0.15 g ai/l; and benomyl+-chlorothalonil at 0.3+0.3 g ai/l. In spinach seed field trials in 1986, the best treatment was benomyl+iprodione at 1.2+1.2 g ai/l, while in 1987 best treatments were chlorothalonil at 4.8 g ai/l and benomyl+chlorothalonil at 0.6+0.6 g ai/l. Minimum rates of fungicides that gave complete control of naturally contaminated/infected slurry treated seed were captan at 2.5 g ai/kg; benomyl and thiram at 5. g ai/kg; and bitertanol at 0.6 g ai/kg of seed.

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THE BIOLOGY OF CLADOSPORIUM VARIABLE CAUSING SPINACH LEAF SPOT. G. Fuentes Davila and R. L. Gabrielson, Washington State University, Puyallup, WA 98371.

Cladosporium leaf spot of spinach is a serious problem in plastic tunnel production in Japan is on occasion a limiting factor in seed production in western Washington. C. variabile grows on potato-dextrose-agar at temperatures ranging from 5-30 C, with an optimum of 20 C. Optimum infection of spinach leaves occurred at 15-20 C with a R.H. above 80° for at least 48 hr. Maximum number of lesions followed inoculation at 1 X 10^4 conidia per ml. These conditions are more likely to be common in plastic tunnel culture and is probably why the disease is serious in Japan and less so in western Washington where day temperatures are usually favorable but the R.H. is too low. Two-4 days following penetration the fungus emerges from the leaf and sporulation is initiated. The fungus persists from season to season on seed and on volunteer spinach plants in old seed fields.

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EFFECTS OF EARLY BLIGHT ON POTATO YIELDS IN NORTHEAST FLORIDA DURING 1987. D. P. Weingartner. University of Florida IFAS Agricultural Research and Education Center. Hastings, FL 32045

Effects of natural early blight (EB) epidemics on tuber yields were compared in nonsprayed (NS), chlorothalonil (CS) and metalaxyl (MS) sprayed plots of 9 potato cvs. Yields of Atlantic, New Superior, La Chipper, Red La Soda and Green Mountain, but not those of Sebago, Ontario, Superior or La Rouge were significantly (P=.05-.10) greater in CS than in NS plots. Regression analysis using yields of Size A tubers as the dependent and % EB on 5 different dates as the independent variables, respectively, revealed significant (P=.01-.05) coefficients of determination (r^2) on 1 or more dates for Atlantic, Ontario, New Superior, La Chipper, La Rouge and Green Mountain, but not Sebago, Superior, or Red La Soda cvs. Dates of greatest r^2 values varied widely among cultivars and may provide a tool for determining the most profitable timing of fungicide applications.

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Influence of Fungicidal Seedpiece Treatments and Tuber-Borne Inoculum on the Development of Four Potato Pathogens. T. A. Zitter and D. E. Halseth, Departments of Plant Pathology and Vegetable Crops, Cornell University, Ithaca, NY 14853.

Daughter tubers saved from field fungicide seedpiece trials and infected at various levels with Rhizoctonia solani (RS), Streptomyces scabies (SS), Helminthosporium solani (HS) and Colletotrichum coccodes (CC) were retreated with experimental or standard fungicides or were left untreated. Tubers were planted in pots using soilless mix and were examined after 3½ mo. HS was infrequently recovered from the immature tubers. Retreatment of seedpieces with tolcolfos-methyl (t-m), flutolanil (f) or CGA 449 (CGA) gave excellent control of RS. CGA additionally controlled SS and CC when applied as a retreatment or applied after the initial treatment with standard fungicides. If maneb treatments followed the initial treatment of CGA, t-m or f, a significant increase in RS occurred and was more severe than if the tubers had been left untreated. Seedpiece treatments can reduce importance of tuber-borne inoculum and improve tuber appearance.

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A RULE-BASED DECISION SUPPORT SYSTEM FOR MANAGING POWDERY MILDEW ON MUSKMELON. R.X. Latin, G.E. Miles, and J.R. Mitchell. Departments of Botany and Plant Pathology and Agricultural Engineering, Purdue University, West Lafayette, IN 47907.

An expert system was developed to assist farmers in making decisions for controlling muskemlon powdery mildew caused by Sphaerotheca fuliginea. The decision rules are based on field research that established a relationship between the timing of initial fungicide sprays, the severity of powdery mildew epidemics, and muskmelon yield losses. Other factors involved in the control decisions are the muskmelon cultivar, the number of weeds from the initial harvest date, the kind, amount, and application dates of fungicides applied previously, and the location of the disease relative to the field in question. The knowledge base was developed using the Personal Consultant Plus (Texas Instruments, Dallas, TX) expert system development shell and operates on an IBM PC compatible computer.

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PYTHIUM DISEASES OF CARROTS IN CALIFORNIA. R. M. Davis, C. M. Liddell, J. P. Guerard, J. Nunez, and E. Vivoda. Department of Plant Pathology, University of California, Davis, CA 95616.

Pythium irregulare and P. ultimum were frequently recovered from carrot fields with histories of carrot root dieback. In greenhouse studies both fungi caused root dieback symptoms in less than 2 wk after seed germination at inoculum densities of 50 or more cfu/g soil. High soil temperatures (27-35 C) or saturated soils aggravated the disease but soils dried to -30 KPa suction did not limit Pythium activity. Symptoms of cavity spot developed on carrots incubated at 15 C and inoculated with P. violae, which was consistently isolated from field carrots. A single soil drench of metalaxyl applied about 50 days after planting or multiple applications throughout the season significantly reduced the incidence of cavity spot in the field. A single preplant application of metalaxyl was generally less effective.

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Reactions of Pepper, Tomato, Strawberry, and Apple Fruit Inoculated with Colletotrichum species. J.F. Hadden and L.L. Black, Dept. of Plant Pathol. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803.

Colletotrichum gloeosporioides, C. capsici, C. acutatum, and C. coccodes incite anthracnose of pepper and tomato. To determine their pathogenicity and virulence, 27 isolates of these four species and 13 isolates of six other Colletotrichum spp. from a wide range of hosts were inoculated by conidial injection into detached pepper, tomato, strawberry, and apple fruit. All isolates of C. gloeosporioides and C. acutatum caused typical anthracnose lesions on all hosts, while isolates of C. capsici and C. coccodes caused typical lesions on pepper and tomato but caused only small or no lesions on strawberry and apple. Colletotrichum truncatum and C. fragariae isolates elicited host reactions similar to those of C. capsici and C. gloeosporioides, respectively. Colletotrichum lindemuthianum, C. destructivum, C. higginsianum, and C. orbiculare isolates caused small or no lesions on these hosts.

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DIFFERENTIATION OF A LIGHT BROWN ROOT DISCOLORATION FROM CORKY ROOT OF TOMATO AND PROPAGULE DENSITY STUDIES OF THE CORKY ROOT FUNGUS IN TOMATO RESIDUE. N. Shishkoff and R. N. Campbell, Dept. of Plant Pathology, University of California, Davis, 95616.

Early symptoms of corky root of tomato, caused by Pyrenochaeta lycopersici, may be confused with a light brown discoloration (LBD) that remains superficial as the plant grows. Fusarium oxysporum has been isolated consistently from LBD and has reproduced LBD symptoms in greenhouse trials. This F. oxysporum has not caused wilt of Pakmor or Earlypak tomatoes or 15 other wilt-susceptible crops. Samples of soil and tomato root debris were collected from November 1987 to May 1988. The number of microsclerotia (ms) estimated by extraction and plating on a semi-selective medium was high in tomato root cortex (10,000 ms/g) and relatively low in the root xylem (280 ms/g), in the 'rhizosphere' soil around the root debris (190 ms/g), and in soil without debris (2 ms/g).

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ROOT ROT OF ONION CAUSED BY PYTHIUM IRREGULARE AND P. COLORATUM. P. C. Vincelli and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Pythium irregulare and P. coloratum were isolated consistently from roots of 8- to 12-week-old onion plants exhibiting root rot and associated foliar symptoms in commercial fields in Orange County, NY. P. coloratum predominated following very wet weather in 1984 and 1986 (13 and 12 cm rainfall during the week prior to symptom appearance, respectively), while P. irregulare was prevalent following moderately wet weather in 1985 (9 cm rainfall). Both species caused root decay on 12-week-old plants (cv. Downing Yellow Globe) which were transplanted into infested vermiculite (100 ml colonized cornmeal:sand mix (9:1)/L vermiculite) and maintained at 14 C for 10 days in a growth chamber under a soil moisture regime of alternating saturation/field capacity. Although a number of Pythium spp. have been reported as causes of damping-off of onion, this is the first report of Pythium spp. causing root rot of mature onion plants in the field.

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IDENTIFICATION OF RESISTANCE TO ROOT KNOT NEMATODES AND VIRUS DISEASES IN CUCUMIS METULIFERUS AND APPROACHES TO HYBRIDIZATION WITH CUCUMIS SATIVUS BY PROTOPLAST FUSION. Z.K. Punja, F.A. Tang, and L.H. Watkins. Campbell Institute for Research and Technology, Davis, CA 95616

The wild African cucumber (C. metuliferus) PI 292190 (2n=24) and pickling cucumber (C. sativus) cv. Calypso (2n=14) were evaluated for response to inoculation with root knot nematodes (M. incognita) (RKN) and to the viruses ZYMV, WMV-1, WMV-2, and CMV. The extent of galling by RKN was rated on a 0-5 scale (0= no symptoms, 5=mortality) six weeks after inoculation. Virus infection was evaluated by symptomatology and ELISA reactions. C. metuliferus was tolerant to RKN (rating of 2) and resistant to ZYMV and WMV-1, while C. sativus was highly susceptible to all pathogens. Mesophyll protoplasts of both species were obtained by enzymatic digestion of leaf strips, and following purification, were plated onto media to induce regeneration. Fusions were attempted using PEG and high Ca/high pH. Plants have been regenerated from C. sativus protoplasts. The presence of somatic hybrids between the species remains unverified.

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A RAPID AND EFFICIENT TECHNIQUE FOR SCREENING CUCUMBER FRUIT FOR RESISTANCE TO RHIZOCTONIA BELLY ROT CAUSED BY RHIZOCTONIA SOLANI AG-4. L.H. Watkins, Z.K. Punja, and G.E. Tolla. Campbell Institute for Research and Technology, Davis, CA 95616

Belly rot is an important disease in commercial fields in MS, NC, and CA. Fruits in contact with soil develop dry, corky, dark-brown lesions which are restricted to the skin and outer pericarp. Of 76 breeding lines evaluated in MS in 1986, nine had lower field disease ratings than Calypso (susceptible check). A laboratory assay was developed to allow rapid screening of this germplasm for incorporation into a breeding program. Inoculum of two R. solani isolates was produced on a sterile quartz sand:corn meal agar:dextrose:water (168:0.6:0.4:26g) substrate. Twelve g was incorporated into 60g of sterile greenhouse soil and cucumber fruit of varying ages, sizes, and genotypes were placed on the soil. Lesion numbers, size, and percent area infected were recorded after 5 days at 25°C. Infection was greater on young fruit than on older fruit. Genotypes varied in their response to lesion development, and two lines were identified with a high level of resistance.

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INHIBITORY REACTIONS AND VEGETATIVE COMPATIBILITY AMONG Streptomyces CAUSING SCAB ON POTATO. Jennifer M. Lorang and N. A. Anderson, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Eighteen Streptomyces isolates were obtained from lesions on potato tubers or garden beet roots and their pathogenicity was confirmed on potato lead-bud cuttings. These cultures were isolated from pit scab, common scab, russet scab, and from suppressive soils, and came from several geographic locations. Isolates were paired in all possible combinations on regeneration media. Five types of inhibitory reactions were observed. Several pairings resulted in inhibition resembling the lethal zygosis reactions known to occur in other Streptomyces species and are thought to be elicited by exchange of plasmid DNA between strains. Of particular interest are the distinct inhibitions of virulent strains by avirulent strains from suppressive soil. Thus far, heterokaryosis occurred only between auxotrophs generated in isolates from identical geographical locations.

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OCCURRENCE AND PATHOGENICITY OF PYTHIUM MYRIOTYLUM RECOVERED FROM CUCUMIS MELONIS WITH SUDDEN WILT. A. B. Amann and W. D. Gubler, Department of Plant Pathology, University of California, Davis, CA 95616.

During October 1987, Pythium myriotylum was isolated consistently from the roots of melon plants exhibiting sudden wilt in the Imperial Valley of California. P. myriotylum was pathogenic on melon seedlings in the greenhouse. It caused pre-emergence and post-emergence damping off of seedlings. Inoculation of greenhouse grown plants at fruit set resulted in root rot and typical sudden wilt at 10 days preharvest. We conclude that P. myriotylum is capable of causing root rot of mature melons and can contribute to the sudden wilt syndrome of melons in the Imperial Valley of California.

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VINE DECLINES OF MUSKMELONS IN SOUTH TEXAS. M. E. Miller, J. M. Amador, Texas A&M University, Weslaco, TX 78596 and B. D. Bruton, USDA-ARS, Lane, OK 74555

A group of diseases resulting in vine declines of muskmelons caused by Macrophomina phaseolina, Didymella bryoniae, Diaporthe melonis, Botryodiplodia theobromae, Meloidogyne incognita, Rotylenchulus reniformis, and a root rot of unknown etiology have become prevalent in south Texas. Symptoms include yellowing and death of crown leaves and gradual decline of the vine as the plant approaches maturity. Melons from affected plants are more likely to sun-burn, full-slip maturity is hastened, and the fruit are generally softer. Diseases in this group incited by M. phaseolina, D. bryoniae, D. melonis, B. theobromae, are characterized by lesions in the crown. All crown lesions typically produce droplets of amber exudate which dry to a dark brown color. Differences in disease severity have been observed among cultivars infected by D. bryoniae and by the unidentified pathogen involved in the root rot of muskmelon.

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BEAN CALICO MOSAIC VIRUS, A NEWLY DESCRIBED GEMINIVIRUS OF BEAN. J.K. Brown, E. Jimenez-Garcia, and M.R. Nelson. Department of Plant Pathology, Univ. of Arizona, Tucson, and SARH-INIFAP, CIANO, Hermosillo, Mexico.

Bean calico mosaic (BCaM), a disease of bean (Phaseolus vulgaris) occurred in whitefly-infested fields in Sonora, Mexico during 1987-88. A bright yellow and green (calico) foliar mosaic and stunting were observed in 100% of the plants in numerous fields. The agent was transmitted to bean by Bemisia tabaci and by mechanical means under greenhouse conditions. Paired, isometric particles (20x30 nm), characteristic of the Geminivirus group, were observed by TEM in partially purified preparations made from symptomatic bean leaves. A wide band of ssDNA was visualized by EtBr staining following agarose gel electrophoresis of preparations made from infected but not from asymptomatic leaves. The DNA band was sized from Hae III, Hha I, and Hpa II restriction fragments, and was estimated to contain 5145-5425 bases.

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FIELD SCREENING METHODS FOR BROWN STEM ROT RESISTANT SOYBEAN. H. Tachibana, K. G. Bidne, and A. Mengistu. Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

Row and hill plot methods were tested for relative efficacy in screening soybeans for both resistance and high yields since low yield was a major problem in early endeavors to develop resistant cultivars. Known resistant and susceptible cultivars were used. Completely randomized block design was used in 4 replicated plots in 1986 and 5 plots in 1987. Highest yields on high infested plots were those of the resistant cultivars and on low infested plots were those of the susceptible cultivars. Yield vs disease slope values obtained for row and hill plots were b = -2.68 and -0.143, respectively, in 1986. In 1987, the values were -2.10 and -1.04. Results indicate that both row and hill plots methods are effective but high yielding resistant soybeans can be more effectively selected if row plots were used. In addition, the results indicate that the highest yielding soybeans, whether resistant or susceptible, are row or hill specific.

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EFFECTS OF SUPPLEMENTAL IRRIGATION APPLIED BY A LINE SOURCE IRRIGATION SYSTEM ON DIPLODIA STALK ROT DEVELOPMENT IN FIVE MAIZE HYBRIDS. M. L. Carson and Z. W. Wicks, III, Plant Science Department, South Dakota State University, Brookings, SD 57007.

The line source irrigation system applies water in a continuous gradient perpendicular to the system. Rows of the five hybrids were planted perpendicular to the line source system in four replications in 1987, and each row divided into six, ten foot segments. Diplodia stalk rot (DSR) ratings for each segment were regressed on the total supplemental water applied to the midpoint of that segment. Pooled residual sums of squares from the regressions of each hybrid were used to calculate the standard error of the difference between two slopes and an LSD statistic used to compare hybrid slopes. Hybrid DSR responses (slopes) to increased supplemental water varied widely. DSR ratings of Pioneer hybrid 3737 increased only slightly with decreasing supplemental water, whereas those of FR23 X A632 increased dramatically.

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THE INFLUENCE OF TIME OF INVASION ON STEM CANKER SYMPTOM DEVELOPMENT. D. V. Phillips and P. L. Raymer, Departments of Plant Pathology and Agronomy, University of Georgia, Georgia Station, Griffin, GA 30223-1797

Three soybean cultivars GaSoy-17, Coker 237, and G81-2057 were monitored for invasion by Diaporthe phaseolorum var. caulivora by culturing the lowest petiole at 2 week intervals between 4 and 10 weeks after planting (WAP). Disease symptoms were rated weekly for 6 weeks before maturity. In the moderately susceptible cultivar GaSoy-17, plants invaded before 6 WAP developed symptoms sooner than plants invaded at 8 or 10 WAP. More GaSoy-17 plants invaded prior to 6 WAP were dead at maturity than those invaded at 10 WAP. In the susceptible cultivar Coker 237, plants invaded before 6 WAP developed symptoms sooner than plants invaded at 10 WAP, but the number of dead plants at maturity was not significantly different. In the highly susceptible cultivar G81-2057, invasion time did not influence symptom development and over 95% of the plants were dead 3 weeks before maturity.

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ASSOCIATION OF FUSARIUM OXYSPORUM WITH A NEW VIRUS-LIKE DISEASE AFFECTING DELAWARE SOYBEANS. T. Weldekidan, R. B. Carroll and T. A. Evans, Univ. of Delaware, Newark 19717-1303.

Fusarium oxysporum (Fo) has resulted in significant blight of Essex soybean in the Delmarva region. In 1985, a new disease was observed that also affects Essex. Symptoms which begin on the first true leaves include stunting, bunched thickened dark-green leaves, superficial stem cankers and reduced numbers of flowers and pods. In 1987, a soil-borne virus-like agent (VLA) was found associated with this disease and Fo was isolated from a high percentage of field-infected plants. Greenhouse studies were initiated to determine any possible interaction between Fo and the VLA. Essex soybeans that were co-infected showed the greatest root damage. A significant reduction in height and fresh weight occurred when plants were co-infected or infected with VLA alone. No significant differences resulted when varieties with resistance to either Fo or VLA were inoculated. The VLA is the most important component of the association and there was no synergistic effect.

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USE OF FOLIAR APPLICATION OF ZOOSPORES OF PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA TO ASSESS TOLERANCE IN SOYBEAN. M. W. Ferguson, Dept. of Plant Science, South Dakota State University, Brookings, SD 57006

Nine cultivars of soybeans were chosen on the basis of prior knowledge of their field tolerance to Phytophthora root rot. They represented maturity groups I, II, and III with a high, moderate, and low tolerant cultivar within each group. Ten and 21 day old plants were sprayed to run off with a zoospore suspension of 10^4 spores/ml in tap water and misted for 24 hours. Plants were rated at 5 and 10 days. Greenhouse ratings related well with field observations. With 10 day old plants, low tolerant lines were killed or severely stunted while lines with high tolerance showed little foliar damage. Ranking of older plants were similar to younger plants, using a slightly different rating scale. Considerable leaf distortion and some shortening of internodes was noted on the older plants.

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PRODUCTION OF MONOCLONAL AND POLYCLONAL ANTIBODIES AGAINST FUSARIUM SPECIES. E.H. Gendloff and J. Leighton-Sands. Agrigenetics Advanced Science Company, 5649 E. Buckeye Road, Madison, WI 53716.

A soluble aqueous extract of Fusarium moniliforme (Fm) and Fusarium graminearum (Fg) was used in the production of monoclonal and polyclonal antibodies against these fungi. Using indirect ELISA, the polyclonal antisera reacted to all Fusarium spp. tested, and to several other fungi. Monoclonal antibodies were produced which reacted to Fg but not Fm, and Fm but not Fg. An indirect sandwich ELISA was developed using a monoclonal antibody against Fg with the rabbit antisera. This assay was specific for Fg, with no cross-reaction to Fm or several other Fusarium spp. tested. There was also no cross-reaction to Colletotrichum graminicola, Cochliobolus heterostrophus, Sclerotinia sclerotiorum, or any of several other fungi tested. This assay is being used to type corn ear and stalk rot infections.

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A NEW RACE OF SUNFLOWER DOWNY MILDEW IN NORTH AMERICA. A. Liubich, T. J. Gulya, and J. F. Miller. Dept. of Pl Path. and USDA-ARS, North Dakota State University, Fargo, ND 58105

A new race of Plasmopara halstedii was identified during greenhouse experiments in the spring of 1988. The new race, designated as race 5, was initially observed on the sunflower inbred Interstate (IS) 2000. This line is completely resistant to races 1, 2 and 3, and with race 4 systemic infection is limited to the cotyledons. Race 5 consistently caused complete systemic infection on IS 2000. In tests with 50 diverse sunflower selections having resistance to races 1 to 4, three additional genotypes were identified which were completely susceptible to race 5. These included a Dahlgren inbred derived from P.I. 406022, two Interstate lines derived from Novinka, and Yugoslavian selections of crosses with Helianthus tuberosus. The USDA lines HA-335 to HA-340, resistant to downy mildew races 1 to 4, were all resistant to race 5.

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HETEROGENEITY AMONG ISOLATES OF RHIZOCTONIA SOLANI FROM PEANUTS. H. H. Fagbenle and D. A. Brown, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078

Rhizoctonia solani is associated with pod, limb, and crown diseases of peanut (Arachis hypogaea L.). Fifteen isolates of this pathogen were evaluated for colony morphology and growth rates on potato-dextrose agar (PDA), nitrate-dextrose agar (NDA), and NDA + 0.2% lithium chloride. Seven lithium chloride-tolerant isolates were identified. Isolates were compared on the basis of anastomosis using AG testers 1, 2T1, 2T2, 3, and 4. Thirteen isolates were 'bridging isolates' which anastomosed with AG's 2T2 and 4. Isolates of these groups interacted with each other to produce putative heterokaryons on PDA containing 1% charcoal. Comparisons of pathogenicity on cabbage and peanut seedlings were made. Eight isolates were pathogenic on cabbage and peanut (cv. 'Early Bunch').

540

INFLUENCE OF FOLIAR CLIPPING TREATMENTS ON PATHOGENESIS OF CRIMSON CLOVER BY SCLEROTINIA TRIFOLIORUM. R. G. Pratt, USDA, ARS, CSRL, Forage Research Unit, Mississippi State, MS 39762.

Crimson clover (Trifolium incarnatum) was grown in a naturally infested soil, with and without additional sclerotia of Sclerotinia trifoliorum, during four winter growing seasons. Foliar clipping treatments were applied individually and in combinations at three stages in the disease cycle: in November, before most apothecium formation; in January, after most apothecium formation; and in February, before major rotting of foliage. Disease severity and forage yields were determined in April. Environmental conditions favored pathogenesis by S. trifoliorum during three growing seasons. Disease was most consistent and severe in plots with added sclerotia. A single clipping of foliage in November increased yields by 57-79% and reduced disease severity by 44-63% in comparison to unclipped plots. Clipping treatments applied in January and February usually did not increase yields over unclipped plots unless preceded by a clipping in November.

541

STAND REDUCTION, GROWTH SUPPRESSION, AND DECREASED ROOT MASS OF FESCUE GROWN FROM SEED IN SOIL INFESTED WITH COCHLIOBOLUS SATIVUS. L. E. Trevathan, Dept. of Plant Pathology and Weed Science, Mississippi State, MS 39762.

Three varieties of tall fescue (Festuca arundinacea) were grown in the greenhouse in sterile and nonsterile soil mix into which a conidial-mycelial inoculum of Cochliobolus sativus was incorporated from cornmeal-sand cultures or was suspended in water and applied directly to seed. To determine if the fungus was responsible for reduced stands, counts of emerged or established seedlings were made weekly through four weeks. Growth of leaves and shoots was measured at the same intervals from the base of the plant to the tip of the uppermost leaf. At the end of the fourth week, plants were lifted from soil and root dry weights were determined. Stands of Fawn, Kenhy, and Kentucky 31 fescue were reduced when inoculum of C. sativus was applied directly to seed or incorporated into the soil. Additionally, plant growth and root dry weights were significantly reduced. Results were similar for plants grown in sterile or nonsterile soil.

542

SEASONAL INCIDENCE OF LEAFSPOT PATHOGENS OF ALFALFA IN THE MOUNTAIN, PIEDMONT, AND COASTAL PLAIN REGIONS OF NORTH CAROLINA. C. Lee Campbell and Jack E. Bailey, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

To determine the prevalence of four leafspot pathogens of alfalfa in the three regions of NC, 10 stems were selected in three fields/county and mailed to us from 23, 22, and 19 counties in fall 1986, spring 1987, and fall 1987, respectively. Two leaflets/stem were disinfested, incubated in moist chambers at room temp, and pathogens identified. When present, occurrence of each pathogen was similar among regions. Leptosphaerulina spp. (mean incidence [MI]: 1986-48%, 1987-56%) and Cercospora spp. (MI: 1986-82%, 1987-69%) predominated in both fall samples, while Leptosphaerulina spp. (MI: 63%) and Phoma spp. (MI: 41%) predominated in spring 1987. Incidence of Stemphylium spp. was relatively low (MI: fall 1986-24%, spring 1987-19%, fall 1987-3%). Phoma spp. were detected in fall samples and Cercospora spp. were identified in the spring sample; however, incidence of both was very low (<3%).

543

EFFECT OF SIMULATED ACIDIC RAIN ON LEAF RUST AND POWDERY MILDEW OF WHEAT. M. J. Munster, C. L. Campbell, and R. I. Bruck, Dept. of Plant Pathology, NC State Univ., Raleigh 27695-7616.

Simulated rains (SR) at pH 2.8, 3.6, 4.2, 4.8, and 5.6 were applied 2x/wk to small field plots of Tyler wheat in which limited epidemics of leaf rust (Puccinia recondita) and powdery mildew (Erysiphe graminis) were occurring. Most ambient rainfall was excluded. Analysis of variance on disease severity showed some interactions between pH and the linear or quadratic effect of time. Only one interaction could be interpreted: mildew increased more rapidly under rains of pH 2.8, 3.6, and 4.2 (vs. pH 4.8 or 5.6) in the last 7 days of the experiment. In vitro germination of P. recondita urediniospores was strongly inhibited by suspension in SR solutions at pH 2.8 or 3.6. In greenhouse tests, disease efficiency of P. recondita was greatest when spores were suspended in SR solutions at pH 4.2, somewhat less at pH 5.6, and much reduced at pH 2.8. The pH of rains applied 2x/wk in the greenhouse, either after infection or both before and after infection, had little or no effect on disease efficiency or latent period of P. recondita.

544

MODELING THE RECOVERY OF AEROSOLIZED BACTERIA IN FIELD RELEASE TRIALS. G. R. Knudsen, Plant Pathology Division, University of Idaho, Moscow, Idaho 83843.

Risk assessment for genetically engineered bacteria sprayed onto crops includes determination of off-site drift and deposition. To help optimize placement of sampling devices, a particle dispersal model was developed to predict recovery on fallout plates and in air samplers around a test site. The microcomputer simulation incorporates equations (estimated from published literature) for particle size distribution, wind speed and direction, turbulence, evaporation, sedimentation, and mortality, with a time step of 0.5 sec. Simulated dispersal of 10^5 representative particles was compared with reported deposition measurements from three field applications of nonrecombinant bacteria. Predicted distributions compared well with observed data (colony counts).

545

EFFECTS OF OZONE AND ACIDIFIED RAIN ON CARBOHYDRATES IN NEEDLES AND ROOTS OF RED SPRUCE SEEDLINGS. Roy L. Patton and Keith F. Jensen, USDA Forest Service, 359 Main Rd., Delaware, OH 43015.

One-year-old red spruce (Picea rubens Sarg.) seedlings were treated with ozone and acidified rain to determine the effects of these treatments on sugar and starch in foliage and roots. The ozone treatments (CSTR chambers) were (1) carbon-filtered air (CF), (2) CF plus 0.15 ul/l ozone for 6 h/d, and (3) CF plus 0.15 ul/l ozone for 6 h/d plus 0.07 ul/l ozone for 18 h/d. The seedlings were treated once each week with 1.25 cm of rain at pH 3.5, 4.0, or 4.5 (2:1 mixture of H_2SO_4 and HNO_3 in deionized water). After 21 weeks, sugar and starch concentrations in roots and needles were determined. Ozone caused total nonstructural carbohydrates (sugar + starch) to increase in current needles and also increased the proportion of starch in those needles. TNC was higher in roots of plants treated with pH 3.5 rain than in roots of plants treated with pH 4.0 rain. The ozone and rain treatments had no effects on sugar or starch concentrations in 1-year-old needles.

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RESPONSES OF ALFALFA AND WHEAT GROWN NEAR +/-500-kV d-c TRANSMISSION LINES. F.J. Crowe, M.R. Schott, T.D. Bracken, and R.J. Raleigh, Oregon St. Univ. Central Or. Exp. Sta. Redmond OR 97756, Oregon St. Univ. Eastern Or. Ag. Res. Ctr. Burns OR 97720, T.D. Bracken, Inc., Portland OR 97202, and Oregon St. Univ. Eastern Or. Ag. Res. Ctr. Burns OR 97720.

Alfalfa hay and winter wheat were produced for two years near span midpoints of +/-500 d-c transmission lines in central Oregon. Strips 3.66 m-wide extending 61 m in both directions beneath and perpendicular to the conductors were divided into 7.62 m plots. An identical set of control plots were 610 m away. As calculated from on-site measurements, the electric field, ion current and ion density at plant height all decreased rapidly at distances greater than about 15 m outward from conductors. From analysis of variance of data from line vs. control plots and of distance and side-of-line treatments, no consistent statistical differences ($P<0.05$) were found for production, seasonal growth stages or heights, hay or grain quality, or foliage dustiness or infectious disease. Wheat tipburn was statistically greater ($P<0.05$) within 15.2 m of the conductors, but averaged less than 2 mm for all distances at all times. Based on associated lab studies, corona may have occurred on wheat leaf tips and awns near conductors as much as 3-19% of the time from jointing through heading, but corona could not be observed even with an image intensifier because of ambient light, even starlight.

547

ARE INJURY SYMPTOMS ON PINE TREES IN THE EASTERN TRANSVAAL (S.A.) AIR POLLUTION RELATED? A.T. Botha*, D.C.J. Wessels* and L.D. Moore**, *Univ. of Stellenbosch and Univ. of the North, S. Africa, respectively; and VPI&SU, Blacksburg, VA, 24061-0331.

Symptoms associated with air pollution injury were first noted on species in managed pine plantations in the Eastern Transvaal Highveld (ETH) in October, 1987. Predominant symptoms were chlorotic and necrotic mottling, often associated with uniform tip chlorosis or necrosis and ranged from little injury on Pinus taeda and P. eliottii, to moderate and severe injury on P. patula. No biotic agents isolated proved responsible for such symptom expressions. In view of the potential pollutant contributions from industries and power stations in the ETH in association with meteorological patterns of the area, it appears likely that air pollution and acid rain or acid mist could be involved in causing the observed symptoms.

549

INCIDENCE-SEVERITY AND INCIDENCE-YIELD RELATIONSHIPS FOR STEWART'S AND GOSS' BACTERIAL WILTS OF SWEET CORN. Suparyono and J. K. Pataky, Department of Plant Pathology, University of Illinois, Urbana 61801.

Incidence-severity and incidence-yield relationships for Stewart's and Goss' wilts were studied in factorial experiments with sweet corn hybrids, pathogens, and disease incidence as main factors. Hybrids, disease incidence, and the hybrid by incidence interaction were significant. Incidence-severity$^{1/2}$ relationships were linear for Miracle (R) and Gold Cup (MR) and were curvilinear for Jubilee (S). At 2-3 wks after inoculation at the 5-7 leaf stage, severity (measured per plot) on Miracle, Gold Cup, and Jubilee was 2, 2, and 6% in plots with 50% incidence and 3, 5, and 61%, in plots with 100% incidence. Severity on Jubilee at 2-3 wks after inoculation at the 3-5 leaf stage was 6 and 36% in plots with 50 and 100% incidence. Yield of Jubilee (weight and marketable ears) decreased about 1.5% for each 10% incidence when inoculation was at the 5-7 leaf stage, and about 5 and 3% (weight and ears) for each 10% incidence at the 3-5 leaf stage.

550

EVALUATION OF SWEET POTATO CULTIVARS AND BREEDING CLONES FOR LEAF BLIGHT (PHYLLOSTICTA BATATAS) REACTION. P. D. Dukes, M. G. Hamilton and Alfred Jones. ARS, USDA, U. S. Vegetable Laboratory, Charleston, SC 29414 and Clemson Univ. Edisto R&E Center, Blackville, SC 29817.

Field evaluations for reaction of sweet potato cultivars and breeding clones to leaf blight incited by Phyllosticta batatas were conducted annually. Annual natural infection by the pathogen was generally consistent and uniform across many replicated experiments and plots of seedlings. A blight index (BI) of 0-10 was used in which "0" equaled no blight and up the scale of reactions to "10" which equaled complete defoliation. Jewel, the most popular cultivar, was used as the disease standard (moderate resistance) that averaged 4 on the BI scale. BI data for many cultivars, breeding clones and other entries were collected. While several older well-known cultivars had lower BI's than Jewel, only one new cultivar, Excel, had a BI of 0 in all plots. Seedlings with a BI larger than 4 were discarded from our breeding program in 1987.

551

AMBIENT TEMPERATURE, RELATIVE HUMIDITY AND PLANT AGE IN RELATION TO LEVEILLULA TAURICA ONSET ON TOMATO. D. G. Kontaxis, University of California, 1700 Oak Park Blvd., Bldg. A2, Pleasant Hill, CA. 94523.

In central California ambient temperature, relative humidity, plant age, and disease onset were monitored in tomato fields for four consecutive years. Climatological data were obtained by weather stations located 0.5 to 1.5 km, away from the tomato fields and/or hygrothermographs placed under the leaf canopy. Each year symptoms of powdery mildew appeared 5

to 8 days after a significant increase in average daily temperature from a low of 18 C. to a high of 29 C. and a simultaneous drop of daily relative humidity from average 86% to average 12%. Symptoms appeared only in fields where the leaf canopy had completely covered the beds. These conditions coupled with mature-fruiting plants triggered symptom expression rather than infection initiation.

552

CHARACTERIZATION OF ISOLATES OF PHYTOPHTHORA CAPSICI FROM NORTH CAROLINA PEPPER FIELDS. J. Beagle-Ristaino, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

Phytophthora capsici was isolated from diseased peppers grown in two fields in eastern and western North Carolina. All isolates were virulent on pepper in greenhouse studies. Linear growth of isolates on cornmeal agar was optimum between 28 and 30 C, and limited growth occurred at 36 C. Mean sporangium lengths ranged from 38.9 to 57.0 µm and widths ranged from 27.5 to 38.0 µm. Sporangium length-breadth (LB) ratios ranged from 1.35 to 1.66 and two significantly different LB ratio groups were evident. None of the isolates produced chlamydospores in V-8 broth medium. Compatibility types were analyzed by crossing isolates with known tester strains of P. capsici. Both A1 and A2 types were present in one field, while all isolates from a second field were type A1. Results confirm that P. capsici is present in North Carolina pepper fields and that oospores may be involved in the overwintering and development of Phytophthora root and crown rot in some fields.

553

IN VITRO TRANSCRIBED RNA TO DETECT SWEET POTATO FEATHERY MOTTLE VIRUS. J. A. Abad and J. W. Moyer. Dept. of Plant Pathology, N. C. State Univ., Raleigh, NC 27695-7616.

In vitro transcribed RNA provides a highly sensitive probe for sweet potato feathery mottle virus (SPFMV) relative to probes using nick translated cDNA from the same clone. The RNA probe was synthesized from a 900 bp SPFMV-cDNA and cloned in an in vitro transcription plasmid, pGem 4-Z (Promega). The probe hybridized with single and double stranded SPFMV-RNA. Plus sense SPFMV-RNA was identified in total nucleic acid extracts from several SPFMV infected Ipomoea spp. including sweet potato. Detection was influenced by virus distribution in host tissues and by the presence of substances such as latex and quinones released from host cells during extraction. Suitability of several nucleic acid extraction methods were compared. Addition of cetyltrimetylamonium bromide (C-TAB) and high salt to various extraction buffers improved the sensitivity of detection by reducing background signal from extracts of 'healthy' plants.

554

ETIOLOGY OF PITH NECROSIS IN FIELD GROWN TRELLISED TOMATOES IN WESTERN NORTH CAROLINA. N.B. Carrol, E. Echandi, and P.B. Shoemaker. Department of Plant Pathology. North Carolina State University, Raleigh, NC 27695-7616.

Field surveys were made during 2 years in western North Carolina to determine incidence and the causal agent of tomato pith necrosis. Occurrence was sporadic and the incidence ranged from 0-11%. Bacteria isolated from necrotic pith of plants in the survey were identified as Pseudomonas corrugata Roberts & Scarlett (Pc) based on morphological, physiological, biochemical and pathogenicity comparisons with the type strain. Isolations yielded predominantly Pc but Erwinia carotovora subsp. carotovora was also present. Inoculations on tomato with Pc isolates from the survey produced typical disease symptoms in tomato plants, including stem lesions, vascular browning, necrotic or hollowed pith, and adventitious roots.

555

A FOLIAR DISEASE ON LITTLE BLUESTEM. J. M. Krupinsky and D. A. Tober, USDA, ARS, Northern Great Plains Research Laboratory, P. O. Box 459, Mandan, ND 58554, and USDA, SCS, Bismarck Plant Materials Center, Bismarck, ND 58502.

A foliar disease was found to be widespread in a little bluestem (Andropogon scoparius Michx.) nursery of plants collected from North Dakota, South Dakota, and Minnesota. In 1984 through 1986, an unknown fungus was consistently isolated from infected leaves. In 1986 the fungus was also isolated from little bluestem plants in a native prairie, from big bluestem (A. gerardi Vitman) plants in a nursery and prairie, and from sand bluestem (A. hallii Hack.) plants in a nursery. The fungus was pathogenic to little bluestem, big bluestem, and sand bluestem in several glasshouse inoculations. Little bluestem isolates varied in aggressiveness when tested on little bluestem plants. The fungus appears to be Phyllosticta andropogonivora Sprague and Rogerson which was described on big bluestem plants.

556

DETERMINATION OF SPORE GERMINATION RATE AND FUNGAL GROWTH IN RESPONSE TO MEDIA SUPPLEMENTS. H. E. Moline, USDA, ARS, Hort Crops Quality Lab, Beltsville, MD 20705.

Parameters were established for minimal substrate and growth factor requirements for the study of germination and growth of fungal spores. Twenty-four well tissue culture plates were used for testing compounds. This allowed the testing of a ug or less in 0.5 ml of medium. Measurements were made using a Bioquant image analysis system attached to a Leitz compound microscope. Where fungal mat weights were needed, spores were placed in dialysis membrane bags and suspended in test media in 50 ml Erlenmeyer flasks containing 10 ml media. At the end of the experiment, fungal mats were washed from the bags and dry weights taken. Botrytis cinerea and several other postharvest pathogens were excellent candidates for study, responding to growth stimulants and inhibitors.

557

EFFECTS OF SELECTED COTTON-LEAF DERIVED VOLATILES ON THE PRODUCTION OF AFLATOXIN IN CULTURES OF ASPERGILLUS FLAVUS. H. J. Zeringue, Jr.,[1] and S. P. McCormick[2]. [1]USDA/ARS, SRRC, 1100 Robert E. Lee, Blvd., New Orleans, Louisiana 70179, and [2]USDA/ARS, NRRC, 1815 N. University St., Peoria, IL 61604.

Volatile organic compounds emitted from the cotton leaves at 2- and 7-day test periods were trapped on Tenax and were tentatively identified using capillary gas chromatography/mass spectrometry after thermal desorption from the adsorbent. Aspergillus flavus, in solid culture, was exposed to individual selected, purified volatile cotton leaf-derived components and the growth pattern of A. flavus and the production of aflatoxin were determined. One of the most bioactive compounds was 3-methyl-2-butanol which inhibited A. flavus growth by 20% but increased production of aflatoxin B_1 by twofold. Other relationships of growth and aflatoxin production resulting from exposure to the bioactive volatiles will be demonstrated.

558

HEAT SHOCK INDUCTION OF TELIOSPORE GERMINATION IN Puccinia graminis. C. A. Griffey and A. P. Roelfs, USDA/ARS Cereal Rust Laboratory, St. Paul, MN 55108.

Teliospores of three asexual and two sexual rust cultures with no winter exposure were plated on 2% water agar and heat shocked in a water bath at 31 to 32 C for 3 days. Germination generally commenced after 3 to 4 days at 18 to 20 C. Variability in germination among teliospores of diverse sources e.g. seedling versus adult plant, controlled versus field production, and cool versus warm growing regimes may reflect variability in teliospore maturity rather than differences in response to heat shock. Teliospores produced in the field were heat shocked for 0 to 5 days. The maximum germination obtained was 19%, after a 2 day exposure period, while 1.3% of the untreated teliospores germinated. Exposure periods exceeding 2 days resulted in decreased germination. The optimum exposure period may vary with teliospore source. Pycnia were produced on barberry bushes which had been exposed to basidiospores from heat shocked telia.

559

EFFECT OF SUPPLEMENTAL APPLE CELL WALL CALCIUM ON DECAY BY BOTRYTIS CINEREA. W. S. Conway and K. C. Gross, USDA, ARS, Hort Crops Quality Lab, Beltsville, MD 20705, C. D. Boyer, Penn State Univ., University Park, PA 16802, and C. E. Sams, Univ. of Tennessee, Knoxville, TN 37996.

To determine if increasing the apple cell wall calcium content decreases decay caused by Botrytis cinerea as it does with that caused by Penicillium expansum, fruit were pressure infiltrated at harvest with solutions of $CaCl_2$ and stored at 0 C for 6

months. Fruit were then inoculated with a conidial suspension of B. cinerea. Polygalacturonase was purified from the decayed area of nontreated fruit which had been inoculated with B. cinerea. Increasing the calcium content of apple cell walls was found to be even more effective in reducing decay caused by B. cinerea than that caused by P. expansum. Polygalacturonase produced by B. cinerea differed somewhat from that produced by P. expansum and may be, in part, the reason that increased calcium affords the cell wall greater protection from maceration by B. cinerea than by P. expansum.

560

EFFECT OF NEMATODE INOCULUM LEVEL ON POPULATION DEVELOPMENT OF HETERODERA GLYCINES AND MACROPHOMINA PHASEOLINA. T.C. Todd and C.A.S. Pearson, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506.

Pasteurized Sarpy loamy sand, placed in PVC tubes (25 cm dia x 76 cm), was infested with four levels of Heterodera glycines (0, 10^2, 10^3, and 10^4) and two levels of Macrophomina phaseolina (-, +). Root densities of the nematode and the fungus were measured at soybean growth stage R6 (full pod) on the cultivars Williams 82 and Pella. On Williams 82, fungal root colonization was positively correlated with initial nematode populations (r=0.56) and with final nematode densities (r=0.65). On Pella, simple linear relationships were not observed. Fungal root colonization was enhanced approximately ten-fold in the presence of H. glycines, regardless of nematode inoculum level. Higher root densities of H. glycines were observed in the presence of the fungus at intermediate levels of the nematode, but not at the highest level.

561

INTACT SOIL PROFILES USED AS MICROPLOTS TO ASSESS SOYBEAN YIELD LOSSES DUE TO CHARCOAL ROT. C.A.S. Pearson[1], F.W. Schwenk[1], and C.W. Swallow[2], Departments of [1]Plant Pathology and [2]Agronomy, Throckmorton Hall, Kansas State University, Manhattan, KS 66506.

A hydraulically-operated soil coring machine was used to extract profiles (25 cm diam x 76 cm) from a Parsons silty loam soil. Two-thirds of the profiles were pasteurized at 75 C for 4 hr; the remaining one-third were not heat treated. Five isolates of Macrophomina phaseolina, grown on sterile oats, were mixed together and incorporated into the top 10 cm of one-half of the heated microplots. Root colonization by M. phaseolina and yields of four soybean cultivars (DeSoto, Douglas, Franklin, and Union) were measured under greenhouse conditions. All four soybean genotypes responded similarly. Plants grown in reinfested and naturally infested profiles yielded 47% and 67% less, respectively, than plants grown in pasteurized microplots. The root densities of M. phaseolina from plants grown in reinfested and naturally infested microplots were not significantly different (P=0.05).

562 Withdrawn

563

DETECTION OF VIRUSLIKE PARTICLES ASSOCIATED WITH ESCAROLE NECROSIS. R.N. Raid, U. of Florida, Belle Glade, FL 33430; L.L. McDaniel and J.H. Tsai, Ft. Lauderdale, FL 33314.

A mechanically-transmissible quasi-spherical viruslike particle (VLP) was identified as the causal agent of a necrotic leaf disease of escarole in Florida. Endive exhibited symptoms similar to those of escarole, with newly formed leaves turning brown and necrotic, resulting in plant death. VLP's in plant sap and a partially purified preparation were slightly pleomorphic (diam. 27 nm ± 0.6). A polyclonal antiserum was developed, using a partially purified preparation of the escarole necrosis VLP (EN-VLP), for use in an indirect ELISA. ELISA tests indicated the EN-VLP is not serologically related to other lettuce viruses known to occur in Florida. The EN-VLP contained RNA's of 4 different sizes and had a capsid subunit Mr of approximately 27 kd suggesting a relationship with members of the ilarvirus group. Disease incidence appeared to be highest in the fall crop with less than one percent of the crop exhibiting visual symptoms.

564

SEROLOGICAL TESTING OF POTATO LEAFROLL DISEASE SAMPLES USING MONOCLONAL ANTIBODIES SPECIFIC FOR BEET WESTERN YELLOWS VIRUS (BWYV) AND POTATO LEAFROLL VIRUS (PLRV). P.J. Ellis and A. Wieczorek, Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B. C. V6T 1X2.

Murine monoclonal antibodies (MCA) prepared against BWYV and PLRV reacted strongly with the homologous virus but exhibited no cross-reactivity when used in heterologous tests. Assays were done using a triple antibody sandwich ELISA procedure; polyclonal antibodies for coating, MCA as the second antibody, and rabbit anti-mouse alkaline phosphatase as the conjugate. A total of 771 potato plants with symptoms of potato leafroll disease, representing 26 cultivars, were tested from 1986 to 1988. Samples were collected from 6 Canadian provinces and 12 states. None of the samples tested positive for BWYV whereas 754 tested positive for PLRV. Seventeen samples tested negative for both viruses; neither BWYV nor PLRV could be recovered from those samples in aphid transmission tests.

565

THE OCCURRENCE OF A COMPLEX OF VIRUSES ASSOCIATED WITH RHIZOMANIA OF SUGARBEET. Hsing-Yeh Liu and James E. Duffus, USDA-ARS, 1636 East Alisal St., Salinas, CA 93905.

Three distinct viral pathogens similar in particle morphology to beet necrotic yellow vein virus (BNYVV), the causal agent of rhizomania of sugarbeet, have been isolated from rhizomania infested fields in California and Texas. These BNYVV-like viruses are vectored by Polymyxa betae Keskin. However, these isolates are distinct from BNYVV in symptom expression, host range, and serology. The distribution of these viruses in the field, their economic importance, and the relationship of these entities to the rhizomania disease of sugarbeet are not yet known.

566

NATURE OF RESISTANCE IN WINTER WHEAT TO BARLEY YELLOW DWARF VIRUS INFECTION AND TRANSMISSION. S.M. Gray, D.M. Smith, and M. Yang. USDA/ARS, Cornell University, Ithaca, NY 14853

The identification of resistance and/or tolerance in winter wheat to barley yellow dwarf is based on symptoms and yield response in field trials. The nature of the resistance is rarely characterized and effects of the putative resistance on epidemiology of the disease are unknown. Partial resistance reported in the cultivars, 'Elmo', 'Caldwell', and 'Auburn', was assessed with respect to effects on virus titer and distribution and aphid transmission. Overall virus titer was similar among cultivars, but significant differences in virus titer and distribution existed when individual leaves and roots were tested at various times after inoculation. Virus titer in 'Elmo' was reduced and virus distribution altered if infection occurred after vernalization. Aphid acquisition and inoculation efficiency were lower in 'Caldwell' and 'Auburn' relative to 'Elmo' or a susceptible cultivar, 'Geneva'. The epidemiological effects of these types of resistance are currently being investigated.

567

IDENTIFICATION AND DISTRIBUTION OF SOYBEAN VIRUSES IN TENNESSEE. B. S. Kennedy and B. B. Reddick. P.O. Box 1071, Knoxville, TN 37901-1071.

Commercial soybean fields from nineteen counties in east, middle, and west Tennessee were surveyed for virus infection in 1987 and 1988. Virus isolates were identified by Protein A Sandwich ELISA. Samples were tested with antisera against alfalfa mosaic (AMV), bean pod mottle (BPMV), bean yellow mosaic (BYMV), cowpea chlorotic mottle (CCMV), cucumber mosaic (CMV), peanut mottle (PMV), peanut stunt (PSV), southern bean mosaic (SBMV), soybean mosaic (SMV), tobacco ringspot (TRSV), tomato spotted wilt (TSWV), and white clover mosaic (WCMV) viruses. Disease incidence ranged from less than 1% to no higher than 10% in most fields sampled. AMV, BPMV, BYMV, CCMV, CMV, SBMV, SMV, TRSV, and TSWV have been detected thus far. Yield reduction due to infection of soybeans with BPMV and SMV will be presented.

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SUBTERRANEAN CLOVER RED LEAF (SOYBEAN DWARF)-LIKE LUTEOVIRUS FOUND IN MISSISSIPPI. M. R. McLaughlin, V. D. Damsteegt, J. E. Duffus, and A. D. Hewings. USDA, ARS, Mississippi State, MS 39762, Frederick, MD 21701, Salinas, CA 93915, and Urbana, IL 61801.

In April 1987, subterranean clover plants, Trifolium subterraneum cv. Meteora, with red leaf margins were collected in the field at Mississippi State, MS. Sap extracts of these plants reacted positively in ELISA with antisera to a Japanese isolate of soybean dwarf virus (SDV) and a California isolate of subterranean clover red leaf virus (SCRLV). The Mississippi isolates were transmitted persistently by Acyrthosiphon pisum, but not by Aulacorthum solani or mechanical inoculation. The isolates induced red leaf symptoms in indicator hosts, T. subterraneum cvs. Geraldton and Mt. Barker. The serology, transmission, and host symptoms, of these isolates appear similar to SCRLV strains of SDV.

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ULTRASTRUCTURAL ASPECTS OF ABUTILON MOSAIC VIRUS. Vania V.B.de Souza and K.S. Kim. Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Variegated Abutilon striatum plants that have been propagated vegetatively in the USA for many years were studied to investigate whether the variegation was caused by abutilon mosaic virus (AbMV), which occurs naturally in South and Central America and Southern USA. Ultrastructural studies revealed the presence of virus-like particles, approximately 15 nm in diameter, and cytopathic effects similar to those of AbMV and other geminivirus infections. The virus was transmitted only by grafting to A. pictum, Malva parviflora, and Sida rhombifolia. Transmission attempts with Bemisia tabaci, the natural vector, were negative. Virus particles occurred only in nuclei of phloem parenchyma cells and in sieve tubes in all three hosts, often in crystalline arrays. The arrangement of particles in tubular structures was different from that observed in malvaceous hosts infected with a Brazilian isolate of AbMV. This virus is considered to be a different strain of AbMV.

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SOME PROPERTIES AND CYTOPATHOLOGY OF A TYMOVIRUS ISOLATED FROM EGGPLANT. V. de Souza, R.C. Gergerich, H.A. Scott, and K.S. Kim. Dept. Plant Path., U of Arkansas, Fayetteville, AR 72701.

An isometric virus measuring 28 nm was isolated from eggplant (Solanum melongena) and mechanically transmitted to Lycopersicon esculentum, Chenopodium quinoa, C. amaranticolor, Petunia hybrida, Nicotiana rustica, N. glutinosa, N. clevelandii, Gomphrena globosa, and Datura stramonium, but not to Sida rhombifolia or Solanum tuberosum. Chloroplasts of infected N. rustica and S. melongena were clustered together and formed tymovirus characteristic double membrane-bound vesicles. Virus purified from N. rustica formed two bands in CsCl and sucrose gradients. SDS-PAGE of the coat protein showed a single polypeptide of about 24.2 kDa. Serologically the virus is related to eggplant mosaic virus (EMV) and tomato white necrosis virus, but not to turnip yellow mosaic, passionfruit yellow mosaic, or desmodium yellow mottle viruses. This virus is related to but differs from previously described members of the EMV subgroup of tymoviruses.

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COMPARISON OF WHEAT STREAK MOSAIC VIRUS (WSMV) ISOLATES IN WHEAT DIFFERING IN REACTION TO WSMV. D. L. Seifers. Fort Hays Branch Agricultural Experiment Station, Hays, Kansas, 67601.

The effect of WSMV isolates on wheat cultivars differing in reaction to WSMV has not been previously assessed. This study was conducted to determine some effects of WSMV isolates on wheat cultivars differing in reaction to WSMV. Virus effects were assessed using: symptom expression, chlorophyll content, relative virus titer, and dry weight. The WSMV isolates caused symptoms of varying severity. All WSMV isolates caused significant decreases in chlorophyll content in all cultivars, but differences in isolate performance resulted bewteen cultivars. Significant differences in titer of isolates was evident within a cultivar and some isolates varied in titer between cultivars. Dry wt of infected plant tissue, within a cultivar, varied with virus isolate and some isolates varied in response between cultivars. These findings indicated that some WSMV isolates differed in their effect on a specific wheat cultivar and the response of an isolate may vary with cultivar.

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ACCURATE DETERMINATION OF PLANT RESISTANCE TO BEETLE-TRANSMITTED VIRUSES. R.C. Gergerich, H.A. Scott, M.C. Langham, and Z. Lin. Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Two comoviruses, cowpea mosaic (CPMV) and cowpea severe mosaic (CSMV), are readily mechanically transmissible to Vigna unguiculata (L.) Walp. subsp. unguiculata 'Monarch' and Phaseolus vulgaris L. 'Black Valentine'. Leaf-feeding beetles, however, rarely transmit either virus to bean, while both viruses are readily transmitted by beetles to cowpea. When an inoculation technique which mimics beetle feeding was used to inoculate bean and cowpea with purified CPMV or CSMV mixed with beetle regurgitant, the results were the same as with beetle transmission. We propose that screening for resistance to beetle-transmitted viruses should be done using an inoculation technique which simulates beetle feeding and gives the same transmission results as the natural vector. Resistance which is inapparent using standard mechanical inoculation is detectable using this procedure.

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DETECTION OF VIRAL RNA IN MEALYBUGS ASSOCIATED WITH MEALYBUG-WILT OF PINEAPPLE. U. B. Gunasinghe and T. L. German, Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822.

The association of double-stranded RNA (dsRNA) and a flexuous rod shaped virus with mealybug affected pineapple has been reported (Gunasinghe and German, 1987. Phytophathology 76: 1073). We have developed a nucleic acid probe to investigate the association of virus with mealybugs (Pseudococcus spp.). dsRNA was isolated from diseased pineapple plants, treated with RNAse A in presence of 2X SSC to digest ssRNA. After phenol extraction and ethanol precipitation the dsRNA was denatured and used as a template for cDNA synthesis using reverse transcriptase, random primers and ^{32}p labeled dCTP. This cDNA was used in dot blot assay to screen for the presence of virus in pineapple plants and mealybugs. Mealybugs collected from bermuda grass and wilt infected pineapple plants reacted positively to the probe. The possible role of mealybugs in transmitting this virus from weed hosts to pineapple is being investigated.

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ELYMUS SP. AS SOURCES OF RESISTANCE TO BARLEY YELLOW DWARF VIRUS. Michael C. Edwards, Roland G. Timian, and Leonard R. Joppa, USDA-ARS Cereal Crops Research, Northern Crop Science Laboratory, North Dakota State University, Fargo, ND 58105.

Although some degree of tolerance to BYDV has been incorporated into the major cereals, a need exists for better sources of resistance. We have evaluated twelve accessions of Elymus canadensis (Canada wild-rye) from at least ten different locations for resistance to BYDV using standard aphid inoculation techniques. A total of 83 plants were assayed for systemic infection and in no instance was any BYDV detectable by either bioassay or ELISA. In only 3 instances were Black Hulless barley checks (to which aphids had been transferred after feeding on the E. canadensis plants) not infected by BYDV. In some experiments, aphids were confined to a limited area of one leaf and that portion of the leaf was later tested via ELISA for evidence of local infection. All results were again negative. Twenty-six plants of Elymus mollis were also evaluated. Only one plant became infected.

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PROPAGATION OF SOYBEAN MOSAIC VIRUS IN SOYBEAN CALLUS CULTURE. P. Chen[1] G. R. Buss[1] and S. A. Tolin[2]. Department of Agronomy[1], and Plant Path., Physiol. & Weed Science[2], Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

The activity and longevity of soybean mosaic virus (SMV) in soybean callus cultures was investigated with eleven SMV cultures from eight strain groups distinguished by differential reactions on soybean cultivars. Calli initiated from hypocotyl tissue cultured in Msoy medium were inoculated with SMV directly by pin-pricking calli soaked in viral inoculum. Infected calli were also established by direct culture of SMV-infected leaves on either Msoy or MS agar medium. Established SMV-callus cultures were maintained under 16 hr photoperiod, subcultured to fresh media as necessary, and assayed periodically for virus. At 10-15C, calli and SMV were active for 16 wks or longer without subculture. Virus titers were comparable with those in infected leaves. Biological integrity of the cultures was maintained. The method is of value for preserving a collection of virus strains in a highly infectious and readily available form and reduces the chance of contamination or loss in viability.

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SOME PROPERTIES OF POTATO VIRUS X SPECIFIC MONOCLONAL ANTIBODIES AND THE HYBRIDOMA CELL LINES. X. W. Xiao, J. Gon, S. H. Cai, J. Liu, L. X. Feng, and H. T. Hsu*, Chinese Academy of Agricultural Sciences, Beijing, and *USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, Maryland 20705.

Seven hybridoma cell lines secreting monoclonal antibodies (McAbs) against potato virus X (PVX) were established by fusing mouse myeloma cells (Sp2/0) with splenocytes of PVX-immunized BALB/c mice. One of the seven hybridoma cell lines secretes McAbs reacting specifically to a common strain of PVX, two secret McAbs reacting to a mild strain and the remaining four secrete McAbs reacting to all of the five strains tested. Three hybridoma cell lines, namely 1-1H5, 1-4C6, and 2-2A7, all secreting IgG3 immunoglobulins were selected and their antibodies were examined. The McAb titers of ascitic fluids were about 300 times that of rabbit antisera measured by indirect ELISA. The reactivities of McAbs were not affected significantly by freeze-drying, repeatedly freezing-thawing, or precipitating with ammonium sulphate.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO BEAN COMMON MOSAIC VIRUS (BCMV). A W Millar, R Surgeoner and P R Mills, Department of Mycology and Plant Pathology. The Queen's University of Belfast, Northern Ireland. BT9 5PX.

Stable hybridoma cell lines secreting monoclonal antibodies against BCMV were produced by fusing spleen cells of mice immunized with a purified preparation of strain NL3 to mouse myeloma cell line P3x63-Ag8.653. One hundred and thirty out of 327 hybridomas secreted antibody specific for NL3. Twenty-eight hybridomas were chosen for further characterization, of which eight were specific for NL3 alone; in ELISA the remaining twenty reacted with both NL3 and the non-necrotic strain NL4. The twenty-eight cell lines have been cloned from single cells obtained by limited dilution and ascitic fluid produced by injection of clones into primed BALB/c mice. Studies are in progress, using these and monoclonals against other strains, to determine the relationship between the eight Netherland BCMV strains (NL1 to NL8).

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PURIFICATION AND MOLECULAR PROPERTIES OF THE TOXIN CODED BY USTILAGO MAYDIS VIRUS P4. C. Ganesa, Y. Chang, W.H. Flurkey, and R.F. Bozarth, and Z. Randawa*. Indiana State University and Pitman-Moore, Inc.* Terre Haute, IN 47809

After growing the fungus to log phase in Ustilago complete medium, the toxin from the clarified culture medium was acetone precipitated and resuspended in 0.50 mM sodium phosphate buffer pH 7 and further purified by passing through CM Sephadex in 25 mM sodium acetate buffer pH 5.5, Sephadex G-50, and G-25. Toxin activity was monitored at each step by assaying on a lawn of sensitive U. maydis strain P2. The purified toxin eluted as a symmetrical band when analyzed by HPLC on W-Porex GP-300. Native toxin produced a single band at approximately 20-25K when analyzed by non-denaturing PAGE and 12.5K when analyzed on SDS-PAGE. These data suggest that the toxin may be composed of two identical subunits. Amino acid analysis indicated that aspartic, glutamic, serine, and threonine accounted for 40% of the AA residues.

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MAIZE NECROTIC LESION VIRUS (MNLV) AND SATELLITE-LIKE PARTICLES O. E. Bradfute and R. Louie*, Dept of Plant Pathology, The Ohio State Univ., OARDC, *(USDA, ARS), Wooster, OH 44691.

MNLV, a newly found soil-borne virus, infects roots of maize (Zea mays L.). Symptoms on rub-inoculated leaves of maize seedlings first appear as chlorotic local lesions that become necrotic after 24-36 hr. Many isometric virus-like particles, ca. 17 and 29 nm in diameter (suggestive of a coinfection of satellite and helper viruses), were found in crude extracts from lesions and in mesophyll cells in thin sections of fixed and embedded chlorotic lesions. Limitation of virus replication to mesophyll cells may account for localized symptoms. Particles of both sizes were located near to each other and to masses of amorphous inclusions and fibril-containing vesicles in the cytoplasm. Close proximity of particles of both sizes at the apparent site of virus replication is consistent with the dependent nature of satellite virus replication. These particles and inclusions were similar to those reported for tobacco necrosis virus and its satellite virus.

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ULTRASTRUCTURE OF TOBACCO LEAF CELLS MIXEDLY INFECTED WITH TOBACCO MOSAIC VIRUS AND ITS SATELLITE VIRUS. K.S. Kim, R.A. Valverde and J.A. Dodds. Depts. of Plant Path., U of A, Fayetteville, AR 72701; LSU, Baton Rouge, LA 70803; and U of C, Riverside, CA 72521, respectively.

Ultrastructural responses of tobacco leaf cells infected with tobacco mosaic virus (TMV) and its satellite virus (STMV) were studied in situ. In cells infected with TMV alone, crystalline arrays of TMV particles and characteristic X-bodies were present in the cytoplasm. In cells infected with both viruses: (1) particles of both viruses occurred in the same cells, (2) STMV particles occurred in either random arrangement or crystalline arrays, (3) unlike TMV particles, STMV aggregates were bounded by a membrane which was also associated with vesicles of 50-80 nm in diameter, (4) no X-bodies were present, and (5) mitochondria possessed abnormal tubular structures. Although STMV occurs in the same cells as its helper virus, its replication and accumulation sites appear to be independent and it induces cytopathic effects that are not induced by TMV alone.

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STRUCTURAL PROTEINS OF SORGHUM CHLOROTIC SPOT VIRUS ATTACH TO CYLINDRICAL INCLUSIONS OF POTATO VIRUS Y IN DOUBLY INFECTED CELLS. W. G. Langenberg[1], S. A. Lommel[2], and T. L. Kendall[3]. USDA/ARS, Dept. of Plant Pathology, University of Nebraska, Lincoln, 68583[1] and Dept. of Plant Pathology, North Carolina State University, Raleigh, 27695[2,3].

Sorghum chlorotic spot virus (SCSV) is a sorghum and corn-infecting furovirus. It is morphologically similar to soil-borne wheat mosaic virus (SBWMV). Potato virus Y (PYV) induces in infected host cells cylindrical (CI) and pinwheel (PW) inclusions typical of viruses in the potato virus Y group. SCSV and SBWMV do not induce CI or PW inclusions. SCSV and PVY can doubly infect Nicotiana benthamiana. In cells infected by both SCSV and PVY, immunogold label showed CI of PVY with attached SCSV structural protein. In cells singly infected by PVY, antibody to SCSV protein did not immunolabel CI. We now show that the structural proteins of both SCSV and SBWMV have the property of attaching to CI of potyviruses.

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CHARACTERIZATION OF MONTANA BARLEY YELLOW DWARF VIRUS ISOLATES TRANSMITTED BY CORN LEAF APHIDS. S.K. Zaske and T.W. Carroll. Plant Pathology, Montana State University, Bozeman, MT 59717.

Three Montana (MT) BYDV isolates vectored by corn leaf aphids were characterized by aphid transmission and/or DAS-ELISA tests. New York (NY) biotypes of Rhopalosiphum maidis (RM), Schizaphis graminum, R. padi (RP), and Macrosiphum avenae (MA) were used with emphasis on transmission by 2n=10 and 2n=8 karyotypes of MT-RM found on barley and corn, respectively. Both karotypes vectored all MT isolates but varied in transmission efficiency. The Valier (V) isolate was transmitted equally well by NY-RM and NY-SG, while the Ft. Ellis (FE) and Chouteau (C) isolates were transmitted less efficiently by NY-SG. Low levels of V and C isolate transmissions occurred with NY-RP. No isolates were transmitted by NY-MA. ELISA tests revealed that the V and C isolates were antigenically similar to NY-RMV. Attempts to serologically characterize the FE isolate were unsuccessful. None of the isolates showed any reaction with antisera to NY-PAV, NY-RPV or NY-MAV.

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MIXED INFECTION WITH PEA MOSAIC VIRUS ALLOWS A NORMALLY NON-APHID-TRANSMISSIBLE ISOLATE OF BEAN YELLOW MOSAIC VIRUS TO BE TRANSMITTED BY APHIDS. H.A. Hobbs and M.R. McLaughlin, USDA-ARS, Crop Sci. Res. Lab., Forage Unit, Mississippi State,MS 39762.

Aphis craccivora transmitted bean yellow mosaic virus-Scott (BYMV-S) from mixed infections with pea mosaic virus (PMV, synonym BYMV-KY204-1), but not from single infections. Transmissions were with 10 aphids per plant and Pisum sativum cv. Dwarf Gray Sugar as acquisition and test host. BYMV-S was transmitted less than half as often as PMV. Highest frequencies of BYMV-S transmission were from acquisition hosts mechanically inoculated with BYMV-S followed by PMV 6-7 days later, and aphid acquisition feeding 9-12 days after PMV inoculation. Transmissions were verified by DAS-ELISA. Tests are underway to determine if aphid transmission of BYMV-S depends on PMV-coded helper component.

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CLOSTEROVIRUS ASSOCIATED WITH DODONAEA YELLOWS DISEASE. W. B. Borth, D. E. Gardner, T. L. German, University of Hawaii, Department of Plant Pathology, 3190 Maile Way, Honolulu, Hawaii 96822.

A recently described lethal yellowing disease affecting Dodonaea eriocarpa in Hawaii superficially resembles diseases caused by mycoplasma-like organisms although the etiology of this disease remains unknown. Virus-like particles have been partially purified from symptomatic stem and leaf tissues of diseased plants. The purification protocol involves homogenization of tissues in Tris buffer + NaCl, precipitation by polyethylene glycol and isopycnic centrifugation in $CsSO_4$/sucrose gradients. Using virions purified by this procedure, the molecular weight of the virion coat protein determined by SDS-PAGE electrophoresis is 23,000 daltons. Double-stranded RNA isolated from symptomatic tissues has a molecular weight of 3×10^6 daltons determined by PAGE. These characteristics suggest a possible closterovirus etiology for the disease.

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THE USE OF ARABIDOPSIS THALIANA CV. COLUMBIA IN PLANT VIRUS INTERACTION STUDIES. L.A. Urban[1], J.L. Sherwood[1], and U. Melcher[2], Dept. of Plant Pathology[1] and Dept. of Biochemistry[2], Oklahoma State University, Stillwater, OK 74078.

Eighteen strains of tobacco mosaic virus (TMV) were found to be systemically infective on Arabidopsis thaliana cv. Columbia (Heyn.). The serologically distinquishable common strain and a petunia strain were chosen for cross-protection studies. Three- to four-week old A.t.plants were inoculated with 50ug/ml of virus or buffer. The plants were challenge inoculated at 3, 7, 10, and 14 days after the first inoculation. Ten days later leaves from the first inoculation, the challenge inoculation, and leaves above those inoculated were tested by protein-A ELISA for the presence of virus. The plants challenge inoculated at 7 and 10 days showed no movement of the challenge inoculum. Regardless of the virus strain inoculated first, this first strain was found in all three leaf samples. The A.t./TMV system is suggested as suitable for studying mechanisms involved in plant virus interactions.

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NATURAL RESISTANCE TO SOYBEAN MOSAIC VIRUS (SMV): EVIDENCE FOR INCORRECT CAPSID PROTEIN PROCESSING IN THE RESISTANT SOYBEAN PI96983. R. J. Clem, L. M. Mansky, J. H. Hill,* R. E. Andrews, and D. P. Durand, Depts. of Microbiology and Plant Pathology,* Iowa State University, Ames, IA 50011.

The soybean (Glycine max) PI96983, which is resistant to SMV, and the susceptible cultivar Williams were inoculated at the primary leaf stage with SMV. Samples of primary and trifoliate leaves were harvested over a 10 day period and analyzed by Western blot using monoclonal antibodies to SMV capsid protein. Infectivity was determined by local lesion assay. In Williams, capsid protein and infectivity were detected in both primary and trifoliate leaves as expected. In PI96983, two proteins which differed in molecular weight from native capsid protein were first detected at 6 days and increased with time in primary leaves only, with no infectivity being detected. Peptide mapping studies are underway to confirm that the two proteins contain native capsid protein sequences.

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A UNIQUE CYTOPLASMIC INCLUSION BODY ASSOCIATED WITH A GRAFT-TRANSMISSABLE VIRUS-LIKE DISEASE OF SESAME. Robert S. Halliwell and M. R. Porterfield, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

A virus-like disease of sesame (Sesamum orientale) has been observed in all cultivars in the Texas Agriculture Experiment Station oil seed experimental plots since 1982. Each year the disease has become more prevalent. The symptoms include a puckering of the leaf surface, twisting of the leaf petiole and severe straping of the leaves formed after infection. Older leaves are epinastic, the plant is stunted and maturity delayed. Healthy young sesame plants chip-budded with a bud from a diseased plant developed symptoms within 21 days, whereas control plants and plants mechanically inoculated with infectious sap remained symptomless. Electron microscopic examination of ultra-thin sections of leaf mesophyll cells from symptomatic leaves revealed numerous unique spiral-shaped cytoplasmic inclusions not observed in healthy controls.

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MITE- AND WHITEFLY-TRANSMITTED VIRUSES SHARE EPITOPES WITH APHID-TRANSMITTED MEMBERS OF THE POTYVIRUS GROUP. R. E. Ford[1] D. D. Shukla[2], M. Tosic[3], and J. Jilka[1]. [1]Department of Plant Pathology, University of Illinois, Urbana, IL 61801, [2]CSIRO, Div. of Biotechnology, Parkville 3052, Australia; [3]University of Belgrade, Yugoslavia 11801.

At least ten viruses with potyvirus-like particles which induce the formation of pinwheel-type cytoplasmic inclusion bodies are transmitted by soil fungi, eriophyid mites, or whiteflies, and not by aphids. The taxonomic status of these viruses is uncertain. An antiserum was produced against dissociated coat protein core (minus N- and C-termini) of a definitive (aphid-transmitted) potyvirus which was found previously to react with all definitive potyviruses tested in this project. Use of this antiserum demonstrated that a mite- (wheat streak mosaic virus) and a whitefly- (sweet potato mild mottle virus) transmitted virus share epitopes with aphid-transmitted potyviruses and are, therefore, definitive members of the potyvirus group.

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VARIANTS OF BARLEY YELLOW DWARF VIRUS FROM A SINGLE ISOLATE. C.H. Lei and R.M. Lister. Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Two-site ELISA tests with unlabelled monoclonal antibodies "MAV1" or "MAV3" as coating antibody, and biotin-labelled MAV1 or MAV3 as detecting antibody, demonstrated that Rochow's Cornell MAV isolate ("C-type") of barley yellow dwarf virus contains virions bearing both MAV1- and MAV3-reacting epitopes. Its current Purdue subculture ("P-type") contains only MAV1-reacting virions, since although C-type gave positive results in all homologous and heterologous tests, P-type reacted positively only in the homologous tests with MAV1. These and earlier findings that P-types can be subcultured from C-type by single Sitobion avenae transfers, indicate that C-type is a mixture including at least 2 serotypes, and P-types are subcultures containing one of these. Sequential feeding tests with single S. avenae showed that both C- and P- serotypes can be acquired simultaneously, and separated during inoculation.

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

ELISA and cDNA probes were used to detect virus and viral RNA, respectively, in studies of cross-protection between two isolates (MAV and P-PAV) of barley yellow dwarf virus (BYDV). The ELISA results indicated that complete reciprocal cross-protection occurred during 10 days following inoculation of the second ("Challenge") isolate when the isolates were inoculated successively, using appropriate aphids, at intervals of from 5-15 days. However, in all the plants, traces of challenge viral RNA were detectable by cDNA probing during this time. At fifteen or thirty days after challenge inoculations, challenge virus and viral RNA were detectable in some plants, but in others, neither could be detected. Other interactions of these two isolates with each other, and with the RPV isolate, will also be described.

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IN VITRO EXPRESSION AND MAPPING OF SOYBEAN MOSAIC VIRUS (SMV) GENES. F. Gadani,[1] L. M. Mansky,[2] D. P. Durand,[2] J. H. Hill,[3] and R. E. Andrews.[2] EniChem SpA, Research and Development, Milan, Italy,[1] Depts. of Microbiology[2] and Plant Pathology,[3] Iowa State University, Ames, IA 50011.

The genomic RNA of SMV was translated in a wheat germ cell-free system to determine the gene products and the strategy of expression of the virus. When SMV-RNA was translated in vitro a set of 10 major polypeptides ranging in molecular weight from 20,000 to 100,000 was observed. Time course experiments revealed that after in vitro translation periods as short as 15 min, or as long as 75 min, all of the bands were present in approximately equal concentration, indicating that proteolytic processing is not a likely source of the smaller polypeptides. To determine the specific location of the coat protein gene as well as other viral cistrons on the genome, partial digestion of the RNA with phosphodiesterase I is being used, followed by immunoprecipitation assays of the translation products.

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HOST RANGE STUDIES AND PARTIAL CHARACTERIZATION OF A COPPER-RESISTANCE PLASMID IN XANTHOMONAS CAMPESTRIS PV. VESICATORIA. D. Malvick and C. Bender, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285

Resistance to copper (Cu) bactericides in tomato strains of Xanthomonas campestris pv. vesicatoria in Oklahoma has been linked to a 190 kb plasmid, pXV10A. In conjugation experiments, pXV10A effectively transferred Cu^r to X. c. pvs. campestris, dieffenbachiae, manihotis nigromaculans, pelargonii, phaseoli, vesicatoria, and vitians. Conjugative transfer of the Xcv Cu^r genes was not detected in the following recipients: X. c. pvs. malvacearum, glycines, translucens, A. radiobacter, A. tumefaciens, E. herbicola, E. rhapontici, P. syringae, P. corrugata, and R. meliloti. After conjugation, colony blotting to these potential recipients using pXV10A as a probe also indicated that pXV10A has a limited host range. Experiments have shown that Cu^r genes on pXV10A are similar to Cu^r genes cloned from a Florida Xcv strain. Further experiments to characterize pXV10A are in progress.

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ANALYSIS OF GENOMIC DNA FROM USA STRAINS OF *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE*. G.-W. Xu and C. F. Gonzalez. Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843.

Genetic similarity among *Xanthomonas campestris* pv. *oryzae* (XCO) strains isolated in Texas (TX) and Louisiana (LA) was analyzed using DNA-DNA hybridization. Twenty-four isolates from five counties in TX and 3 isolates from one parish in LA were analyzed. Total DNA was isolated and digested with *Eco*RI and separated by agarose gel electrophoresis. Southern blot transfers were probed with pJEL101, a pUC18 clone containing a 2.5-kb fragment of repeated DNA from a race 2 isolate of XCO (J. E. Leach, unpublished). Autoradiographs revealed hybridization profiles which separated the isolates into four groups. The three LA isolates comprised one distinct group while the TX isolates comprised the remaining groups. Analysis shows the probe sequence to be differentially conserved. Probe analysis may be useful for the detection of XCO and identification of genetic variability.

597 Withdrawn

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ISOLATION OF GENES FOR THE BIOSYNTHESIS OF FUSAROMYCIN A, AN ANTIBIOTIC ACTIVE AGAINST *FUSARIUM* AND *THIELAVIOPSIS*. W. T. Tucker, S. J. Abbene, and N. Gutterson. Advanced Genetic Sciences, Inc., 6701 San Pablo Avenue, Oakland, CA 94608.

Pseudomonas fluorescens strain NP77, a derivative of the oomycin A-producing strain Hv37a, produces an antifungal antibiotic which we have termed Fusaromycin A. Chemical mutants of NP77 which have lost the ability to inhibit *Fusarium* were isolated. A cosmid library of NP77 in the vector pRK7813 was used to complement these mutants. Complementing cosmids were analyzed by subcloning, insertion mutagenesis and by the construction of transcriptional gene fusions.

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ANALYSIS OF pTiC58 CONJUGAL TRANSFER GENES. S. Beck von Bodman and S.K. Farrand, Department of Plant Pathology, University of Illinois, Urbana, IL. 61801.

Conjugal transfer of the *Agrobacterium* Ti plasmid pTiC58 depends upon induction by the opines agrocinopine A and B. We used two Tn*5* mutagenesis strategies to identify genes important to conjugation in pTiC58. First transfer-constitutive mutants of wild-type strain C58 were isolated which no longer depend upon opine induction. Of three such mutants all showed insertions in the Ti plasmid mapping to a 3.6 kb region close to *acc*, the opine catabolic locus. Unlike previously described spontaneous Tra^c mutants, each of these remained inducible for *acc* functions. Second, C58 harboring a spontaneous Tra^c Ti plasmid was mutagenized to generate insertions affecting conjugal transfer. Eight Tra^- mutants were isolated, all with insertions mapping to one of two regions on the Ti plasmid. The first, about 13 kb in size, maps close to *acc*. The second region, approximately 4

kb in size, maps between the T-region and *noc*. The results show that conjugal transfer of pTiC58 may be regulated by two elements and that the genes involved in Tra comprise two separate loci.

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CONSTRUCTION OF A STABLE PROMOTER-PROBE PLASMID TO STUDY PATHOGENICITY AND AVIRULENCE GENE EXPRESSION IN XANTHOMONAS CAMPESTRIS IN INTACT PLANTS. S. Swarup, R. DeFeyter and D. W. Gabriel. Plant Pathology Department, University of Florida, Gainesville, 32611.

Several previously described promoter-probe vectors were evaluated for use in a number of pathovars of Xanthomonas campestris. None of these vectors proved entirely satisfactory in gene expression assays in whole plants. Many were unstable, some were highly strain dependent for conjugation, and activity assays in several were difficult to carry out in different leaf tissues. To eliminate these difficulties we constructed a highly stable, wide host-range promoter-probe vector from the plasmid Sa, using the Vibrio fischeri bioluminescence lux gene as a reporter of promoter activity. This plasmid, pSS500, has a kanamycin resistance gene, multiple cloning sites, and a transcriptional terminator upstream of the reporter sequence. It was possible to screen for expression of lux genes in bacterial strains growing both locally and systemically in intact plants and also to carry out similar screening in microtiter plate assays. This suggests a possible use of this broad host-range vector for assaying pathogenicity and avirulence gene expression in a non-disruptive manner in susceptible, resistant and non-host plants.

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SPECIES-SPECIFICITY AND CROSS-REACTIVITY OF MONOCLONAL ANTIBODIES GENERATED TO SPIROPLASMA CITRI AND S. KUNKELII. R. L. Jordan, M. Konai, I-M Lee, AND R. E. Davis. USDA-ARS, Plant Sciences Institute, Beltsville, Maryland 20705.

A panel of 46 monoclonal antibody-secreting hybridomas was developed from mice immunized with a mixture of Spiroplasma citri and S. kunkelii strains. Forty of the monoclonal antibodies (McAbs) recognized antigenic sites found on Ig-trapped intact spiroplasmas and purified membrane preparations, but the remaining 6 McAbs reacted only with disrupted spiroplasma cells in indirect ELISA. In ELISA tests using 36 strains of spiroplasmas from group I and groups III-XI, seventeen McAbs reacted only with strains of S. citri, another 17 McAbs reacted only with S. kunkelii strains. The remaining 12 McAbs detected antigenic sites common in 11 to 26 of the 26 group I spiroplasmas tested, including insect and flower spiroplasmas and the periwinkle spiroplasma, S. phoeniceum. These McAbs will be useful in providing information on the antigenic relationships among newly recognized spiroplasmas.

603 Withdrawn

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EVALUATION OF EFFECTS OF FOREIGN DNA ON FITNESS OF COCHLIOBOLUS HETEROSTROPHUS. N. P. Keller, O. C. Yoder, and G. C. Bergstrom. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The genetic load of integrated foreign DNA was investigated using a near-isogenic system of transformed and progenitor strains of the southern corn leaf blight fungus, Cochliobolus heterostrophus. The genome of C. heterostrophus was marked with plasmids containing the Escherichia coli hygromycin B phosphotransferase gene fused to a fungal promoter. Transformed strains which stably maintained plasmid DNA were used in a competition study with the wildtype by inoculating mixtures of transformant and wildtype conidia onto maize leaves. Frequency of hygromycin B-resistant conidia recovered from lesions was measured over five successive disease cycles. The proportion of conidia showing hygromycin B resistance decreased over time in most mixtures. These results suggest that the presence of foreign DNA in the genome exacts a fitness cost on C. heterostrophus.

605

MOLECULAR CLONING AND RESTRICTION ANALYSIS OF RIBOSOMAL RNA GENES (rDNA) FROM SEVERAL ANASTOMOSIS GROUPS OF RHIZOCTONIA SOLANI. R. Vilgalys and D. Gonzalez. Department of Botany, Duke University, Durham, NC 27706.

The plant pathogenic fungus Rhizoctonia solani is comprised of at least nine biologically diverse anastomosis groups (AG). To study the detailed structure and organization of ribosomal genes in R. solani, we cloned a single rDNA repeat unit from AG 4. The 8.7 kb repeat contains coding regions for all major rRNAs (5S, 17S, 5.8S, 25S). This cloned rDNA repeat was used to survey restriction fragment-length polymorphisms in 80 additional isolates. Each of the nine AG are characterized by one or more unique rDNA restriction patterns. Preliminary estimates for the size of the rDNA repeat from different AG range from 8.7 to 10.0 kb. Interestingly, individual multikaryotic isolates within some AG may possess more than one rDNA length variant. The pattern of rDNA variation which we observe in R. solani provides further support that AG represent independent evolutionary lines.

606

NUCLEASE ACTIVITY IN THE EXTRACELLULAR SPORE MATRIX OF *COLLETOTRICHUM* SP., Beth A. Snyder and Ralph L. Nicholson, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Colletotrichum sp. produce a water soluble, extracellular mucilage during the process of conidiation. The mucilage is a mixture of glycoproteins containing various enzymes. We have determined that the mucilage also contains a nuclease with the ability to degrade double-stranded DNA and wheat germ tRNA. DNase activity was detected in crude mucilage (1 µl) using agarose gel electrophoresis (0.6%) after incubation with 250 ng λ DNA. DNase activity was also detected with a qualitative microtiter plate assay with calf thymus DNA and by the use of DNase test agar plates. RNase was assayed by agarose gel electrophoresis after incubation with 1 µg/µL wheat germ tRNA. Four species of *Colletotrichum* and 23 isolates of *C. graminicola* were screened for DNase. All isolates and species contained activity. The apparently ubiquitous nature of this enzyme(s) in the extracellular spore mucilage may indicate a role in the disease development process of *Colletotrichum* sp.

608

CONSTRUCTION OF A cDNA LIBRARY FROM ASPERGILLUS PARASITICUS mRNA ISOLATED AT THE TIME OF FIRST EXPRESSION OF ENYZMES CATALYZING AFLATOXIN BIOSYNTHESIS. T. E. Cleveland and D. Bhatnagar USDA/ARS/SRRC, P. O. Box 19687, New Orleans, LA 70179.

A cDNA library was constructed using mRNA's isolated during the transitional period between the active and stationary growth phases of mycelia from an aflatoxin (AF)-producing strain of Aspergillus parasiticus; about 2.5 X 10^4 clones per µg cDNA were obtained. Activity and Western blot assays of mycelial proteins demonstrated that one of the gene products/enzymes catalyzing a step in the AF biosynthetic pathway, an O-methyltransferase (MTase) (Bhatnagar et al., 1988, Prep. Biochem., in press), is produced de novo during the above transitional period (1 to 2 days growth; liquid culture); AF accumulation begins during this period. The cDNA library will be screened for the MTase cDNA using serological techniques and/or with oligonucleotide probes derived from the amino acid sequences of MTase protein. The molecular regulation of the MTase gene and other AF genes will be examined with serological and cDNA probes developed during these investigations.

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FURTHER CHARACTERIZATION OF GENETIC RELATEDNESS AMONG DOUBLE-STRANDED RNA's (dsRNA) IN RHIZOCTONIA SOLANI. N. Bharathan and S. M. Tavantzis. Department of Botany and Plant Pathology, University of Maine, Orono, Maine 04469.

Nucleotide sequence relationships among dsRNA's found in 51 isolates of R. solani were established using nucleic acid hybridization. Cultures were obtained from the United States and Japan, and included members of anastomosis groups (AG) 1, 2, 3, 4 and 5. Hybridization experiments were conducted under high stringency conditions. Sequence homology (up to 50%) among dsRNA's of American and Japanese isolates was limited to members of AG 3, which is the major cause of Rhizoctonia disease in potato in both of these geographic areas. DsRNA bands within each AG of American isolates were classified into 3 to 4 sequence homology (SH) groups. Percent homology varied from 40 to 80% within a SH group. The possibility of dsRNA sequence homology among isolates belonging to different AG's was examined. The only cross hybridization involved 3 hypovirulent isolates from AG 2 3 and 5.

610

CHARACTERIZATION OF CELL SURFACE COMPONENTS OF TRANSPOSON TN5 GENERATED HR$^-$ MUTANTS OF PSEUDOMONAS SYRINGAE PV PHASEOLICOLA. W. F. Fett, S. F. Osman, J. M. Wells and M. F. Dunn, USDA-ARS, ERRC, 600 E. Mermaid Lane, Philadelphia, PA 19118

Eight independent, prototrophic transposon Tn5 insertion mutants of P. syringae pv. phaseolicola and the wild-type strain NPS 3121, (strains supplied to us by Drs. P. Lindgren and N. Panopoulos) were tested for cell surface hydrophobicity and charge as well as for lipopolysaccharide (LPS), exopolysaccharide (EPS) and whole cell fatty acid composition. All eight mutants were HR$^-$ on tobacco and either nonpathogenic or of reduced virulence (Vir$^-$) on bean. Cells of one mutant strain (NPS 4005, Vir$^-$) were more hydrophobic than the parent strain, while no mutants differed in charge. Initial studies indicated that strain NPS 4005 had an altered LPS chemotype, but this could not be confirmed in later experiments. All other mutant strains had LPS composition similar to the wild-type. The parent and mutant strains could also not be differentiated by whole cell fatty acid or EPS compositions.

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Cellular Fatty Acid Analysis of Plant-Pathogenic Coryneform Bacteria. N.C. Gudmestad and P.J. Henningson. Department of Plant Pathology. North Dakota State University. Fargo, ND 58105

Cellular fatty acid composition of plant-pathogenic coryneform bacteria was determined using gas-liquid chromatography. Several strains each of fifteen different species or subspecies of bacteria belonging to the genera Clavibacter, Rhodococcus, Curtobacterium, and Arthrobacter were studied. Subspecies of Clavibacter were particularly high in 12-methyl-tetradecanoic acid (a15:0), 14-methyl-hexadecanoic acid (a17:0) and 14-methylpentadecanoic acid (i16:0). The major fatty acids of Curtobacterium sp. were a15:0 and a17:0. Cellular fatty acids of Rhodococcus fascians were qualitatively different from the other bacterial strains. The major fatty acids for this species included trans-9-hexadecenoic acid (16:1t), hexadecanoic acid (16:0), cis-9-octadecenoic acid (18:1c) and 10methyl-octadecanoic acid (10Me19:0). Quantitative fatty acid differences, expressed as ratios of various fatty acids, differentiated all bacterial species and subspecies.

612

PATHOLOGICALY OPENED STOMATA: A MECHANISM FOR TISSUE DESICCATION IN BACTERIAL HYPERSENSITIVITY? S. Pike and A. Novacky, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Cotyledons of cotton and cucumber were inoculated with the incompatible pathogen Pseudomonas syringae pv. syringae to induce the bacterial hypersensitive reaction. Symptoms, mainly on the adaxial surface, were followed macroscopically and light microscopically. Stomata were found to open near the time of macroscopic tissue collapse. Opening did not appear to be light related. Stomatal opening and tissue collapse first occurred around the inoculation wound site. The amount and pattern of opening appeared to be related to the availability of H_2O. Stomata not located near major veins nor a border with healthy tissue did not open as widely and closed by 48 h. Open and closed stomata appeared in close proximity. We hypothesize that stomatal opening depends upon the availability of leaked K^+ and the companion processes of senescence and remobilization of nutrients.

613

SEQUESTERED SUPEROXIDE RADICALS IN MECHANISMS OF PLANT DEFENSE. T. J. Jacks, O. Hinojosa and E. B. Lillehoj, Southern Regional Research Center, USDA/ARS, P. O. Box 19687, New Orleans, LA 70179.

Extraction of whole leaves of cotton and other plants for short periods with aprotic solvents yielded extracts that contained superoxide radicals. Periods of contact sufficiently long to also extract water or addition of water to the former extracts resulted in destruction of the radicals. Extraction of superoxide was enhanced by tetrabutylammonium ion (cationic stabilizer) and by crown ethers (coordination complexers). The results suggest that superoxide is always present in healthy tissues and is normally sequestered in aprotic sites, such as membranes, in vivo. The availability of superoxide for instant release suggests a role for the toxic radical in front-line plant defense.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF EXTRACELLULAR ENZYMES PRODUCED BY SNOW MOLD FUNGI. F. Mehdizadegan and J. H. McBeath. Agricultural and Forestry Experiment Station, University of Alaska, Fairbanks, AK 99775-0080

Enzymes produced by Sclerotial low temperature basidiomycete (SLTB) and Sclerotinia borealis were concentrated by adding acetone to culture filtrates, collecting the precipitate after centrifugation (20,000g, 20 min.), and resuspending the pellets in acetate buffer. Activities were assayed on agarose diffusion plates and stained with Congo red and ruthenium red to detect cellulases and pectinases, respectively. Approximate molecular weights (MW) of enzymes were estimated by gel filtration. SLTB produced 3 molecules (3.9, 5.2 and 7.1x10^4 MW) and S. borealis produced one (7.9x 10^4) showing cellulytic activities. Pecteolytic enzymes from SLTB had a 5.8x10^4 MW, and those from S. borealis were 4.3 and 6.0x10^4 MW. Combinations of cellulytic enzymes produced by SLTB increased intensity of activities, but combining pectinases produced by S. borealis had no synergistic effect.

615

IMMUNOASSAY FOR NAPHTHAZARIN PHYTOTOXINS PRODUCED BY Fusarium solani. D. Phelps, S. Nemec, R. Baker, and R. Mansell. U.S. Department of Agriculture, Orlando, FL; USDA, Winter Haven, FL; and Biology Dept., Univ. of S. Florida, Tampa.

Cultures of F. solani from root-rotted citrus roots produce 11 known naphthazarin toxins and are negative for fusaric acid and T-2 trichothecene. Napthazarins have not been extracted from naturally infected citrus tissue in sufficient amounts for detection by conventional methods. Therefore, an ELISA immunoassay was developed using isomarticin as a hapten. Using carbodiimide, isomarticin was coupled to bovine serum albumin to produce an antigen for antibody production in rabbits, and coupled to alkaline phosphatase to produce a tracer. This assay detected as little as 5 ng isomarticin per well and other naphthazarin toxins at less than 10 ng per well. Selectivity of the test was demonstrated by it being 3 orders of magnitude less sensitive to a number of other phenolic compounds including closely related naphthoquinones. Methods for applying this assay to plant materials are being developed.

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THE PRODUCTION OF MULTIPLE PEAKS OF mRNA ACCUMULATION IN RESPONSE TO ELICITORS. C. S. Tepper and A.J. Anderson.

Department of Biology, Utah State University, Logan, UT 84322-5305.

Plant tissues respond to elicitor treatment by accumulating mRNAs corresponding to genes which code for phenylalanine ammonia-lyase and chalcone synthase enzymes which are involved in the biosynthesis of isoflavanoid phytoalexins. Crude and purified elicitors from the fungal bean pathogen Colletotrichum lindemuthianum induced the accumulation of these mRNAs in cotyledons of incompatible cultivars. Multiple peaks of accumulation were observed over a ten-hour time course. Pectic fragments also induced the same response. Treatment of the cotyledons with water, or elicitor treatment of a compatible cultivar, did not result in multiple peaks of mRNA accumulation. This wave pattern is distinct from the single peak of accumulation that is observed in elicitor-treated suspension cultured bean cells.

617

HYDROGEN PEROXIDE FORMATION BY WOOD DECAY FUNGI IN LIQUID MEDIUM. B. L. Illman and T. L. Highley, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, Wisconsin 53705-2398.

Hydrogen peroxide(H_2O_2)-derived hydroxyl radical ($OH^{\cdot}$) has been proposed to be a cellulose-degrading agent in the decay of wood by brown-rot fungi. Nitrogen-limiting liquid culture medium containing the chromogen 2,2'-azino-di(3-ethyl benzthiazoline-6-sulphonic acid) diammonium salt (ABTS) with horseradish peroxidase was used to detect the production of H_2O_2 by brown-rot fungi. Additionally, the effect of carbohydrate (cellobiose, glucose, galactose, arabinose and mannose) and nitrogen (ammonium tartrate) concentration on H_2O_2 production by Postia placenta was determined. Hydrogen peroxide was detected in all nitrogen-limiting medium and in nitrogen-amended medium with a low concentration of carbohydrate. High nitrogen and carbohydrate concentrations suppressed H_2O_2 production.

618

PRODUCTION OF PHYTOALEXINS IN RACE CLONE INTERACTIONS OF ALFALFA AND COLLETOTRICHUM TRIFOLII C. J. Baker, N. R. O'Neill and G. R. Bauchan, USDA, ARS, Beltsville, MD 20705 and J. R. Tomerlin, EPA, Washington, DC 20460.
Evidence suggests that phytoalexins are involved in resistance interactions between C. trifolii and alfalfa. We have examined nine specific race/clone interactions. Stem sections of seedlings inoculated with conidia were incubated for 5 days and analyzed for phytoalexin and fluorescent compounds. Results suggest that production of specific fluorescent compounds and phytoalexins is a function of the specific race/clone interaction indicating a specific response rather than a general stimulation of these compounds. When callus was incubated with conidial suspensions, compatible and incompatible interactions were distinguishable, however specificity in phytoalexin accumulation was not observed.

619

RELEASE AND DISTRIBUTION OF ARACHIDONIC ACID FROM PHYTOPHTHORA INFESTANS SPORES IN INOCULATED POTATO TISSUE. K. E. Bretschneider and R. M. Bostock, Dept. of Plant Pathology, University of California, Davis, 95616.

Arachidonic acid (AA), a component of the lipids of P. infestans, is an elicitor of hypersensitive resistance in potato. Spores of P. infestans incorporate exogenous ^{14}C-AA, and the sites of AA incorporation in the spores and in potato tissues inoculated with these spores can be visualized by microautoradiography. During infection by an incompatible race (race 0 vs. cv. Kennebec), radioactivity is released into the host tissue and can be detected as early as 15 hr after inoculation. Initial observations of tissues inoculated with a compatible race (race 1.2.3.4) did not show significant release of label into host cells at 15 hr. This method should be useful for determining the relationship between release of AA from fungal tissue and host cell responses occurring during hypersensitivity expression.

620

HEAT-LABILE MOLECULES SECRETED BY PYRICULARIA ORYZAE KILL PLANT CELLS. P. Bucheli, S.H. Doares, K.S. Ham, A. Darvill and P. Albersheim, CCRC, University of Georgia, P.O. Box 5677, Athens, GA 30613.

Dialyzed filtrates from the fungus Pyricularia oryzae grown either on pectin or rice cell walls contain a heat-labile activity that kills suspension-cultured plant cells. Initial purification on CM-Sephadex C-50 yielded a partially purified fraction with killing activity. When the active CM-Sephadex C-50 fraction was purified on an FPLC Superose 12 column, a highly active fraction was obtained in which various enzymatic activities including pectin lyase, pectinmethylesterase and protease were detected. We have evidence that none of the enzymes secreted by the fungus when assayed independently kill suspension-cultured plant cells and therefore suggest more than one molecule secreted by Pyricularia oryzae may be required to kill plant cells. (Supported by DOE grant (#DE-FG09-85ER13425) and NSF grant (DMB-8518488) and a Swiss National Science Foundation post-doctoral fellowship (PB)).

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PURIFICATION OF PECTIN LYASE FROM CULTURES OF RHIZOCTONIA SOLANI AG 2-2 AND EXTRACTS OF INFECTED SUGAR BEET ROOTS. W. M. Bugbee, USDA-ARS, Northern Crop Science Laboratory, Box 5677 University Station, Fargo, ND 58105-5677

An isolate of Rhizoctonia solani, anastomosis group 2-2, pathogenic on sugar beet roots, was cultured in broth. The carbon source was sugar beet root cell walls. Inoculum for root inoculation was produced by culturing the same isolate on sterile barley. Extracts from the cell wall broth culture and rotted roots were fractionated by affinity batch chromatography on cross-linked sodium polypectate followed by gel permeation chromatography on an agarose based gel. Pectin lyase was interactive with the agarose gel and eluted in the bed volume which resulted in a final effective purification. Pectin lyase was the most prevalent and active pectolytic enzyme from in vitro and in vivo sources. The purified enzyme caused wilt when injected into Rhizoctonia susceptible sugar beet plants but not when injected into resistant plants.

622

CHANGES IN BARLEY LEAF PEROXIDASES FOLLOWING INOCULATION WITH ERYSIPHE GRAMINIS F. SP. HORDEI. K. Kerby and Shauna C. Somerville. DOE-MSU Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

Peroxidase is involved in the terminal steps of lignin biosynthesis. Lignification of tissue has been implicated as a response to fungal infection. Changes in the intercellular leaf peroxidase activity were monitored in two congenic barley lines which differ from each other at the Ml-a locus. One line (C1-16137) is resistant and the other (C1-16138) is susceptible to Eryisphe graminis f. sp. hordei, race 3. Four days after inoculation, the specific activity of peroxidase increased over 13 fold in the resistant host and over 18 fold in the susceptible line. Isoelectric focusing of acetone-treated intercellular wash fluid revealed that within 24 hours after exposure to the fungus, a novel peroxidase isozyme appeared. The timing of the appearance of this isozyme was compared with the temporal events of fungal infection.

623

IN VITRO PRODUCTION AND PARTIAL PURIFICATION OF A TOXIN FROM PYRENOPHORA TRITICI-REPENTIS. A. Tomas, J. E. Leach, and W. W. Bockus, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Culture filtrates of Pyrenophora tritici-repentis, causal organism of tan spot of wheat, contain a toxic activity which appears to be a major determinant of the disease in vivo. The activity was assayed by infiltration of wheat leaves with serial dilutions of culture filtrates. Toxic activity was first detected after 10-12 days of culture and correlated with a decrease in pH. Filtrates from cultures grown in the light had higher toxicity than those from cultures grown in the dark. Toxic activity was partially purified from concentrated filtrates by G50 chromatography. The activity was heat stable but was lost after treatment with proteinase K. Analysis of the toxin-containing G50 fractions by SDS polyacrylamide gel electrophoresis revealed a protein of apparent MW 13,000. The association of this protein with toxicity is being investigated.

624

EVIDENCE FOR CALCIUM INVOLVEMENT IN ACTIVATION OF K^+/H^+ EXCHANGE IN TOBACCO BY PSEUDOMONAS SYRINGAE PV. SYRINGAE. M. M. Atkinson, L. D. Keppler, C. J. Baker and C. F. Mischke. Microbiology and Plant Pathology Laboratory, USDA-ARS, Beltsville, MD 20705.

An early event in the hypersensitive response of tobacco to P. s. syringae is the sustained activation of a plasmalemma K^+ efflux/net H^+ uptake exchange response (XR). We are investigating whether Ca^{++} functions as a second messenger during this cellular response. When added to tobacco cell suspensions, La^{3+}, a calcium channel blocker, prevented XR initiation and inhibited ongoing XR. Similar results were obtained with EGTA. Rate of $^{45}Ca^{++}$ uptake by tobacco cells increased 2 - 3 fold at the onset of XR and remained elevated for at least 2.5 h thereafter. These results indicate that external Ca^{++} and possibly plasmalemma Ca^{++} influx are required for initiation and continuation of K^+/H^+ exchange.

625

IONTOPHORETIC MICRONINJECTION OF LUCIFER YELLOW INTO HYPHAL CELLS OF Erysiphe graminis f. sp. hordei. V. M. Russo and W. R. Bushnell, USDA/ARS, Cereal Rust Laboratory, University of Minnesota, St. Paul, MN 55108.

Lucifer yellow was injected iontophoretically into hyphae of Erysiphe graminis f. sp. hordei grown on a monolayer of coleoptile epidermis from Hordeum vulgare. For injection, 24-48 hr-old colonies on the host surface were submerged in 0.01M $Ca(NO_3)_2$ and injected with pipettes partially filled with 0.25% lucifer yellow and backfilled with 3M KCl. Inside diameters of pipette tips were 0.2-0.6 µm. The dye was delivered with a train of 0.5 volt pulses (one 0.5 sec pulse/sec) applied for 15-60 sec with a WPI M-707A Microprobe System. Cells collapsed after injection but lucifer yellow was found in adjacent cells which remained alive and often produced new hyphal branches. The results show that dye moved from iontophoretically injected cells into neighboring hyphal cells which maintained growth potential. DNA and other ionized substances possibly can be introduced into hyphae in a similar way.

626

ALTERATIONS IN POTATO GENE EXPRESSION AFTER APPLICATION OF ARACHIDONIC ACID TO TUBER TISSUE. B.A. Stermer[1] and R.M. Bostock[2], [1]The Noble Foundation, Box 2180, Ardmore, OK 73402 and [2]University of California, Davis, CA 95616.

Early changes in protein synthesis and translatable mRNA populations were investigated in potato after elicitation of hypersensitive resistance with arachidonic acid (AA). In vivo labeling of potato tubers with [^{3}H]leucine showed that the rate of net protein synthesis nearly doubled within 6 h of application of AA. This is one of the earliest active responses to the elicitor known. Application of other poly-unsaturated fatty acids to tuber tissue also caused transient increases in protein synthesis, however AA always induced the largest and most prolonged increases. Electrophoretic analysis of the polypeptides from the in vitro translation of tuber poly(A)$^+$ RNA indicated that AA caused changes in mRNA populations. These results clearly show that AA has marked effects on the RNA and protein metabolism of potato tubers within 24 h of its application.

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A NOVEL C_{32} STERYL SULFATE PRODUCED BY SPECIES IN THE GENUS FUSARIUM. Harland R. Burmeister and Ronald F. Vesonder, Northern Regional Research Center, USDA-ARS 1815 North University Street Peoria, IL 61604.

A novel C_{32} steryl sulfate was discovered in Fusarium spp. Methods were developed for its identification, and representative strains of the genus were screened for its presence. It was found in seven species. Production and purification were carried out with F. graminearum NRRL 13166 as the producing strain. The C_{32} steryl sulfate is one of the more abundant and easily attainable microbial steroids. It inhibits the growth of some fungi, gram-positive bacteria, and germinating seeds. The discovery of steryl sulfates in fungi provides an additional biological system to assist in the elucidation of the biochemistry of sulfated steroids.

628

CHANGES IN PROTEIN PATTERNS OF LIVE PEANUT KERNELS AS INDUCED BY *ASPERGILLUS FLAVUS* AND *A. PARASITICUS*. J. B. Szerszen and R. E. Pettit, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

The level and duration of protein synthesis induced by exposure of sound mature peanut kernels and isolated live cotyledons to *Aspergillus flavus* Fl 102 and *A. parasiticus* NRL 2999 were measured by changes in SDS-PAGE, IEF, and two-dimensional electrophoretic separations. The trial included 13 peanut cultivars known to differ in degree of susceptibility to *Aspergillus* sp. Pathogenesis related proteins (PR) were detected 18 hrs after infection in cotyledons and after 24 hrs in kernels. The PRs occurred within a tightly grouped constellation of protein spots in a basic region of 2-D gels. Patterns of PRs differed with cultivars. Mapping of PRs showed enhanced synthesis of 4 and 6 PRs at 30 and 36 hrs respectively after inoculation of cultivars J-11, SN 55-437, Toalson, and TX 798736 with *A. flavus*. PRs were only detected from cotyledonary tissue.

629

PROTEINS INDUCED BY *ASPERGILLUS FLAVUS* AND *A. PARASITICUS* IN PEANUT KERNELS FROM PLANTS GROWN UNDER DROUGHT STRESS AND NORMAL IRRIGATION. J. B. Szerszen and R. E. Pettit, Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843-2132.

Peanut kernels from 5 cultivars grown under drought stress (DS) (lasting 100 days from planting until harvest) and kernels from plants grown under normal irrigation (NI) were inoculated with conidia of *Aspergillus flavus* or *A. parasiticus* and incubated 5 days at 95% RH. One and two-dimensional electrophoretic separations of kernel proteins were performed at 6-hr intervals and silver stained. These revealed synthesis of 4 pathogenesis-related proteins (PR) 24 hrs after inoculation in kernels from all cultivars tested and grown under NI. Kernels from DS plants of each cultivar lacked PRs except from cultivar TX 798736, which had 5 PRs. The level of these PRs increased within the next 12 hrs. Images of PRs became diffuse 42 hrs after inoculation. These PRs have potential use as molecular markers in the search for DS/*Aspergillus* resistance.

630

THE NATURE OF MITOCHONDRIAL PLASMIDS IN GLIOCLADIUM VIRENS, A BIOCONTROL FUNGUS. Sue Mischke, USDA-ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705

Gliocladium virens is a biocontrol fungus which antagonizes soilborne plant pathogens such as Pythium ultimum and Rhizoctonia solani. More than 70% of the 20 G. virens strains examined contain one or more plasmids in their mitochondria. It is important to determine the nature and function of these plasmids since they may contain genetic material related to biocontrol capabilities. Strain G-1 contains a single plasmid in high copy number which has been shown by nuclease digestions to be double-stranded DNA. This plasmid appears to exist in a circular form with a size of 3.2 $\pm$ .5 kb. Experiments are in progress to clone and map this plasmid and to determine its relationship to plasmids in other G. virens strains. At least five G. virens strains exhibit phenotypic instability that can be induced or suppressed by changing growth media. Preliminary results suggest that phenotypic instability in strain G1-3 may be related to its plasmid.

631

PETASOL - THE FATE OF THIS PHYTOHORMONE-MIMIC IN MONOCOTS. G. Bunkers, D. Kenfield, F. Sugawara, and G. Strobel. Dept. of Plant Path., MSU, Bozeman, MT 59717.

Drechslera gigantea, the causative agent of zonate-eyespot disease on grasses, produces several bioactive molecules which mimic the properties of known phytohormones. These compounds, known as eremophilanes, are able to delay senescence in detached leaves of monocots, at 10^{-5} to 10^{-6}M concentrations. ^{14}C-petasol applied to detached oat leaves and incubated for 3 days, is converted into a compound which exhibits different chromatographic properties than petasol and is not bioactive. Mass spectrometry and NMR analyses indicate that the petasol moiety is present in the conversion product. Hydrolysis of the conversion product led to the recovery of petasol from it. Our results indicate that the plant is capable of modifying this eremophilane to form a petasol adjunct.

632

RESISTANCE OF THE ROOT COLONIZING BACTERIUM PSEUDOMONAS PUTIDA TO ACTIVATED OXYGEN SPECIES. J. Katsuwon and A.J. Anderson. Department of Biology, Utah State University, Logan, UT 84322-5305

Plant root surfaces are aggressively colonized by beneficial

bacteria of the Pseudomonas putida - fluorescens group. The plant root surface possesses peroxidases which have the capacity to produce activated oxygen species, superoxide anion $O_2^{-\cdot}$ and hydrogen peroxide, H_2O_2. Survival of P. putida on the rhizoplane may depend on the enzymes superoxide dismutase (SOD) and catalase which degrade $O_2^{-\cdot}$ and H_2O_2 respectively. P. putida produces both SOD and catalase. A single isozyme of SOD has been detected by native PAGE and activity levels are similar throughout the growth phase. Catalase activity increased four-fold during growth phase; in stationary phase a second catalase isozyme was detected.

633

TIME OF APPLICATION AND SURVIVAL OF PSEUDOMONAS FLUORESCENS ON INFECTION BY SEPTORIA TRITICI. F. Mehdizadegan and F. J. Gough. Agricultural and Forestry Experiment Station, University of Alaska, Fairbanks, 99775-0080; Oklahoma State University and USDA-ARS, P.O. Box 1029, Stillwater, 74076.

Antagonist P. fluorescens (PFCNS), resistant to streptomycin and naladixic acid, and its culture filtrate were applied to wheat seedlings 24 h before, simultaneously, and 24 h after inoculation with S. tritici under greenhouse conditions. Populations of PFCNS on wheat leaves were determined at 0, 1, 3, and 6 days, after application. Incidence of S. tritici infection was determined by measuring numbers of pycnidia per gram of dry leaf. In all cases, PFCNS populations declined sharply initially but stabilized after the third day. PFCNS reduced the incidence of S. tritici, but was most effective when applied simultaneously. Application of PFCNS filtrate reduced infection when applied simultaneously or 24 h after inoculation with S. tritici.

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EVALUATION OF INA-MINUS DELETION MUTANTS FROM STRAWBERRY IN FIELD TRIALS ASSESSING ENVIRONMENTAL FATE AND EFFICACY. T.V. Suslow, L. Joe, A. Moayeri, M. McCarty, J. Hayashi, D. Misumi, J. Lindemann, D. Matsubara, P. Continente. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA 94608.

Two field experiments with recombinant strains of *Pseudomonas syringae* (RGP36R2) and *P. fluorescens* (GJP17BR2) have been conducted towards evaluating the environmental fate, competitiveness and efficacy of the introduced strains. Parental strains of the test isolates were originally obtained from healthy strawberry. In most recent tests, randomized complete blocks (n = 4) of strawberry cv 'Douglas' were established in Brentwood, CA in duplicate with the objectives of evaluating dose, timing, frequency and formulation, in addition to the above mentioned objectives. Worker exposure, aerosol dispersal, persistence in soil and on non-host plants, dispersal by pollinator bees, and other aspects of environmental fate and risk were quantitated. In summary, RGP36R2 was more environmentally competent under ambient conditions spanning 14 December 1987 to 23 April 1988 and more successful at pre-emptive exclusion of endemic INA+ strains than was GJP17BR2. Introduced strains were found to have a low or negligible propensity to disperse and establish beyond the treated test area.

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INDUCED RESISTANCE AGAINST BIOTROPHIC FUNGI AND ITS DEPENDENCE ON PRECONDITIONING TEMPERATURE CONDITIONS. A.-G. Falkhof and F. Schönbeck, Institute of Plant Pathology, University of Hannover, 3000 Hannover 21, W.-Germany.

Resistance against powdery-mildew, downy-mildew and rust can be induced in plants by the application of metabolites excreated by a strain of Bacillus subtilis into the nutrient solution. On induced resistant plants infection densities as well as the size of the few developing colonies are reduced; the vegetative and generative reproduction of the pathogens is decreased considerably. This type of induced resistance requires a special conditioning of the plants by environmental conditions acting prior to the application of the inducer. If plants are grown at natural temperature conditions outdoors or in greenhouse or at frequently alternating temperatures in a light and humidity controlled growthchamber, the infection densities are efficiently reduced after the treatment with the inducer. In contrast to this the disease intensities are not reduced on plants grown at constant temperatures in growthchambers.

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INDUCED RESISTANCE AND YIELD RESPONSE OF FIELD GROWN BARLEY. U. Steiner, E.-C. Oerke, and F. Schönbeck, Institute of Plant Pathology, University of Hannover, 3000 Hannover 21, W.-Germany.

Resistance can be induced against diseases caused by biotrophic pathogens by treating the plants with metabolites of a selected isolate of Bacillus subtilis. With winter and spring barley varieties differing in disease susceptibility and productiveness the effect of induced resistance on infection with powdery mildew and grain yield were examined. Furthermore, the efficiency of disease reduction and yield response were studied in relation to improved nitrogen fertilization, increasing the susceptibility for powdery mildew. On induced resistant plants the pathogens developed less sporulating colony area, sporulation rate and formation of cleistothecia were reduced. The degree of disease reduction varied with fertilizer level and host genotype. Although protection was not complete, yield response partially exceeded that of fungicide treated plants. Disease reduction and increase of grain yield were not strongly correlated after biological control by induced resistance.

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VARIABILITY IN BIOCONTROL OF FRUIT ROTS AMONG ISOLATES OF THE YEAST, DEBARYOMYCES HANSENII. M. Wisniewski, C. Wilson, USDA, ARS, AFRS, Kearneysville, WV, 25430, and E. Chalutz, ARO, Volcani Center, Bet Dagan, Israel

Recent experiments have demonstrated that a yeast, D. hansenii (US-7) can control postharvest decay on citrus caused by Penicillium digitatum, Penicillium italicum and Geotrichum candidum. Biocontrol of postharvest decay of apples, caused by Botrytis cinerea, has also been documented. In this study, performance of 8 isolates of D. hansenii, obtained from the American Type Culture Collection, was assessed in comparison to the biocontrol activity of US-7, isolated from citrus fruit at AFRS. Pathogens and isolates of D. hansenii were applied to wounded fruit and lesion development was monitored over a 12 day period. Water/pathogen inoculations served as controls. In apples, challenged with Botrytis, US-7 reduced lesion size by 63% compared to controls. Performance of other isolates ranged from a 12-37% reduction. Similar results were obtained on grapefruit challenged with P. digitatum. In coculturing experiments, US-7 significantly reduced the growth of Botrytis to a greater extent than the other isolates tested. In general, data indicated that US-7 exhibited the greatest biocontrol activity and that biocontrol activity was strain specific.

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CERCOSPORIN, FUNGISTATIC TO SOYBEAN PATHOGENS, RECOVERED FROM CERCOSPORA KIKUCHII. M. A. Pathan, J. B. Sinclair and R. E. Wagner, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801-4709.

Cercosporin, a nonspecific fungistatic compound was recovered from dry soybean seed coats of cv. Amsoy 71 inoculated in the field with isolate PR of C. kikuchii. Cercosporin activity was tested by plating different fungi on PDA containing 1.0 or 10.0 uM concentrations. Mean colony diameter (cm) of Colletotrichum truncatum, Fusarium sp., Phomopsis longicolla, P. sojae, and Rhizoctonia solani was significantly (P=0.05) inhibited below the controls at 1.0 uM and completely inhibited at 10.0 uM cercosporin. It was fungitoxic to C. kikuchii, P. longicolla, R. solani and fungistatic to C. truncatum, Fusarium sp., and P. sojae when transferred back to nonamended PDA. The percent inhibition of radial growth (PIRG) ranged from 22-50% for P. sojae and 31-69% for P. longicolla on PDA with 125, 250, or 500 ug/ml cercosporin.

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IN VITRO COLONY INTERACTIONS AMONG SPECIES OF TRICHODERMA GROWN AT TWO TEMPERATURES. J. L. Reaves and R. H. Crawford, USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331

Interactions between paired colonies of Trichoderma spp. in culture at two temperatures are reported. Interaction types (e.g. zones of inhibition, demarcation lines, ridges of conidia, overgrowths, and intermingling) in the zones between isolates were not consistent at 10 or 25°C. Except for intermingling, all types of interactions occurred more frequently in interspecific pairings at either temperature. Microscopic examination of hyphae in the interaction zone between colonies revealed that anastomosis occurred only in self- and intraspecific pairings whereas coiling occurred in all pairing types. Terminal and intercalary chlamydospores formed in hyphae in the interaction zone in a number of dual cultures.

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IMPACT OF D-FACTOR MYCOVIRUSES ON INFECTION BY THE ELM PATHOGEN OPHIOSTOMA ULMI. J.F. Webber, Forest Research Station, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK.

D-factor (ds RNA) mycoviruses usually cause reduced rates of growth and conidial germination of O. ulmi in culture. Field experiments now show that d-factors also greatly reduce the capacity of O. ulmi to infect healthy elms via scolytid feeding grooves. Thus, whereas only c. 1000 healthy conidia could produce a xylem infection, as many as 50,000 spores were required with d-factor carrying isolates. This loss of pathogenic potential is probably caused both by low spore viability, and by reduced ability to compete with other micro-organisms. Further experiments have also indicated that the transfer of d-factors between infected and healthy isolates of O. ulmi can occur in beetle feeding grooves, as well as during the saprophytic phase of the pathogen in diseased elm bark. However, the extent to which this occurred was moderated by the vegetative compatibility system of the fungus.

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BIOLOGICAL CONTROL OF HYDRILLA VERTICILLATA (L.F.) ROYLE WITH AN ENDEMIC FUNGAL DISEASE. G. F. Joye CEWES-ER-A, U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS

An endemic fungal pathogen was recently isolated from Hydrilla foliage collected in Lake Houston near Houston, TX. The fungus grew well in V8 broth but did not sporulate. Test plants were grown to 100 cm in a eutrophic water column at 25 C. Symptoms of the disease appeared on foliage within seven days after inoculating test plants with a mycelial concentration of 10^5 cfu/ml. Plants were completely destroyed within three weeks after inoculation. The rapid death of inoculated Hydrilla plants exemplifies the potential of this fungus as a bioherbicide. The name of the causal fungus has not been determined.

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PROPERTIES OF A GERMINATION STIMULATOR OF TELIOSPORES OF CANADA THISTLE RUST EXTRACTED FROM SOIL OF POTTED CANADA THISTLE. R. C. French and A. R. Lightfield, USDA-ARS, Bldg. 1301, Ft. Detrick, Frederick, MD 21701

Canada thistle (Cirsium arvense (L.) Scop.) is occasionally devastated by the spermagonial/aecial stage of the rust, Puccinia punctiformis, initiated in the root by germinated teliospores. We have previously described a hexane extract of steam-distilled thistle roots which stimulated teliospore germination. Inoculation of root cuttings with teliospores produced plants with systemic infections. Water collected from the soil of potted thistle plants stimulated teliospore germination on 1% water agar in 7 days at 18 C. Water from soil without thistle plants was not stimulatory. The active material was collected and concentrated on C-18 silica gel columns. The concentrated extract is volatile and stimulated germination across an air space. Stimulatory volatiles were also collected from live washed roots. Research is in progress on characterization of the active substance.

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LIMITED FIELD EVALUATION OF PUCCINIA CARDUORUM FOR BIOLOGICAL CONTROL OF MUSK THISTLE. W. L. Bruckart, USDA-ARS, Frederick, MD 21701; A. B. Baudoin, Dept. Pl. Path.; R. Abad and L. T. Kok, Dept. Ent.; VPI&SU, Blacksburg, VA 24061.

After six years of evaluating P. carduorum in a containment greenhouse for biocontrol of musk thistle (Carduus thoermeri), permission was granted by the Animal and Plant Health Inspection Service (APHIS) for a limited field study with an isolate from Turkey. This is the first exotic plant pathogen to pass review by APHIS and the second released in the continental U.S. for weed control. Transplanted musk thistles were artificially inoculated in October, 1987 at an isolated site near Blacksburg, VA. Uredinia and telia were observed in December, 1987. Uredinia were seen on new growth in April, 1988, indicating the pathogen had overwintered. Non-target Cynara scolymus (artichoke) and native North American Cirsium spp., slightly susceptible in greenhouse tests, were planted between plots in May and rated for susceptibility to P. carduorum in the field.

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SCREENING POTENTIAL BIOANTAGONISTS AGAINST PATHOGENS OF TURF. A.L. O'Leary, D.J. O'Leary, and S.H. Woodhead, Ricerca, Inc., P.O. Box 1000, Painesville, OH 44077, and R. Battershell, Fermenta Plant Protection Co., P.O. Box 8000, Mentor, OH 44061

A technique was developed to evaluate potential bioantagonists against Pythium and Rhizoctonia diseases on turf. 'Penncross' bentgrass was grown in 288-plug plastic trays. Fourteen grass plugs were planted, evenly spaced, in 14 X 17 X 6 cm fiber pans of pasteurized soil. Grain infested with R. solani or P. aphanidermatum was placed in a center hole in the soil, a grass plug was planted in the hole, and infested grain was placed on the plug. Potential bioantagonists were either incorporated in the soil prior to planting the plugs, or were sprayed on the plugs after planting. Treatments were evaluated by counting the number of dead and symptomatic grass plugs. For example, in pans inoculated with R. solani, 20% of plugs were dead when sprayed with conidia of a Trichoderma sp. compared to 100% of the plugs which were not sprayed.

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COLONIZATION OF SOYBEAN ROOTS BY FUNGI ISOLATED FROM CYSTS OF HETERODERA GLYCINES. C. M. Stiles and D. A. Glawe. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Fungi isolated from cysts of Heterodera glycines were tested for the ability to colonize soybean roots. Camposporium pellucidum, Cylindrocarpon destructans, Diheterospora chlamydosporia, Fusarium oxysporum, Fusarium solani, Gliocladium catenulatum, Mariannaea elegans, Paecilomyces lilacinus, Paecilomyces marquandii, Paraphoma radicina, Pyrenochaeta terrestris, Stagonospora heteroderae, and Trichoderma koningii were grown in liquid culture. Mycelial suspensions were used to inoculate roots of 10-day-old soybean seedlings (cv. Williams 82). Fungi were reisolated weekly for three weeks. Each fungus was successfully reisolated. Plants inoculated with P. terrestris sometimes had pink-discolored roots, but no symptoms were detected in other plants. The ability of cyst-colonizing fungi to infect soybean roots suggests that research is needed to determine if fungi can grow into cysts from previously colonized roots.

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BIOLOGICAL CONTROL OF THE POSTHARVEST PATHOGEN *PENICILLIUM DIGITATUM* ON EUREKA LEMONS. D. J. Appel, R. Gees and M. D. Coffey, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Isolates of *Myrothecium roridum* and *M. verrucaria* controlled the postharvest pathogen *Penicillium digitatum* on lemons (cv. Eureka). Ripe lemon fruit were wound inoculated with 1 x 10^6 conidia/ml of *P. digitatum* 18 h prior to treatment with the *Myrothecium* spp. isolates. The lemons were incubated at room temperature at high relative humidity for 18 h to allow the pathogen to germinate in the wound. Each antagonist was applied by dipping the lemons into a suspension of conidia (1 x 10^8/ml) for 30 sec. In repeated experiments, the *Myrothecium* spp. isolates reduced disease incidence by ≥87% compared to the control. When the antagonists were tested for control capabilities at different temperatures, the degree of control was ≥85% at all temperatures tested (12,15,18,21 and 24°C).

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Inhibition of germination of sclerotia of Sclerotinia sclerotiorum by chitinase. O. Anas, I. Alli and R. Reeleder. Dept. of Plant Science, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada H9X 1C0.

Sclerotia of Sclerotinia sclerotiorum (Lib.) de Bary fed upon by larvae of Bradysia coprophila Lintner have a decreased ability to germinate. Germination of sclerotia was enhanced after they were washed with distilled water. Analysis by gel electrophoresis and ion-exchange HPLC indicated that two homogeneous protein fractions were present in salivary secretions deposited upon sclerotia by larvae. These proteins exhibited chitinase activity. Damaged or undamaged sclerotia soaked for one hr in 1.5 µg/ml chitinase exhibited an increase in eruptive mycelial germination when compared to those soaked in distilled water. Soaking in 50, 100 and 150 µg/ml chitinase decreased all types of germination. Carpogenic germination was more frequent after treatment with distilled water than after treatment with chitinase.

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HYPHAL GROWTH FROM ALGINATE GRANULES CONTAINING TRICHODERMA HARZIANUM. G. R. Knudsen and D. J. Eschen, University of Idaho, Moscow, Idaho 83843.

Hyphal growth rates were quantified for an isolate of Trichoderma harzianum formulated in sodium alginate granules containing 2 mg mycelium (mean dry weight) and either 0 or 2.5 mg bran. Autoclaved wheat seeds were placed at 0-10 cm distances from granules in glass tubes or dishes containing sand or silt loam soil (0.3 bar, 15 or 25 C). Hyphal growth, branching, and colonization of seeds over time were monitored visually and by selective isolation of T. harzianum from seeds. Growth rates were fastest (>0.5 cm/day) at 25 C with bran. These results were incorporated into a microcomputer simulation model of mycelial growth patterns, then used to predict colonization of wheat seeds and sclerotia of Sclerotinia sclerotiorum by T. harzianum applied at planting in a pea field.

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USE OF BIOLUMINESCENCE FOR MONITORING ENTEROBACTER CLOACAE POPULATIONS IN BIOCONTROL OF PYTHIUM ULTIMUM. D. R. Fravel, D. P. Roberts, and R. D. Lumsden, Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, MD 20705.

The luciferase genes on plasmid pUCD607 enable various bacteria to bioluminesce (J. J. Shaw and C. I. Kado, 1986. Bio/Technol.). Plasmid pUCD607 was mobilized into the biocontrol agent Enterobacter cloacae (E6) by conjugation and the resultant strain E6(pUCD607) was bioluminescent. Biocontrol of P. ultimum by E6(pUCD607) on lettuce was similar to that of E6 ($P = 0.01$). Populations of E6(pUCD607) in soilless potting mix and on lettuce roots were estimated over a 12 day period using a scintillation counter to measure bioluminescence. There was a positive, linear correlation between populations determined by dilution plating and bioluminescence. Dilution plating was more sensitive than scintillation counting in detecting populations. Bioluminescence is a promising technique for the study of population dynamics in biocontrol.

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PSEUDOMONAS STRAIN AMMD ADVERSELY AFFECTS APHANOMYCES ZOOSPORES AND CYSTS. J. L. Parke and E. B. King. Department of Plant Pathology, University of Wisconsin-Madison, Madison WI 53706

The nonfluorescent Pseudomonas strain AMMD applied to captan-treated pea seeds increases pea emergence and yield in fields naturally infested with Aphanomyces euteiches f. sp. pisi. Although the mechanism of biological control is not known, the bacterial culture adversely affects the development of zoospores and cysts in vitro. Motility of synchronously released zoospores is immediately reduced from 96% to 0% upon addition of a broth culture of Pseudomonas diluted to 1% (10^8 CFU/ml). Primary and secondary cysts treated with the broth culture fail to release zoospores and do not germinate directly. Non-inoculated broth or cell-free culture filtrate do not affect either zoospore motility or cysts, yet heat-killed cultures have the same effect as live cultures. These results suggest that physical interaction between the bacterium and the fungus may be required to affect motility and germination.

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MOISTURE STRESS TOLERANCE AND AGGRESSIVENESS AS POSSIBLE INDICATORS OF BIOCONTROL CAPABILITIES IN SAPROPHYTIC STRAW-COLONIZING FUNGI. W. F. Pfender, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Several straw-colonizing fungi were tested for traits considered relevant to biological control of Pyrenophora tritici-repentis, a residue-borne wheat pathogen. These traits are: chitinase production, rapid growth, growth at moderately low water potentials (-7 MPa), ability to invade dead straw already inhabited by primary colonizers, and inhibition of Pyrenophora ascocarp formation in nonsterile leaves. Common pioneer colonizers lack chitinase but can grow at -7 MPa in nonsterile leaves; they do not inhibit Pyrenophora ascocarp formation. Most soil-borne invaders of straw produce chitinase but are sensitive to water stress; some inhibit ascocarp formation under wet conditions. Several residue-associated fungi produce chitinase and can grow at -7 MPa. These fungi, and some drought-sensitive but fast-growing chitinase formers, may be capable of biocontrol of residue-borne pathogens.

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REDUCED FUNGICIDE APPLICATIONS FOR CONTROL OF POD AND STEM BLIGHT OF SOYBEAN. J.S. Gershey, G.T. Berggren, and J.P. Snow. Dept. of Plant Path. and Crop Phys., La. Ag. Expt. Sta., LSU Ag. Center, Baton Rouge, LA 70803.

Fungicide evaluations were conducted over a six year period to compare single, high rate applications to multiple, low rate applications for the control of pod and stem blight, caused by *Diaporthe phaseolorum* var. *sojae*. Single applications were made at either the R3 or R5 growth stage. Multiple applications consisted of a single treatment at both R3 and R5. The cultivar 'Davis' and fungicides benomyl (Benlate 50 WP), thiophanate-methyl (Topsin-M), and chlorothalonil (Bravo) were used throughout the duration of the study. A highly significant ($P = 0.01$) yield increase over the nontreated control was observed for the single applications of benomyl at R3. Significant disease control was obtained with all fungicides applied at higher rates over nontreated controls. Disease ratings were taken at R8 using the Southern Soybean Disease Workers 0-9 rating system.

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EVALUATION OF FUNGICIDE SMOKE DEPOSITION WITH COMPUTER-CONTROLLED ELECTRON BEAM ANALYSIS. C. R. Krause[1], C. C. Powell[2] and J. M. Ichida[1]. [1]Nursery Crops Research Laboratory, USDA-ARS, 359 Main Rd., Delaware, OH 43015 and [2]Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Deposition of vinclozolin particles onto Rhododendron leaves as well as onto inert specimen stub surfaces within a greenhouse were evaluated. Vinclozolin applied as a self-dispersing smoke, was detected with computer-controlled electron beam analysis (CCEBA) using a combination of scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDXA) and automatic image analysis (AIA). Particles were catagorized for shape and size with SEM, chemically characterized with EDXA and quantified per unit area with AIA. Vinclozolin-rich particles as well as extraneous contaminants were quantified. Distribution of vinclozolin particles on both surfaces was uniform when measured in regular increments from the point of ignition.

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IN VITRO EVALUATION OF FUNGICIDES TO INHIBIT THE GROWTH AND DEVELOPMENT OF Stemphylium vesicarium, CAUSAL ORGANISM OF

PURPLE SPOT OF ASPARAGUS. J. Ciborowski and J. Percich, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Ten-day old fungal discs of Stemphylium vesicarium were placed on potato dextrose agar (PDA) plates amended with the following fungistatic compounds: Imazalil (IM) and iprodione (IP) at 0 and 10 ug/ml, mancozeb (MA) and propaconizole (P) at 0 and 25 ug/ml, and myclobutanil (MY), IP, MA and P at 0, 50 and 100 ug/mL. The fungus was incubated on amended PDA at 25C with a 12 hour light/dark period. After 15 days, all fungicides resulted in significant (P=.01) suppression of mycelial growth and conidial production when compared with the control. Propaconizole and IP at both 50 and 100 ug/ml resulted in significantly greater inhibition of mycelial and conidial development when compared with MY at the same concentrations. Imazalil at 10 ug/mL and MA at 50 ug/mL resulted in no mycelial growth.

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CONTROL OF SPHAEROTHECA FULIGINEA ON CUCURBITS WITH AN OIL EXTRACTED FROM THE AUSTRALIAN TEA TREE. M.W. Olsen, J. Cassells and D. Cross, Environmental Research Lab, Univ. of Arizona, Tucson, AZ 85706

Powdery mildew of cucurbits caused by Sphaerotheca fuliginea is a serious problem in Arizona and occurs throughout the year in greenhouses. In closed systems where fungicides cannot be used, alternatives for disease control have been sought. A non-toxic oil extracted from the Australian Tea Tree (Melaleuca alternifolia) was found to be effective against S. fuliginea in the greenhouse. When the oil, emulsified with 2.5% Tween 20, was applied weekly in a 1% aqueous solution, the mildew was controlled at least 90% on Crook Neck squash and Toska cucumber. No phytotoxicity was observed at concentrations as high as 1.5%

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COMPARISON OF PROPICONAZOL RATES FOR CONTROL OF FUNGAL BROWN SPOT ON CULTIVATED WILD RICE. C. M. Huot and J. A. Percich, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Wild rice (Zizania palustris 'K-2') was inoculated with conidial suspensions of Bipolaris oryzae. Propiconazol rates were 50, 75 and 100 g a.i./ha at boot and heading, and 100 and 125 g a.i./ha at boot only. Plants treated with 50 or 125 g a.i./ha did not have significant (p =.01) yield increases or decreased disease severity when compared with the non-treated control. Plants treated with either 75 or 100 g a.i./ha at boot and heading had significant yield increases of 29 and 94%, respectively when compared with the control. Plants receiving only one application of propiconazol at 100 g a.i./ha resulted in a significant yield increase of 52% above the control. With the exception of the 125 g a.i./ha treatment (phytotoxic), propiconazol on the average reduced leaf infection by 62, 56 and 30% on the flag, second and third topmost leaves, respectively.

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THE EFFECT OF IPRODIONE, MANCOZEB AND PROPICONAZOL ON YIELD AND FUNGAL BROWN SPOT SEVERITY OF CULTIVATED WILD RICE. J. A. Percich, and C. M. Huot, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Wild rice (Zizania palustris 'K-2') was inoculated in the field with a conidial suspension of Bipolaris oryzae. Iprodione at 0.56 and 1.13 kg a.i./ha and mancozeb at 1.8 kg a.i./ha were each applied five times on a 7-day schedule beginning at the boot stage of plant development. Propiconazol was applied at 100 g a.i./ha during boot and again 14 days later at heading. Iprodione at 0.56 and 1.13 kg a.i./ha, and mancozeb at 1.8 kg a.i./ha, resulted in significant (p = .01) yield increases of 54, 163 and 156%, respectively, above the control. Propiconazol at 100 g a.i./ha resulted in a significant yield increase of 51% above the control. All fungicide treatments on the average resulted in 40, 60 and 28% less leaf infection on the flag, second and third topmost leaves, respectively, when compared with the control.

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SCREENING FUNGICIDES FOR CONTROL OF BLACK SIGATOKA ON BANANA AND COFFEE RUST IN THE GROWTH ROOM. J.C. Davis, A.L. O'Leary and S.H. Woodhead, Ricerca, Inc., P.O. Box 1000, Painesville OH 44077

Techniques for screening fungicides for control of Black Sigatoka (Mycosphaerella difformis f.sp. fijiensis) and Coffee Rust (Hemileia vastatrix) on young plants were developed. Banana plants (4-5 leaf stage) were inoculated by spraying leaves with a suspension of conidia. Coffee plants (4-8 leaf stage) were inoculated by spraying plants with a suspension of urediospores. Inoculated plants were incubated in humidity chambers and then placed in growth chambers until lesions developed. Inoculated leaves on control plants typically had 40% and 73% diseased tissue for Black Sigatoka and Coffee Rust, respectively. Disease severity was adjusted by varying the concentration of propagules. The screens can be used to evaluate protective and curative activity. Tenacity of chemicals can be evaluated on whole plants exposed to varying levels of simulated rain with our rain table.

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ASSESSMENT OF FIELD PERFORMANCE AND SOFT ROT RESISTANCE IN A POPULATION OF PROTOPLAST-DERIVED POTATO CLONES. R.J. Taylor, C.L. Ruby and G.A. Secor, Dept. Pl Path, NDSU, Fargo, ND 58105.

Tuber yield was evaluated for 65 clones regenerated from potato (cv. Crystal) mesophyll protoplasts. Clones, planted in randomized complete blocks replicated 4 times, were compared to 2 Crystal standards; the mother clone (MC) from which the protoclone population was derived and a commercially produced selection (CP). Yield was equal to MC in 30 clones and significantly reduced in 35 clones (p=0.05). Yield, relative to the CP standard, was reduced in 25 clones, similar in 38 clones and significantly greater in 2 clones. The 30 high yield clones (yield=MC) were tested for resistance to Erwinia carotovora subsp. carotovora (Ecc) by inoculating disks of tuber tissue. Susceptibility to Ecc was expressed as weight of rotted tissue removed from the disks. Protoclones with significantly greater resistance than MC (2) and CP (1) were identified while susceptibility to Ecc was greater in nearly 40% of the clones.

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CHARACTERIZATION OF SELECTED WHEAT LEAF RUST RESISTANCES. Beatriz A. Perez, and A. P. Roelfs, Cereal Rust Laboratory, USDA/ARS and Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Resistance to wheat leaf rust (Puccinia recondita f. sp. tritici) was characterized by severity and type of host response in field tests. Area under disease progress curves (AUDPC), severities and rates of disease increase were useful to distinguish Lr13, Lr34, and Lr34&T3 from Lr12, Lr16, and LrT3, but were inadequate to distinguish between Lr13, Lr34, and Lr34&T3. Lr16 had a unique moderately resistant response. Benito, Buck Poncho, Chris, Ciano 67, Columbus, Cruz Alta Inta, Era, Fletcher, Frontana, Fronteira, Kenyon, Klein Aniversario, Klein Cartucho, Klein Titan, Las Rosas Inta, Marshall, Pampa Inta, Rio Negro, Surpresa, Veranopolis, Wheaton, TcLr13, TcLr34 and TcLr34&T3 had a significantly lower severity and AUDPC than the check. TcLr34 had a low severity until maturity when it increased sharply. Disease progress curves (DPC) best distinguished between Lr13 and Lr34 resistances in the field.

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EVALUATION OF WHEAT FOR REACTION TO TAN SPOT CAUSED BY *PYRENOPHORA TRITICI-REPENTIS*. L. Lamari and C.C. Bernier. Plant Science Dept., Univ. of Manitoba, Winnipeg, MB, R3T 2N2.

A total of 695 wheat accessions were evaluated for reaction to *P. tritici-repentis* in the growth room and categorized, using a rating system based on lesion type. A range of reactions was observed and high levels of resistance were identified in the di-, tetra-, hexa- and octoploid wheats. Resistance was characterized by small dark brown to black lesions (primary lesions) without, or with slight amounts of, tan necrosis or chlorosis, and susceptibility by primary lesions surrounded by either tan necrosis or chlorosis. About 5% of the entries developed primary lesions and extensive chlorosis, that expanded to cover the entire leaf. Reactions obtained in tests at the 2 leaf stage were similar to those obtained at the 4-6 leaf stage in the growth room and on mature plants in the field. Cultivars with the lowest lesion type scores had the lowest disease severity rating in inoculated plots and in plots sown into infected wheat stubble.

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EFFECT OF CHLORIDE APPLIED IN IRRIGATION WATER ON WHEAT TANSPOT IN A GROWTH CHAMBER. G. W. Buchenau, S. S. A. Rizvi, and P. E. Fixen, Plant Science Department, South Dakota State University, Brookings, SD 57007.

Spring wheat was watered daily with modified Hoagland's solution adjusted with $CaCl_2$ to provide chloride concentrations of 0 to 200 mg l^{-1}. Primary leaves were inoculated with Pyrenophora tritici-repentis conidia. Chloride reduced tanspot rating and number of lesions per leaf. Butte required higher chloride concentrations for tanspot reduction than Guard, which required more than Marshall, Stoa or Thatcher. Marshall had less tanspot than Thatcher in untreated pots, but applied chloride eliminated differences between these two cultivars. Soil chloride at the end of the experiment was a linear function of total chloride applied in the water. Plant tissue chloride in Butte and Guard was also a linear function of applied chloride when chloride concentration of the irrigation water exceeded 20 mg l^{-1}. Butte tissue accumulated more chloride than did Guard tissue.

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CHANGES IN WHEAT LEAF RUST SEVERITY AND REACTION TYPE BY CHLORIDE AMENDMENT. S. S. A. Rizvi, G. W. Buchenau, and P. E. Fixen, Plant Science Department, South Dakota State University, Brookings, SD 57007.

Five spring wheat cultivars and five near-isogenic lines were grown in washed sand in 4.5 X 4 cm plastic pots and watered daily with 5 ml of Hoagland's solution modified with $CaCl_2$ to provide Cl concentrations ranging from 0 to 100 mg-L^{-1}. Primary leaves were inoculated with leaf rust isolates of known virulence. Chloride reduced rust intensity of both isolates on the two types of wheats. For example, against isolate US 17, a rust intensity of 100% on Lr 3b (0 ppm Cl) was reduced to 58% (100 ppm Cl). Similar results occurred on Lr 1, Lr 16, Lr 24, Butte, Guard, Marshall, Stoa, and Thatcher. In chloride treatments, pustule type was also reduced from susceptible to resistant or intermediate eg. susceptible on Lr 1 (0 ppm Cl) to moderately resistant to resistant (100 ppm Cl) on the same line. Chloride seems to affect the phenotypic expression of the tested genes and cultivars.

666

SEVEN NEW MULTIPLE DISEASE RESISTANT PROCESSING TYPE BEANS. D. J. Hagedorn and R. E. Rand. Dept. of Plant Pathology, 1630 Linden Drive, University of Wisconsin, Madison, WI 53706.

Diseases of processing beans, Phaseolus vulgaris, are severe production constraints. Control efforts have emphasized breeding for disease resistance with primary focus on bacterial brown spot (BBS) Pseudomonas syringae pv syringae, and root rot (RR) Aphanomyces euteiches pv phaseoli and Pythium spp. Recently we have developed 7 processing-type bean breeding lines with multiple disease resistance (MDR)-resistance to BBS, RR, bean common mosaic virus (BCMV), and halo blight (HB), Pseudomonas syringae pv phaseolicola. These beans have new and unique combinations of resistance plus very good horticultural characteristics. They have been given the following Wis. (MDR) numbers: 101, 108, 201, 214, 302, 309 and 315. They may be the most disease resistant processing type beans developed.

667

EVALUATION OF BROCCOLI AND CAULIFLOWER PLANT INTRODUCTIONS FOR DOWNY MILDEW RESISTANCE. C. E. Thomas and E. L. Jourdain. ARS, USDA, U. S. Vegetable Laboratory, Charleston, SC 29414.

The 240 available U. S. Plant Introductions (PI) classified as Brassica oleracea var. botrytis (consists of both broccoli and cauliflower types) were evaluated for resistance against downy mildew. Plants at the two expanded leaf stage were inoculated with a suspension of 5.0×10^3 conidia per ml of a local isolate of Peronospora parasitica. Inoculated plants were incubated in dew chambers at 16C for 24 hours and were then placed in a 22C growth chamber with a 12 hr photoperiod. On the seventh day after inoculation, plants were returned to the 16C dew chamber for 30 hr and ratings for downy mildew reaction classes were made at nine days on a 0-9 scale of increasing disease severity. A disease index (DI) was calculated for each line. No lines had a high level of resistance (DI $\leq$ 3). PIs 181860, 204765, 204768, 204772, 204773, 204775, 204779, 241612, 291567, 343481, 462225, 264656, and 373906 were moderately resistant (DI of 3.1-5).

668

VIRULENCE OF CERCOSPORIDIUM PERSONATUM ISOLATES ON PEANUT AT THREE TEMPERATURES. B. B. Shew and M. K. Beute, Depts. of Crop Sci. and Plant Path., N. C. State Univ., Raleigh 27695.

Virulence of a North Carolina (NC) and a Thailand (T) isolate of C. personatum was compared on four peanut genotypes with low to high resistance to the pathogen. Detached leaves were inoculated with either isolate and incubated 6 da at 26/18, 30/22, or 34/26 C (day/night). Both isolates produced fewer lesions on all genotypes as temperature increased, but T always caused more infections than NC. Post-infection symptom development was studied by inoculating leaves, incubating them 6 da at 20 C, and transferring them to 26/18, 30/22, or 34/26 C. Symptom development on genotypes with low, moderate or high resistance was similar for both isolates at each temperature combination. Although fewer lesions of either isolate sporulated at higher temperatures, sporulating lesions were present on all genotypes at 34/22. The T isolate produced lesions with fewer spores than the NC isolate at all temperatures.

669

MATERNAL INFLUENCE AND EFFECTS OF HOST TRAITS ON THE RESPONSE OF SWEET CORN LINES TO FUSARIUM MONILIFORME. J. M. Headrick and J. K. Pataky. Dept. Plant Path., Univ. Ill., Urbana 61801

In 1987, sweet corn inbreds resistant or susceptible to kernel infection by F. moniliforme were crossed (with reciprocals) to determine the inheritance of resistance. A strong maternal influence was observed in crosses with the resistant inbred IL125b. The reaction of silk-inoculated F_1 hybrids approximated that of the female parent when F. moniliforme incidence was measured as symptomless kernels in vitro or as apparent infection. The maternal effect may be due to factors in female tissues or cytoplasm. In 1987, silks of resistant inbreds remained actively growing up to 21 days after pollination while silks of susceptible inbreds rapidly senesced. Thus, dead silk tissue may serve as an infection court to facilitate invasion of developing kernels. Further study of the silk mode of entry and testing of the generations necessary to distinguish endosperm, silk (pericarp) or cytoplasmic effects are underway. The effects of endosperm genotype and sugar content on infection by F. moniliforme also will be discussed.

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WOUND-HEALING PROCESS IN PEAR FRUIT AS RELATED TO INFECTION BY BOTRYTIS CINEREA and PENICILLIUM EXPANSUM. W. Janisiewicz and J. Jones, USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

Wounds of harvest-mature (20 lb pressure) 'Bartlett' pears became resistant to infection by Botrytis cinerea and Penicillium expansum if wounded fruit were incubated at 24 C and 75 ± 5% RH for a minimum of 120 hr prior to inoculation with the pathogens. This wound-healing response was not observed on more mature (12 lb) pears kept under the same conditions and on fruit at both stages of maturity kept at 2 C between wounding and inoculation. On these fruit, regardless of healing time, rots developed after inoculation with these fungi. This wound-healing response may determine a minimum period during which biological control agents must be effective in order to control wound-invading pathogens. Also, if properly managed, the wound-healing could have a significant effect on the reduction of postharvest spoilage of pears.

671

PUCCINIA RECONDITA AVIRULENT ON WHEAT CULTIVAR THATCHER. J. Huerta-Espino and A.P. Roelfs, USDA-ARS, Cereal Rust Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Triticum aestivum cultivar Thatcher has been widely used as a susceptible host and background parent for the near isogenic lines used in evaluation of resistance to leaf rust. Thatcher is known to have adult plant resistance to a few North American leaf rust cultures. As a part of a global monitoring of virulence in Puccinia graminis f. sp. tritici and P. recondita, several cultures of leaf rust from Ethiopia were found to be avirulent on seedlings of Thatcher. These cultures were obtained from durum wheats (T. turgidum), in the Addis Ababa area. The cultures are virulent on the durum cultivar Glossy Hugenot, and a few are avirulent on bread wheat cultivar

Morocco which is widely used susceptible check. A wide range of bread wheat cultivars evaluated were resistant to these cultures.

672

A COMPARISON OF ELISA METHODS USING COMBINATIONS OF MONOCLONAL AND POLYCLONAL ANTIBODIES TO DETECT THE ENDOPHYTE OF TALL FESCUE. R. A. Shelby and V. C. Kelley, Departments of Plant Pathology and Botany and Microbiology, Auburn University, AL 36849.

Rabbit polyclonal (RP) and mouse monoclonal (MM) antibodies were developed against mycelial homogenates of the tall fescue endophyte, Acremonium coenophialum. Initial screening of MM hybridoma cultures for specific antibody production utilized a modified indirect ELISA with infected and noninfected seed and plants as well as cultures of the target antigen and other nonrelated seed and plant contaminants. MM antibodies were not superior to RP antibodies in this test, however there was considerable variation in the performance of MM clones. Of the four immunoglobulin subclasses tested, IGG-1 was superior. Further enhancement of sensitivity was obtained when both RP and MM antibodies were used in a modified sandwich ELISA with a commercial anti-rabbit or anti-mouse conjugate.

674

DETECTION OF THE MAIZE BUSHY STUNT (MBS) MYCOPLASMALIKE ORGANISM (MLO) IN MAIZE USING ^{32}P-LABELED CHROMOSOMAL AND EXTRACHROMOSOMAL DNA PROBES. L. L. McDaniel, M. J. Davis, J. H. Tsai, R. L. Cox, and N. A. Harrison. University of Florida, IFAS, REC; 3205 College Avenue; Fort Lauderdale, FL 33314.

DNA was isolated from Zea mays cv. Aristogold Bantam inoculated with a Florida isolate of the MBS-MLO and probed by dot hybridization with ^{32}P-labeled recombinant plasmids pMBS11B and pMBS18B containing extrachromosomal and chromosomal MBS-MLO DNA, respectively. MLO DNA was detected in stalks 1 wk prior to symptom expression with both the extrachromosomal probe and a mixture of probes but was not detected with the chromosomal probe alone until initial disease symptoms (leaf reddening) were expressed 2-3 wk after inoculation. The minimum detection limits corresponded to 20 mg and 300 mg of tissue for the extrachromosomal and chromosomal probes, respectively. More MLO DNA was detected in stalk than in leaf tissue. MLO DNA was also detected in tassels.

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SEROLOGICAL DETECTION OF PSEUDO-CURLY TOP VIRUS (PCTV) USING AN ENZYME-LINKED IMMUNOSORBENT ASSAY. L. L. McDaniel and J. H. Tsai. University of Florida, I.F.A.S., R.E.C.; 3205 S.W. College Avenue; Fort Lauderdale, FL 33314.

The viral etiology of the pseudo-curly top disease of tomato (Lycopersicon esculentum) was demonstrated. A polyclonal antiserum to the geminivirus-like particles of PCTV was prepared to partially purified PCTV from infected nightshade (Solanum nigrum) plants. Using this antiserum in a double antibody sandwich enzyme-linked immunosorbent assay, the virus was detected in eggplant (Solanum melongena), lettuce (Lactuca sativa), nightshade and tomato plants, as well as in the treehopper vector, Micrutalis malleifera. However, the PCTV was not detected in inoculated groundcherry (Physalis floridana) or sugarbeet (Beta vulgaris). Using agar immunodiffusion assays, a relationship between PCTV and the beet curly top virus and bean golden mosaic virus was demonstrated. Nucleic acid extracted from partially purified PCTV was degraded by DNase I but not RNase A.

676

ELISA DETECTION OF PRUNE DWARF AND PRUNUS NECROTIC RINGSPOT VIRUSES IN EXTRACTS OF DORMANT BUDS AND YOUNG LEAVES OF PEACH AND PRUNE TREES. C. F. Luhn and J. K. Uyemoto, USDA-ARS, Dept of Plant Pathology, University of California, Davis, CA, 95616.

ELISA was used to test for prune dwarf (PDV) and Prunus necrotic ringspot (PNRSV) viruses. Dormant buds (in February) and young leaves (April) were collected from peach (P. persica) and prune (P. domestica) trees. Tissues were extracted in carbonate buffer, pH 9.6. For 2-year-old Carson peach trees, 17/80 bud extracts were PDV positive (avg ELISA value=1.25) and 16 trees were doubly infected with PNRSV (0.56). There were 20 trees with both viruses with leaf extracts, including all of the February sampling. Prune collections (12 mature trees) yielded one PDV- (0.18) and 12 PNRSV- (0.59) positives and 11 PDV- (0.59) and 12 PNRSV- (1.72) positives for dormant bud and leaf tissues, respectively. Controls consisting of virus-infected and healthy (h) plants (Cucumis sativus) gave values of 1.18 (PDV) and 0.05 (h) and 0.80 (PNRSV) and 0.25 (h) with PDV and PNRSV antisera, respectively. These results suggest that young leaves are the tissue of choice for ELISA.

677

BROAD-SPECIFIC DETECTION OF ANDEAN POTATO LATENT VIRUS BY DOT IMMUNOBINDING ASSAY. C. Lizárraga and C. Barrera. International Potato Center, Apartado 5969, Lima, Peru.

Direct DAS-ELISA is highly specific for the detection of Andean Potato Latent Virus (APLV) of which three serological strain groups (Hu, Caj-Col and CCC) have been described. Only the Hu and the Caj-Col groups have been found so far in Peru. A simple dot immunobinding assay (DIBA-ELISA) in which crude sap extracts are spotted onto nitrocellulose membranes detected isolates of these two serological strain groups using antiserum to Hu. Similarly, an antiserum against Caj-2, an isolate from a native potato cultivar that did not react in direct DAS-ELISA with Hu antiserum and which was shown to be a member of the Caj-Col group by gel diffusion and intragel adsorption tests, was able to detect isolates of APLV in the Hu and Caj-Col groups. The homologous and heterologous reaction spots had almost the same intensity.

678

EVALUATION OF FRENCH EXPERT SYSTEMS FOR DIAGNOSIS OF TOMATO AND POTATO DISORDERS. W. R. Stevenson and S. S. Adams, Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

In 1984, an extensive program to develop expert systems for plant disease diagnosis was initiated by the National Institute of Agronomic Research (INRA) in France. Rule-based systems have been written to diagnose a wide range of plant disorders affecting 18 field, ornamental, fruit, and vegetable crops. We are currently involved with INRA in a cooperative evaluation of the tomato and post-harvest potato modules. The tomato and potato modules consider 42 and 67 disorders, respectively. Twenty-five disorders for each crop have been tested. In each case, 17 disorders were diagnosed with 7-15 questions and 7 required 16-35 questions. Currently, 11 potato and 25 tomato disorders found in the U.S. are not considered by these expert system modules. In addition to determining the accuracy of diagnoses, we are identifying ambiguities, deficiencies, and inconsistencies in the knowledge bases. These modules appear to have potential for use in U.S. extension programming.

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LOSS OF ENDOGENOUS CARBON BY CONIDIA OF COCHLIOBOLUS SATIVUS AND ITS EFFECT ON GERMINATION AND VIRULENCE. C.A. Jasalavich, M. Hyakumachi, and J.L. Lockwood, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Cochliobolus sativus conidia progressively lost endogenous carbon when incubated on natural or sterilized Capac loam at -10 mb and -100 mb matric potentials for 50 days. Loss of ^{14}C was detected as $^{14}CO_2$ evolved from soil and conidia and as residual ^{14}C in the soil. Conidia lost more carbon when incubated on sterilized (25% of total label) than on natural (12% of total label) soil, but there were no differences in loss due to matric potential. Conidia germinated on sterilized, but not on natural soil. Conidia did not lose

nutrient independence for germination after incubation on either sterilized or natural soil. Virulence of conidia also did not decline even after 50 days of incubation on sterilized or natural soil. These results contradict previous reports for this fungus with respect to loss of nutrient independence and virulence.

680

EFFECT OF CULTURAL PRACTICES ON COMMON ROOT ROT OF SPRING WHEAT. B. Salas and R. W. Stack. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

The influence of tillage, fertility and crop rotation on the development of common root rot in spring wheat was studied in the field. Treatments were arranged as main plots or subplots in a randomized complete block design. Incidence and severity of common root rot was determined by the subcrown internode index. Among the known root rot pathogens isolated from diseased crowns and roots, Helminthosporium sativum was predominant followed by Fusarium avenaceum. Other cereal root pathogens were recovered at much lower rates. Nitrogen level or added phosphate did not affect any of the parameters measured. Root rot incidence and severity were greater under conventional tillage than under reduced tillage and were greater in rotations including sunflowers. Recovery of H. sativum paralleled root rot disease measurements.

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ROOT-INFECTING FUNGI ASSOCIATED WITH WHEAT IN THE WEST CENTRAL REGION OF MOROCCO. A. Lyamani and D.C. McGee, Aridoculture Center, B.P. 290. Settat, Morocco, and Dept. of Plant Pathology, Iowa State University, Ames, IA. 50011

Wheat field surveys were carried out in the 1986 and 1987 growing seasons in the west central region of Morocco. Ten root-infecting fungi were isolated Fusarium acuminatum, F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. monoliforme, F. oxysporum, F. solani, Helminthosporium sativum, and Stemphylium sp. H. sativum and F. equiseti were the most prevalent species, while F. acuminatum, F. avenaceum, F. graminearum and Stemphylium sp. the least. Isolations from seeds, soils and plants indicated that root rot in the surveyed area was chiefly caused by soil-borne inoculum.

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PATHOLOGY OF PLASMOPARA LACTUCAE-RADICIS, A ROOT-INFECTING DOWNY MILDEW OF CULTIVATED LETTUCE. M. E. Stanghellini, J. E. Adaskaveg, and S. L. Rasmussen. Dept. of Plant Pathology, Univ. of Arizona, Tucson, Az, 85721.

Plasmopara lactucae-radicis, a newly described downy mildew (Mycotaxon 31:395-400), is reported for the first time as a root pathogen of hydroponically-grown lettuce. This unique fungus, which colonizes only roots, caused yield reductions of ca. 50% in greenhouse pathogenicity tests. Direct penetration of roots by zoospore cysts, systemic invasion of roots by intercellular hyphae and intracellular haustoria, and sporulation on infected roots occurred at root temperatures between 16 and 25 C. No infection occurred at root temperatures of 12, 14, or 30 C. Oospores formed in necrotic roots ca. 10 days after inoculation.

683 Withdrawn

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A CONCEPTUAL MODEL OF ROOT GROWTH. J.H. Bowers[1], G.H. Smerage[2], and D.J. Mitchell[1]. [1]Dept. of Plant Pathology and [2]Dept. of Agricultural Engineering, Univ. of Florida, Gainesville, FL 32611.

A process-oriented model is presented for the description of root growth in dicotyledonous seedlings with taproots. This model is being developed in a study with the objective to describe the relationship between root growth and root infection by Phytophthora. The pepper root system was used to develop the model. The model is based on processes depicting the initiation and development of root segments. A root segment is defined as that portion of a root between successive branches or from the most terminal branch to the root tip. Each segment is identified by its root order, according to the morphometric root analysis system, and root type. Three root types are recognized; taproot, primary and secondary lateral roots, and basal roots (roots initiated in the basal region of the hypocotyl). The model begins with planting, progresses through seed germination and taproot formation, primary and secondary lateral branching, and basal root initiation and branching. Branching transformations initiate first-order roots and define second- and third-order segments. The delays between successive stages are major determinants of the dynamics of the model. The temporal response of a delay process corresponds to the probability density function of the transit time between branching events. The model is adaptable to fibrous root systems and monocotyledonous seedlings.

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INDUCTION OF DISEASE SYMPTOMS ON WHEAT BY TOXIC METABOLITE(S) FROM PYTHIUM ARRHENOMANES. H. Mojdehi and L. L. Singleton. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Pythium arrhenomanes (Drechs.) isolates were grown in a glucose-glutamic acid liquid medium at pH 6 for 18 days (25C, dark). Cell-free filtrates inhibited root hair formation, as well as root elongation, and caused root browning, all of which are characteristic symptoms of Pythium root rot. Filtrates were separated into various molecular weights (M.W.) by ultrafiltration and dialysis. The toxic metabolite was found to consist of at least two components. The smallest component was <1000 in M.W., and the largest component >50,000, but <100,000 in M.W. Both components were needed to reproduce whole culture filtrate effects. Effects of culture filtrates on wheat were reproduced by the addition of the larger toxic component into medium alone. This may indicate that the smaller component could be a medium constituent.

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LOSS OF VIABILITY OF CONIDIA OF COCHLIOBOLUS SATIVUS FOLLOWING GERMINATION ON SOIL TREATED WITH ATRAZINE. T. Isakeit and J.L. Lockwood, Dept. Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824.

The effect of atrazine (25 µg/g) on conidia of Cochliobolus sativus exposed to fungistatic conditions on Boyer sandy loam (-1 kPA matric potential) was monitored by placing conidia on polycarbonate membranes on soil and examining for germination on soil and subsequent viability on carrot agar. After ten days, 18% of the conidia had germinated and viability of condiia had dropped from 95% to 80%. The viability of conidia that had germinated on soil was 25%, while the viability of conidia that had never germinated on soil was 96%. 82% of conidial germ tubes lysed within one day, and 100% lysed within three days after germination on soil. Earliest germination occurred 18 hours after exposure to atrazine, but maximum germination occurred between 1 and 2 weeks. These results demonstrated that death of conidia on atrazine-treated soil is associated with their previous germination on soil.

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EFFECT OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM CULMORUM ON ROOT ROT AND YIELD OF FOUR DURUM WHEAT VARIETIES. A. Lyamani and D. C. McGee; Aridoculture Center, B. P. 290, Settat, Morocco; and Dept. of Plant Pathology, Iowa State University, Ames, IA. 50011

Seed of four durum wheat varieties was inoculated with either H. sativum or F. culmorum and planted in 1986 and 1987 growing seasons in plots in which the soil was similarly inoculated. Non-inoculated seed and soil plots were also included as comparative checks. Emergence, root rot severity, incidence of deadheads, straw and grain yield were measured. F. culmorum was more virulent and damaging than H. sativum to measured parameters. The variety Cocorit was very susceptible to F. culmorum and slightly susceptible to H. sativum. Kyperounda was moderately susceptible to both pathogens. Marzak and Karim were moderately susceptible to F. culmorum and showed good resistance to H. sativum.

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CLONING AND SEQUENCING OF THE COAT PROTEIN CISTRON OF MAIZE DWARF MOSAIC VIRUS, STRAINS A AND B. J. Jilka and J.M. Clark, Jr. Department of Biochemistry. University of Illinois, Urbana, IL 6180].

We are cloning and sequencing the viral RNAs of maize dwarf mosaic virus, strains A and B. Our initial efforts have focused specifically upon the characterization of the coat protein cistron of these viruses. After isolation of viral RNAs from these viruses, we have used two methods to characterize the 3'-terminal regions of these RNAs. First, we have used oligodT primers and reverse transcription to generate complimentary cDNA from the RNAs. The cDNAs were inserted into a plasmid vector and sequenced in the denatured plasmid DNA by the dideoxy sequencing method. Second, we used a primer deduced from a highly homologous central region of the coat protein of other potyviruses and reverse transcriptase to sequence a central portion of the coat protein cistrons.

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EVIDENCE FOR A PROTEIN BOUND TO THE dsRNA OF CRYPHONECTRIA PARASITICA. Y. Wu and N.K. Van Alfen, Molecular Biology/Biochemistry Program,Department of Biology, Utah State University, Logan, 84322-5305.

The double-stranded (ds) RNA associated with hypovirulence of Cryphonectria (Endothia) parasitica does not have a protein capsid. It is compartmentalized within host membrane vesicles. The dsRNA appears to be of viral origin, but the lack of infectivity and a protein capsid creates difficulties in its classification as a virus. Associated with the dsRNA within the fungal vesicles is a polymerase, the products of which are primarily plus sense single-stranded RNA. To understand the nature of this virus and its replication strategy, we have been attempting to isolate replicative intermediates of the virus. The dsRNA isolated from vesicles has been shown to be in two forms, one associated with a protein(s) and another free of the protein. Gel retardation of dsRNA isolated from fungal vesicles, and the abolishment of the retardation by treatment of the complex with pronase or proteinase K, suggest that a protein(s) bound to the dsRNA is responsible for its slower migration in agarose gels. When the dsRNA is isolated using phenol extraction, gel retardation is not observed. The possibility that the protein bound to the dsRNA is a polymerase is being explored.

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DOT BLOT DETECTION OF TOMATO SPOTTED WILT VIRUS USING CLONED cDNA PROBES. T. L. German and D. Rice, Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822.

We have prepared a library of clones specific for tomato spotted wilt virus RNA. E. coli was transformed with recombinant pBR322 containing cDNA prepared from partially purified viral RNA. Colonies were selected which would hybridize with cDNA prepared from the RNA in sucrose gradient fractions of infected plants corresponding to partially purified virus preparations but not with cDNA prepared from RNA in identical fractions of uninfected plants. DNA complementary to viral RNA was excised from plasmid DNA with a restriction endonuclease (Pst I), separated by agarose gel electrophoresis and ^{32}P labeled without further purification using DNA polymerase and random primers. When used as a probe in a standard nucleic acid dot blot assay the test could easily distinguish between TSWV infected and uninfected plants of several genera collected from a variety of locations. These include tobacco, tomato, lettuce, chrysanthemum, and Emelia.

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CLONING OF ZUCCHINI YELLOWS MOSAIC VIRUS. Rebecca Grumet, Horticulture Department, Michigan State University, East Lansing, Michigan 48824.

Zucchini yellows mosaic virus (ZYMV), a ssRNA virus of the potyvirus group, causes serious crop losses in cucurbit species. We seek to use a pathogen-derived resistance approach to genetically engineer resistance to this disease and so have produced cDNAs to the virus. The virus was purified from infected zucchini leaves using 2 cycles of PEG precipitation and ultracentrifugation in a 0-1.2 M sucrose-cesium sulfate gradient. Coat protein prepared from SDS-degraded virions migrated as a single band in SDS-PAGE and was used for antibody production. Viral RNA was isolated from SDS-degraded virions in a linear sucrose gradient and was used as a substrate for cDNA synthesis using an oligo dT primer and reverse transcriptase. ds cDNA was blunt-end ligated into SmaI cut Bluescript vector. Several transformants containing inserts of 0.5-5 Kb were identified. Results of antibody screening and restriction mapping will be presented.

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EXPRESSION IN BACTERIA OF THE PUTATIVE POLYMERASE GENE OF BEAN GOLDEN MOSAIC VIRUS. A. J. Howarth and J. G. Utermohlen. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

A plasmid (pAZ18) containing the putative polymerase gene of bean golden mosaic virus (BGMV) was constructed by cloning the 1452 basepair HindIII-EcoRI of DNA1 in Bluescript(+). The pol gene was fused with the plasmid-borne lacZ gene by deletion of 5'-upstream sequences with exonuclease III and mung bean nuclease following linearization of the plasmid by digestion with KpnI and HindIII. Deletion mutants were screened by restriction analysis and nucleotide sequencing. One construction, pAZ18d304, was an in-frame fusion of lacZ and pol. A novel protein of 40.7 kDa was produced in E. coli under control of the lac promoter.

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PHYLOGENY OF FOURTEEN GEMINIVIRUSES. A. J. Howarth and J. G. Utermohlen. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

The amino acid sequences of the putative polymerases of fourteen geminiviruses were aligned and the minimum mutation distance was computed. All possible pairwise comparisons were used to construct a phylogenetic tree. The two main branches correlated with the host range specificities of the viruses to either monocots or dicots. No correlation was seen for vector specificity or number of genomic DNAs. The branch of dicot-infecting viruses divides geographically into Old World and New World viruses. The polymerase gene of the dicot-infecting viruses is a single open reading frame, while in the monocot-infecting viruses the gene is in two overlapping open reading frames.

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The Mapping of the Five Prime Leader Region of Viral Transcripts to Bean Golden Mosaic Virus Genome. J. G. Utermohlen and A. J. Howarth. Department of Plant Pathology, University of Arizona, AZ 85721.

The 5' untranslated leader sequence of the viral transcripts from bean golden mosaic virus (BGMV) were mapped to its genome. Using primer extension analysis with oligonucleotide primers derived from the viral genome, the leader sequences of six viral transcripts were deduced. RNA preparations from both infected and non-infected plants were compared and complementary DNA (cDNA) bands were detected in both reactions. The reactions with RNA from BGMV-infected plants contained unique cDNA species. The results of these comparisons indicate that the leader sequences of genes I through VI are 84, 83, approximately 96, 126, 205, and 109 bases in length, respectively. Appropriate promoter signals are located upstream from these transcription-initiation sites in the viral genome.

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EXPRESSION OF FOREIGN GENES BY TOBACCO MOSAIC VIRUS IN NON-SOLANACEOUS HOSTS. D. Lewandowski, P. Bubrick, P. R. Desjardins, and W. O. Dawson, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

We report development of tobacco mosaic virus (TMV) based vectors for the expression of foreign genes. The bacterial gene chloramphenicol acetyltransferase (CAT) was inserted into TMV cDNA either 5' (CAT-CP) or 3' (CP-CAT) of the coat protein (CP) gene to produce virion-based vectors, or in place of the CP gene (S3-CAT-28) to produce a free RNA vector. Infectious *in vitro* RNA transcripts of the chimeric viral constructs were produced which replicated and expressed CAT activity. CAT activity was detectable in tobacco and several non-solanaceous hosts. Low levels of CAT activity were associated with CP-CAT suggesting that the location of the foreign gene may influence expression levels.

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SITE-DIRECTED MUTAGENESIS OF TOBACCO MOSAIC VIRUS LEADING TO ALTERED DISEASE SYMPTOMS. J. N. Culver and W. O. Dawson, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Site-directed mutagenesis was utilized to create a number of point mutations in the coat protein (CP) gene of tobacco mosaic virus (TMV). Single base changes were selected so as to alter the amino acid sequence of the CP. These changes were based on the CP amino acid sequence of a number of TMV symptom mutants previously described (Funatsu and Fraenkel-Conrat, Biochemistry, 3:1356). Single base changes were incorporated into a subcloned portion of the CP gene via M13 *in vitro* mutagenesis. Base changes were confirmed by DNA sequencing and the mutant CP genes reassembled into full length cDNA clones of wild type TMV. Infectious transcripts of mutant cDNA induced a hypersensitive symptom on the normally systemic *Nicotiana sylvestris*.

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EPITOPE SPECIFICITY OF STRAIN-, VIRUS-, SUBGROUP-SPECIFIC AND POTYVIRUS GROUP CROSS-REACTIVE MONOCLONAL ANTIBODIES. Ramon Jordan and John Hammond. USDA-ARS, PSI, Beltsville Agricultural Research Center, Beltsville, Md. 20705

A panel of 42 monoclonal antibodies (McAbs) generated to a mixture of distinct potyviruses were evaluated by comparative antigenic analysis, in antigen competition assays, and in tests with biochemically manipulated intact virus and protein subunits. The McAbs define at least 27 potyviral coat protein epitopes based on their differential reactivities with 47 isolates/strains of 25 distinct (definitive) potyviruses. One McAb recognizes an epitope conserved on all 66 isolates, of 35 definitive potyviruses, so far tested. In general, the virus-specific McAbs react with trypsin-cleaved N-terminal peptides or other surface-located sites, whereas the cross-reactive McAbs recognize sites found within virus particles. Comparison of McAb virus- and coat protein polypeptide fragment(s)-specificity with amino acid sequence data more closely defines specific epitopes and their putative amino acid sequences.

700

Ti PLASMID TRANSFORMATION AND REGENERATION OF NICOTIANA EDWARDSONII - A USEFUL HOST FOR MANY PLANT VIRUSES. J. M. Kiernan, K.-B. Goldberg, J. E. Schoelz, S. Gowda, and R. J. Shepherd. University of Kentucky, Lexington, KY 40546.

Nicotiana edwardsonii Christie and Hall, a hybrid of N. clevelandii x N. glutinosa, is a host to at least eleven plant viruses, including three caulimoviruses. Experiments with several culture media to determine their ability to regenerate shoots from N. edwardsonii leaf discs, showed that MS medium containing either 50μM 2iP and 1.7μM IAA or 12.3μM 2iP and 4.6μM kinetin was most effective. Gene VI of CM1841 and D4 strains of cauliflower mosaic virus and genes I and VI of figwort mosaic virus were successfully introduced into N. edwardsonii using either pGA472 or pKYLX-7 Ti plasmid gene vectors bearing a selectable marker for kanamycin resistance. Selection of transformed shoots was achieved by using kanamycin in these media. Presence of viral genes was confirmed by DNA dot blot and western blot analyses.

701

GENETIC ANALYSIS OF GENE VI OF CAULIFLOWER MOSAIC VIRUS STRAIN W260, A GENE WHICH DETERMINES SYSTEMIC INFECTION OF NICOTIANA BIGELOVII. E.J. Anderson and J.E. Schoelz, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Cauliflower mosaic virus (CaMV) strains W260 and CM1841 have common hosts in the Cruciferae but can be distinguished by their ability to cause systemic symptoms on Nicotiana bigelovii. CaMV strain W260 is able to systemically infect N. bigelovii, while CM1841 does not induce any response in this host. Previous work has demonstrated that a 496 bp region in the first half of gene VI has a role in determining systemic infection of N. bigelovii by strain W260. We report here the nucleotide sequence of the first half of gene VI from strain W260. Sequence comparisons between W260 and CM1841 revealed seven amino acid differences within the first half of gene VI. We also present a strategy for determining precisely which amino acid substitutions are responsible for the observed host range differences.

702

CHARACTERIZATION OF A TEMPERATURE INDUCED RNA IN TOMATO BUSHY STUNT VIRUS INFECTIONS. A.L. Goldstein and T.J. Morris. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

A temperature induced symptom attenuation, similar but not identical to that induced by defective interfering RNAs, has been observed in Nicotiana clevelandii inoculated with tomato bushy stunt virus (TBSV). Nucleic acid analysis of infected N. clevelandii plants grown between 28 and 32 C revealed an additional viral-specific dsRNA not evident in plants grown between 16 and 21 C. This species corresponded in size to an additional ssRNA species of about 3.5 kilobases also present in the tissue. Northern analysis demonstrated that the 3.5 kb RNA was 3' co-terminal with genomic TBSV and that it was not a DI species in that it failed to hybridize to 5' terminal cDNA clones. The possibility that the 3.5 kb RNA represents a third subgenomic species is under investigation.

703

SEQUENCE HOMOLOGY BETWEEN RNA2 OF ARABIS MOSAIC VIRUS AND RNA1 OF STRAWBERRY LATENT RINGSPOT VIRUS. A. Hadidi, R. C. Walsh, J. M. Kaper, M. E. Tousignant, and P. Piazzolla. USDA, ARS, PSI, National Plant Germplasm Quarantine Center, Glenn Dale, MD 20769.

SP6-generated cRNA transcripts of cloned cDNA of arabis mosaic virus (AMV)-RNA2 were used as hybridization probes to detect the AMV genome and related sequences. In northern blot hybridization experiments, ^{32}P-labeled cRNA hybridized to AMV-RNA2. The probe did not hybridize to nucleic acids isolated from healthy Chenopodium quinoa plants or to RNA isolated from purified tomato ringspot virus, tomato bushy stunt virus, tobacco ringspot virus, tobacco mosaic virus, cucumber mosaic virus or to purified RNA2 of strawberry latent ringspot virus (SLRV). SLRV-RNA1, however, hybridized to the probe. These results indicate a sequence homology between SLRV-RNA1 and AMV-RNA2 and support the definitive assignment of SLRV to the nepovirus group.

704

CHARACTERIZATION OF THE COAT PROTEIN GENE OF TOMATO BUSHY STUNT VIRUS. P.Q. Hearne, B.I. Hillman and T.J. Morris. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

We have cloned and sequenced most of the genome of the cherry strain of tomato bushy stunt virus (TBSV) and have identified and mapped the position of two subgenomic RNAs, approximately 2.2 and 1.0 kb as co-terminal with the 3' end of the viral genome. The 5' terminus of the 2.2 kb subgenomic RNA has been mapped to a position 2156 nucleotides from the 3' terminus of the viral genome. The translation of the first open reading frame (ORF) of the 2.2 kb subgenomic RNA was compared with published capsid protein amino acid sequence of the BS-3 strain. These results confirm that the 2.2 kb subgenomic RNA is the messenger RNA for the capsid protein and that it is located internally on the genome of TBSV.

705

TURNIP CRINKLE VIRUS INFECTION FROM IN VITRO TRANSCRIBED INOCULUM. L.A. Heaton, and T.J. Morris. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

We have recently determined the complete nucleotide sequence of the 4.0 kb genomic RNA of turnip crinkle virus (TCV). In order to take advantage of this relatively simple, genetic system, we constructed cDNA plasmids from which infectious RNA can be synthesized in vitro. To facilitate subsequent

sub-cloning and run-off transcriptions, Sma I and Xba I recognition sequences were introduced at the 3' and 5' ends, respectively. Infectious RNAs were synthesized in vitro by ligation of full- length TCV cDNAs to both E. coli and T7 RNA polymerase promoters, linearization with Xba I, and transcription by the polymerases. The absence of satellite RNAs was used to demonstrate that the TCV infections were derived from cloned cDNA.

706

DETECTION OF STRAWBERRY CRINKLE VIRUS USING LECTIN PROBES. B.G. Hunter, T.J. Morris, and A.O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

Glycoprotein-specific, biotinylated lectins have been used as probes for a Western blot type of analysis to monitor the purification of strawberry crinkle virus (SCV), a plant rhabdovirus. Partially purified SCV preparations from infected Physalis tissue contain a major protein of about 75,000 daltons when analyzed by SDS-PAGE. We believe this disease-specific protein is SCV glycoprotein because it binds concanavalin A, wheat germ agglutinin and Pisum sativum agglutinin. These results are the first convincing demonstration of the ability to detect SCV using an in vitro procedure.

707

STRUCTURE AND RELATIONSHIPS OF PANICUM MOSAIC VIRUS SATELLITE RNAS. A. O. Jackson, D. S. Sopher, C. Masuta, and D. Zuidema, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Panicum mosaic virus (PMV) and related strains have RNA genomes of 4600 NT and capsid proteins 30,000 MW. They can support replication of satellite viruses that enhance symptoms on graminaceous hosts. The physicochemical properties of the satellites have been studied by electrophoresis, RNA hybridization and sequence analysis. Satellite PMV RNA is 826 NT long and the 17,000 MW coat protein is encoded by the 5' most open reading frame (ORF). Three isolates of the related St. Augustine decline virus contain the 800 NT RNA plus small RNAs of 380 NT that are encapsidated in satellite virus particles. One of these strains also contains an additional RNA of about 420 NT. Since these small RNAs fail to cross hybridize with satellite virus probes, we postulate that they are satellite RNAs that depend on PMV for replication, but use the satellite virus capsid protein for encapsidation.

(See page 1618 for Abstract 708)

709

MOLECULAR CHARACTERIZATION OF DEFECTIVE INTERFERING RNAS OF TOMATO BUSHY STUNT VIRUS. D.A. Knorr and T.J. Morris. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

Defective interfering RNAs have been generated by high multiplicity passage of tomato bushy stunt virus. We are in the process of generating complete cDNA clones to a number of these de novo generated DI species. We have constructed a plasmid containing the 3' terminus of TBSV joined to a T7 RNA polymerase promoter. Manufacture of this plasmid as single-stranded DNA and precise linearization between the promoter and 3' complementary region allows priming of cDNA synthesis to generate a linearized ssDNA plasmid containing the desired TBSV DI sequences. Second strand synthesis is primed by utilizing a synthetic oligonucleotide complementary to both the T7 promoter region and the 5' terminal cDNA of TBSV.

710

DE NOVO GENERATION OF TOMATO BUSHY STUNT DEFECTIVE INTERFERING RNAs. T.J. Morris, R. Mullin, P. Hearne and B. Hillman. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

Tomato bushy stunt virus (TBSV-cherry) was inoculated to a local lesion host and 12 single lesion isolates were passed on either of two systemic hosts, N. clevelandii and N.benthamiana. Six of the single lesion isolates were serially passed in each host without dilution (high m.o.i.) and two were passed at a sap dilution of 1/200 (low m.o.i.). Symptom attenuation was evident in the high m.o.i. inoculations as early as passage 3 and all high m.o.i. isolates developed attenuated disease. None of the 4 low m.o.i. isolates displayed reduced virulence by passage 12 when the experiment was terminated. In all cases, the appearance of symptom attenuation was coincident with the appearance of defective interfering RNA molecules in the plants as monitored by northern hybridization.

711

BIOLOGICAL ACTIVITY OF IN VITRO TRANSCRIPTS DERIVED FROM cDNA CLONES OF BARLEY STRIPE MOSAIC VIRUS. I.T.D. Petty, B.G. Hunter & A.O. Jackson, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Barley stripe mosaic virus (BSMV) is the type member of the hordeivirus group and has a tripartite plus-sense RNA genome. We have devised a unique one-step cloning strategy which allows full-length viral cDNA to be directionally inserted behind a bacteriophage T7 promoter. Full-length cDNA clones comprising the complete genome of both the Type and ND18 strains of BSMV have been isolated, and appropriate mixtures of in vitro transcripts derived from these clones are infectious when inoculated onto barley seedlings. These clones, together with those derived from other BSMV strains, will allow definitive pseudorecombination studies to map the genetic determinants for viral phenotypes such as seed transmission and ability to form local lesions on various plant hosts.

712

COMPARISONS OF THE GENOMIC SEQUENCE AND ORGANIZATION OF MELON NECROTIC SPOT VIRUS AND CUCUMBER NECROSIS VIRUS. C. J. Riviere and D. M. Rochon. Plant Science Department, University of British Columbia, Vancouver, B. C. V6T 2A2, and Agriculture Canada Research Station, Vancouver, B. C. V6T 1X2.

Melon necrotic spot virus (MNSV) and cucumber necrosis virus (CNV) are monopartite, ssRNA viruses with genomes of ca. 1.5 x 10^6 d and isometric particles of ca. 30 nm. These viruses are both transmitted by the soil fungus Olpidium radicale and infect members of the Cucurbitaceae. We have determined the complete nucleotide sequence of CNV (a Tombusvirus) and greater than 95% of the nucleotide sequence of MNSV. Our data indicate that MNSV is a Carmovirus. Similarities and differences between the genomes and predicted gene products of MNSV and CNV will be summarized. Regions of the genome, currently under investigation for their possible involvement in the Olpidium transmission of these viruses, will be presented.

713

MOLECULAR CLONING OF cDNA TO POTATO VIRUS Y RNA. D. W. Thornbury, Hans Dessens, and T. P. Pirone. University of Kentucky, Lexington, KY 40546-0091.

The RNA of potato virus Y (PVY) was purified and complementary DNA (cDNA) was cloned into the bacterial plasmid pUC119. Nucleotide sequence analysis showed that a specific, less than full length cDNA was generated by oligo dT priming at an internal site in addition to the 3' terminal poly (A) tail of the viral RNA. Comparison of the nucleotide sequence of a 1.5 Kb region of the PVY genome with that of tobacco vein mottling virus (TVMV) and tobacco etch virus (TEV), two other members of the potyvirus group, showed 40% sequence homology with the TVMV cylindrical inclusion (CI) gene and 30% homology with TEV-CI. Comparison of the amino acid sequence deduced from the nucleotide sequence showed 64% and 66% homology for TVMV and TEV respectively. This suggests that the gene for CI protein represents a highly conserved region of the genome.

714

DETECTION OF TOMATO RINGSPOT VIRAL GENOME BY HYBRIDIZATION WITH SP6-GENERATED cRNA PROBE. A. Hadidi, R. C. Walsh, and C. A. Powell. USDA, ARS, PSI, National Plant Germplasm Quarantine Center, Glenn Dale, MD 20769.

DNA fragments complementary to RNA of the Prunus stem pitting strain of tomato ringspot virus (TmRV) were cloned into pBR322 and subcloned into SP6 transcription vector pSP64. A high specific activity ^{32}P-labeled cRNA probe was generated by SP6 RNA polymerase. In northern blot hybridization analyses, the probe hybridized with RNA from TmRV but not with RNA from tobacco ringspot virus, arabis mosaic virus, strawberry latent ringspot virus, or cucumber mosaic virus. In dot blot hybridization experiments, the cRNA probe hybridized with total nucleic acids isolated from

TmRV infected woody and herbaceous hosts. No hybridization was obtained with total nucleic acids isolated from healthy controls.

715

RECONSTITUTION OF TURNIP CRINKLE VIRUS WITH IN VITRO SYNTHESIZED RNA TRANSCRIPTS. N. Wei, L.A. Heaton and T.J. Morris. Department of Plant Pathology, University of California, Berkeley, CA., 94720.

Reconstitution of structurally intact and infectious turnip crinkle virus (TCV) particles has been achieved by reassembling isolated viral coat protein and phenol extracted viral RNA. High affinity coat protein binding sites have been sequenced and mapped on the viral RNA in an effort to locate sites of initiation of virus assembly. Complete cDNA clones of the viral genome have been constructed and infectious RNA transcripts have been reconstituted with coat protein subunits using specific conditions for reassembly with native viral RNA. Mutagenesis studies are now in progress to identify regions of the genome which participate in the reassembly process.

716

cDNA CLONES OF TOMATO SPOTTED WILT VIRUS RNA. D. J. Rice and T. L. German, Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822.

Tomato Spotted Wilt Virus (TSWV) was partially purified from a plant homogenate by differential and surcrose gradient centrifugation. When RNA was purified from this preparation and analyzed by denaturing formaldehyde agarose gel electrophoresis, it was estimated that the RNA preparation consisted of 1% viral RNA. The RNA was cloned into E. Coli using standard methods and screened for infection specific colonies by differential colony hybridization. Infection specific colonies were further characterized by the ability of the cloned insert to hybridize to RNA purified from healthy, mock inoculated, or tobacco mosaic virus infected plants. Northern blot analysis revealed that the cloned inserts are specific to several TSWV RNA bands.

717

ENZYMES ASSOCIATED WITH GLYCOLYSIS AND KREBS CYCLE IN SPIROPLASMAS. Jianchi Chen and C. J. Chang. Department of Plant Pathology, University of Georgia, Griffin, GA 30223

Log-phase spiroplasma cells were harvested after centrifugation at 19,600g for 30 min. Pellets were washed twice with 1X Kappa buffer supplemented with 0.23M sucrose. Crude enzyme, obtained by osmotic lysis of washed pellets in osmolytic buffer containing 10 mM HEPES buffer (pH 7.4), 2 mM 2-mercaptoethanol, and 1 mM $MgCl_2$, were fractionated into cytosol and membrane fractions by centrifugation at 40,000g for 40 min. Activities of lactate dehydrogenase, NADH oxidase, and malate dehydrogenase were detected in all four spiroplasmas: S. citri, S. melliferum, S. apis, and S. floricola. Enzymes not detected were citrate synthase, aconitase, succinyl-CoA synthetase, fumarase, and three dehydrogenases, i.e., isocitrate, α-ketoglutarate, and succinate. Spiroplasmas apparently do not utilize the Krebs cycle for energy, yet the pathway that involves malate dehydrogenase warrants further investigation.

718

METABOLISM OF XYLELLA FASTIDIOSA ASSOCIATED WITH PIERCE'S DISEASE OF GRAPEVINES. C.J.Chang, Department of Plant Pathology, University of Georgia, Griffin, GA 30223-1797.

Cell extracts of two aerobically grown Pierce's disease strains of Xylella fastidiosa and one strain of Xanthomonas campestris pv campestris were examined for 11 different enzyme activities associated with glycolysis, the Krebs cycle or pentose phosphate shunt. Phosphohexose isomerase was detected in both strains of X. fastidiosa and X.c.pv campestris, whereas lactate dehydrogenase was detected only in X. c.pv campestris. Glucose-6-phosphate dehydrogenase and eight enzymes associated with the Krebs cycle, namely citrate synthase, aconitase, fumarase, succinyl-CoA synthetase, and four dehydrogenases i.e., isocitrate, α-ketoglutarate, succinate, and malate were detected. Both X. fastidiosa and X.c.pv.campestris derive energy through the Krebs cycle and pentose phosphate shunt. Based on these results, X.c.pv campestris has a glycolytic pathway for energy whereas X. fastidiosa does not.

719

MOLECULAR CLONING OF AND SCREENING BY A NEW METHOD FOR DNA FRAGMENTS FROM ELM YELLOWS (EY) AND TOMATO BIG BUD (BB) MYCOPLASMALIKE ORGANISMS (MLOs). I.-M. Lee, R. E. Davis, and N. D. Dewitt. USDA, ARS, Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705.

DNA fragments from EY and BB infected plants were cloned in sp6 plasmid vectors. A procedure was developed to screen for MLO-specific recombinant plasmids by using non-radioactive, biotinylated DNA preparations from healthy or MLO-infected plants. Sixty and 88 recombinant plasmids were found specific to EY and BB diseases, respectively. Biotinylated probes, constructed from several of the EY- and BB-specific recombinant plasmids, hybridized in dot blot hybridizations with nucleic acid extracts from EY and BB infected plants of periwinkle, respectively, but not from healthy plants.

720

CURRENT EXPERIMENTAL HOST RANGE OF THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT. Golino, D. A.*, Oldfield, G. N.**, and Gumpf, D. J.***. USDA-ARS, Dept. of Plant Pathology, Univ. of Calif., Davis, California*; USDA-ARS, Boyden Lab** and Dept. of Plant Pathology, Univ. of Calif., Riverside, California***.

The leafhopper vector, Circulifer tenellus, was used to inoculate 57 species of plants with the Beet Leafhopper Transmitted Virescence Agent, a mycoplasma-like organism (MLO). Of them, 37 species developed symptoms of infection. No non-symptomatic hosts were found. None of the monocots tested developed symptoms. Several species known to be the hosts of economically important MLOs were demonstrated to be hosts of BLTVA. The host induction response, the induction of flowering in plants grown under environmental conditions which would normally be non-inductive for flowering, was seen in 8 host species.

721

SEASONAL DETECTION OF XYLELLA FASTIDIOSA IN CITRUS WITH BLIGHT. D. L. Hopkins[1], F. W. Bistline[2], L. W. Russo[2], and C. M. Thompson[1]. Central Florida Research and Education Center[1], University of Florida, Leesburg, FL 32748 and Coca Cola Foods[2], Plymouth, FL 32768.

Small stems and roots from citrus trees with blight were collected monthly from three groves in Florida. Segments of the roots and stems were vacuum infiltrated with succinate-citrate-phosphate buffer to extract bacteria. The extracts were concentrated by centrifugation and resuspension of the pellet in a small volume of buffer. Enzyme-linked immunosorbant assay (ELISA) was used to detect Xylella fastidiosa in the extracts. In all three groves, X. fastidiosa populations were detected in the trees in two peak periods. One peak occurred in mid-summer (June-August) and the other in mid-winter (December-February). All trees tested negative in April and May. In December, 75% of the trees in one sample tested positive. X. fastidiosa was also cultured from a few of the extracts to confirm the positives.

722

ISOLATION AND GROWTH OF THE PIERCE'S DISEASE BACTERIUM ON SIMPLE BACTERIOLOGICAL MEDIA. S. M. Fry, R. D. Milholland, and P.-Y. Huang, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Sap from petioles of grapevines infected with the Pierce's disease bacterium (PDB) was plated on PD2 medium (Phytopathology 70:425-429), nutrient agar (NA) and nutrient agar supplemented with 1.0, 1.5, and 2.0% sucrose (NAS). Isolations of two PDB strains (FC and C) were successful on all media tested. Dilution plating revealed that single colonies of three strains of PDB (FC, C, and CHC) could grow on all media, however, NA and NAS media supported significantly fewer colonies than the PD2 medium. The FC and C strains were serially transferred on the PD2 medium and 2.0% NAS once a week and were inoculated into French Colombard plants at ~2 mo intervals. After being transferred for 9 mo on both media, the PDB caused symptoms and were reisolated from inoculated plants, demonstrating that the PDB remained pathogenic.

723

ELECTROPORATION FOR TRANSFECTION OF SPIROPLASMA CITRI WITH SPIROPLASMAVIRUS SVIS2 DNA. S. L. McCammon and R. E. Davis, USDA,

ARS, Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705.

Electroporation was employed to obtain transfection of Spiroplasma by the 6.5 kb circular single-stranded DNA from Spiroplasmavirus SVIS2. The experiments were performed at field strengths of 3750 to 10000 volts per cm for 50 to 100 msec using approximately 10^9 Spiroplasma cells per ml in 10% sucrose. Transfection through electroporation of susceptible host Spiroplasma citri strain M200H yielded 5 X 10^5 transfectants per ug DNA, 1 X 10^{-4} transfectants per per colony forming unit, and 3 X 10^{-6} transfectants per DNA molecule. The transfection frequencies were similar to those obtained using polyethylene glycol (PEG), but transfection per ug DNA using electroporation was approximately three to five-fold greater than that obtained using PEG. A major advantage of electroporation over PEG for transfection experiments is convenience of sample handling and reduced experimental time.

724

GENETIC AND SEROLOGICAL RELATIONSHIPS BETWEEN THE WESTERN X-DISEASE MYCOPLASMA-LIKE ORGANISM (WX-MLO) AND OTHER MOLLICUTES. B. C. Kirkpatrick, J. D. Fraser, and G. A. Fisher, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

WX-MLO-specific antisera and 24 cloned fragments of the WX-MLO genome were used in ELISA, western blots, and DNA hybridization assays to examine the relatedness of WX-MLOs to other plant and animal associated mollicutes. WX-MLOs are antigenically and genetically very similar to X-disease MLOs present in the Pacific Northwest, Utah, and Eastern Canada. Eighteen or more of 24 WX-MLO DNA probes hybridized with DNA from plants with plum and apricot proliferation, walnut and pecan bunch, and peach yellows diseases. Two of 24 WX-MLO DNA probes hybridized with DNA from plants infected with western aster yellows, peach rosette, and elm yellows (EY) MLO. Low, but consistently positive ELISA values were obtained with EY-infected periwinkle; western blots revealed a common 29.2 Kd protein in WX- and EY-MLO-infected plants. No hybridization was detected between WX-MLO DNA probes and 7 culturable Mycoplasma species and 4 Spiroplasma species.

725

EVIDENCE FOR HOMOLOGY BETWEEN PLASMIDS FROM WESTERN ASTER YELLOWS MLO AND PLASMIDS FROM MAIZE BUSHY STUNT MLO. C. R. Kuske[1], M. J. Davis[2], and B. C. Kirkpatrick[1]. Depts. of Plant Pathology, 1) Univ. of California, Davis, CA 95616, 2) Univ. of Florida, Fort Lauderdale, FL 33314.

^{32}P-labeled plasmids from the severe strain of western aster yellows MLO (SAY-MLO) were used to probe a Southern blot of maize bushy stunt MLO (MBSM) DNA derived from infected corn. SAY plasmid probe hybridized with at least six bands of MBSM extrachromosomal DNA under stringent conditions. A cloned 4.1 kb fragment of MBSM plasmid DNA was used to probe Southern blots of DNA from three strains of Western AY-MLO (severe, dwarf and Tule Lake). MBSM plasmid and SAY plasmid probes produced identical hybridization patterns with DNA from AY-MLO infected plants and leafhoppers, however, SAY plasmids and MBSM plasmids differ in number and in apparent size. No hybridization with healthy plant or leafhopper DNA was observed with either probe. These results indicate that SAY-MLO plasmids and MBSM plasmids share some sequence homology, suggesting that the two MLO's may be genetically related.

726 Withdrawn

727

PROTOPLAST FUSION IN THE MYCOPARASITE *GLIOCLADIUM ROSEUM*. C. M. Kenerley and M. D. Thomas, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station and USDA ARS SCRL, College Station, TX 77843.

Hyphae of *G. roseum* do not readily undergo vegetative anastomosis to form heterokaryons. Hence to genetically modify several strains, protoplasts were isolated and a series of multifactor fusions were performed. All four pairwise fusions involving two Met$^-$ Leu$^-$ and two His$^-$ Asn$^-$ parents resulted in progeny that grew on minimal medium. Nonparental fusion progeny were recovered at a frequency of 1 x 10^{-3}. Stable recombinants were isolated only after several successive, single conidial isolations on selective media. Fifteen stable His$^-$ Asn$^+$ and three stable His$^+$ Asn$^+$ fusion products were isolated. Prototrophic recombinants also contained a differential color marker from the His$^-$ Asn$^-$ parent. No reversion has been observed in the parental markers. The mutants' and fusion products' parasitic activity on sclerotia of *Phymatotrichum omnivorum* are being tested.

728

COMBINATION OF BROADLY EFFECTIVE RUST RESISTANCES INTO DRY BEAN GERMPLASM LINES. J. R. Stavely, J. R. Steadman, and K. F. Grafton, USDA, ARS, MPPL, Beltsville, MD 20705, Dept. of Plant Pathol., Univ. of Nebraska, Lincoln, NE 68503 and Dept. of Agronomy, North Dakota State Univ. Fargo, ND 58105.

Germplasm lines combining resistance to the 33 available North American races of the bean rust pathogen, Uromyces appendiculatus, are being released for the Great Northern and pinto classes of dry beans in 1988. From one to three genes control resistance to each race. Several genes or complex loci of linked genes effective against multiple races of the pathogen are combined in the new lines. The Great Northern lines, Belneb -1 and -2, combine resistance genes from black seeded line B-190 (Mexico 309), pinto cv. Olathe, and Great Northern 1140. The pinto lines, Beldak -1 and -2, combine resistance genes from black seeded Compuesto Negro Chimaltenango, pinto Olathe, and red seeded Mexico 235.

729

THE EFFECT OF HORMONES ON THE PRESENCE OF SPINES ON LEAVES OF THE RED SPANISH PINEAPPLE VARIETY. Rosa-Márquez, E., L. J. Liu, and E. Lizardi. Agricultural Experiment Station, University of Puerto Rico, Río Piedras, PR 00927

The presence of spines on the leaves of the pineapple variety Red Spanish is a very serious problem in the commercial production of this cultivar. We were able to produce spineless plants of the stated variety by culturing meristems in Murashige and Skoog's basal medium plus 0.1 mg/2.4-D and 0.5 mg/lBA. The results obtained indicate that the probability of producing spineless pineapple plants from meristems of spiny plants is 32.4% and that of producing a spiny pineapple plant from spineless ones is 4.1%. As leaves developed, some of the induced pineapple plantlets reversed to the original type. Of the 2,318 plants examined, 72.9% of the spiny Red Spanish pineapple and 85.8% of the spineless Red Spanish pineapple remained unchanged. The above mentioned hormones could be used successfully for propagating spineless Red Spanish pineapple for commercial production.

730

MAPPING COMPONENTS OF PARTIAL RESISTANCE TO CHROMOSOME ARMS IN THE MAIZE/EXSEROHILUM TURCIUM PATHOSYSTEM. V. A. Brewster and M. L. Carson, Plant Science Department, South Dakota State University, Brookings, SD 57007.

F_2 populations were constructed by crossing and selfing a series of Northern Corn Leaf Blight susceptible translocation stocks with the resistant inbred MO17. The translocations were marked with the recessive gene waxy and the F_2 seeds were identified as waxy or non-waxy prior to planting. Plants were inoculated in the field using ground leaf tissue and in the greenhouse with a spore suspension. Once an epidemic had been started data were recorded for infection efficiency, latent period, lesion length, sporulation and disease severity. Ratings from waxy and non-waxy plants for each translocation F_2 were compared using t-tests. A significant difference between ratings for waxy and non-waxy plants should occur if that component of partial resistance is associated with the chromosome arm(s) involved in that translocation.

731

DEVELOPMENT OF GREENHOUSE INOCULATION TECHNIQUES FOR WESTERN GALL RUST ON RADIATA PINE, WITH SOME EARLY RESULTS. M.-M. Chen, W. J. Libby, B. Kinloch, F. W. Cobb, D. Vogler. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Greenhouse inoculations of Pinus radiata host clones with single-gall isolates of Peridermium harknessi showed varying success rates using different inoculation techniques. First symptoms appeared within 30 days, and these increased in number and certainty through 90 days, becoming stable by 120 days. Some infections produced aeciospores within 11 months after inoculation. The isolates appear to vary substantially in pathogenicity, though all have the same isozyme genotype; host clones, varying substantially in susceptibility, have the same relative susceptibility in the field and in greenhouse inoculation (with the possible exception of those from Guadalupe Island). Isolate origin on resistant or susceptible hosts in the field was not predictive of relative pathogenicity in greenhouse inoculation.

732

USING DENSITOMETRY TO ESTIMATE GENOTYPIC FREQUENCIES FOR PHOSPHOGLUCOSE ISOMERASE ALLOZYMES IN POPULATIONS OF THE BEAN RUST FUNGUS. D. C. Linde, J. V. Groth, and A. P. Roelfs, Dept. of Plant Pathology, Univ. of Minn. and USDA/ARS Cereal Rust Laboratory, St. Paul, MN 55108.

Phosphoglucose isomerase (PGI) is a dimeric, polymorphic enzyme in germinating urediniospores of Uromyces appendiculatus. Three distinct bands occur in heterozygous isolates after starch gel electrophoresis. Transmission densitometry was used to estimate the frequency of the homozygous slow (s), homozygous fast (f), and heterozygous (h) individuals in mixed preparations of the three. Areas under each peak were transformed to genotypic frequencies by comparison with standard 1:1 (f:s) and 100% h preparations. Observed and expected frequencies in prepared mixtures were linearly related for all genotypes. Correspondence of observed with expected frequencies was high, generally differing by 10% or less. The method was applied to 10 field collections of the fungus and revealed polymorphism for PGI allozymes in three of them.

733

COMPARISON OF VIRULENCE FREQUENCY AND DIVERSITY OF BEAN RUST (UROMYCES APPENDICULATUS) ISOLATES FROM DOMINICAN REPUBLIC AND NEBRASKA. M. R. Miles and J. R. Steadman, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln NE, 68583.

The frequency of virulent reactions of bean (Phaseolus vulgaris) rust isolates from Nebraska (NE) and Dominican Republic (DR) on 19 differentials were compared. Virulence, pustules >300 µm, was found on all 19 differentials, frequencies varied by year. Most isolates were virulent on 3 differentials and avirulent on 1. Virulence on the remaining differentials varied by specific location. DR isolates were more virulence than NE isolates. Principal component analysis indicated diversity of virulence combinations among isolates. Further, correlation of the isolate reactions indicated that virulence on one differential usually was not highly correlated with virulence on others. Both results have implications for rust resistance.

734

CLUSTER ANALYSIS OF BEAN RUST (UROMYCES APPENDICULATUS) ISOLATES COLLECTED IN NEBRASKA AND COLORADO, 1979 - 1986. M. R. Miles and J. R. Steadman, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln NE, 68583.

Fifty six single pustule bean rust isolates, on pinto and great northern cultivars were inoculated onto 19 bean rust differentials, which represent major sources of resistance. The most common primary leaf reaction from these isolates and previously reported U.S. races 38 - 57 were used in a cluster analysis. The 56 isolates and 20 races differed in virulence. Clusters were independent of year and location. However, the most similar isolates tended to come from two locations in any one year, or within the same location in different years. Eight races representing other US locations were clustered with Nebraska isolates. None of the 19 differentials were resistant to all isolates, indicating the presence of unnecessary virulence within the pathogen population.

735

CLUSTER ANALYSIS OF BEAN RUST (UROMYCES APPENDICULATUS) ISOLATES COLLECTED IN THE DOMINICAN REPUBLIC, 1982 - 1985. M. R. Miles and J. R. Steadman, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln NE, 68583.

Seventy single pustule bean rust isolates, from Phaseolus vulgaris under commercial cultivation, were inoculated onto 19 bean rust differentials which represent major resistance sources. The most common primary leaf reaction from these isolates and previously reported U.S. races 38 - 57 were used in a cluster analysis. The 70 isolates and 20 described races differed in virulence. The Dominican isolates tended to cluster by field collection or location within year. Nine U.S. races were clustered with Dominican isolates, indicating some similarity in virulence between temperate and tropic bean rust populations.

736

PRODUCTION AND SPECIFICITY OF MONOCLONAL ANTIBODIES TO GLOMUS MOSSEAE. P.D. Millner and S.R. Reynolds. USDA-ARS, Soil-Microbial Systems Laboratory, Beltsville, Md. 20705.

Sporocarps of Glomus mosseae (INVAM 156) served as antigen and were prepared by crushing cleaned, surface-sterilized sporocarps in sterile saline, and ultrasonicating the mixture for 15 min. Hybridomas from mouse spleen and SP2/0-Ag14 mouse myeloma cell fusions were screened by ELISA for antibody production to crushed sporocarps. Antibodies to G. mosseae (GM 156) did not cross-react with crushed spore antigens from Acaulospora longula, Endogone pisiformis (hyphae), Glomus aggregatum, G. etunicatum, G. macrocarpum, Gigaspora margarita, and Scutellospora coralloidea, spore washings, and extracts of uninfected and G. mosseae infected roots. Antibodies to GM 156 did react in ELISA and immunofluorescence tests with the soluble spore contents of other isolates of G. mosseae and G. monosporum. This supports other evidence of conspecificity between these two Glomus species.

737

SPORE GERMINATION OF VA MYCORRHIZAL FUNGI AFTER STORAGE IN SOIL AT FIVE MATRIC POTENTIALS. D.D. Douds & N.C. Schenck, Plant Pathology Dept; Univ of Florida; Gainesville, FL 32611.

Spores of 4 vesicular-arbuscular mycorrhizal (VAM) fungi were incubated at room temperature for up to 4 mos in soil at 5 matric potentials (Ψm). They were removed and incubated 1 mo in moist soil to assay germination and hyphal growth. The difference in germination between that which occurred in storage and that obtained after the subsequent mo in moist soil (net germination), was zero for Gigaspora margarita. However, hyphae continued to grow and additional germ tubes were produced. Net germination of Glomus intraradices increased with decreasing storage Ψm, independent of storage duration. Net germination of Glomus mosseae was inversely proportional to storage duration and independent of Ψm. Net germination of Acaulospora longula increased with duration of storage, independent of Ψm. Each species studied had a different response to storage and Ψm, reflecting the complexity of the problem of storage of VAM fungi.

738

MYCORRHIZAE IMPROVE DROUGHT RESISTANCE OF MAIZE. D.M. Sylvia and L.C. Hammond, Soil Science Dept., and J.M. Bennett, Agronomy Dept., University of Florida, Gainesville, FL. 32611.

Mycorrhizae affect the water relations of plants grown in chambers and greenhouses; however, there are few data on the response of mycorrhizal plants to drought stress in field environments. A field plot of Millhopper fine sand (loamy, siliceous, hyperthermic Grossarenic Paleudult) was fumigated with methyl bromide, fertilized with N-P-K, and two inoculation treatments applied to subplots of three water management treatments (0, 8, and 17 cm of seasonal irrigation). Inoculation treatments were noninoculated (NI) and inoculated (I) with ca. 1 propagule of Glomus etunicatum per cm of row. Percentage root colonization by VAM fungi (surface 30 cm) was 0 and 41% in NI and I plots after 6 wk, and 18 and 45% in NI and I plots after 13 wk, respectively. Maize response to mycorrhizal inoculation was inversely related to drought stress: grain yield was 4, 16, and 33% greater in I vs NI plots in the 17-, 8-, and 0-cm irrigation treatments, respectively.

739

VARIATION IN FATTY ACID CONTENTS BETWEEN VIRULENT AND AVIRULENT, STREPTOMYCIN RESISTANT AND SUSCEPTIBLE STRAINS OF ERWINIA AMYLOVORA. T. van der Zwet, USDA, ARS, AFRS, Kearneysville, WV, 25430, J.M. Wells, USDA, ARS, Plant Science Research Unit, Eastern Regional Research Center, Philadelphia, PA 19118, and R.N. Goodman, University of Missouri, Columbia, MO 65211.

Nine streptomycin-resistant (SR), and 9 strep-susceptible (SS) strains of Erwinia amylovora were analyzed for fatty acid content, using standard gas liquid chromatography (GLC) analysis of 25-50 resolved components. Three of the SR strains and 1 of the SS strains were avirulent; all others were virulent. All isolates were grown on trypticase soy agar for 3 days at 27C. Fatty acids were also examined for 6 virulent SS (check) isolates using standard (< 25 components) and long chromatographs (> 50 components). Of the 7 fatty acid classes, the percentage cyclic acids were consistently lower (3.1% of total fatty acids) for the SR strains than for the SS strains (7.1%). The reverse was observed for unsaturated acids (SR strains with 37.5% and SS strains with 32.9% of total fatty acids). These differences were reflected in the saturated to unsaturated acid ratios, which for SR strains was 1.2 and for SS strains 1.5. Strep resistance of E. amylovora isolates may be predicted by determining such ratios using GLC analysis.

740

DETERMINATION OF CORYNEBACTERIUM SEPEDONICUM CELL POPULATIONS IN POTATO DURING THE GROWING SEASON WITH A MONOCLONAL IMMUNOFLUORESCENCE PROCEDURE. S.H. De Boer and M. McCann.

Agriculture Canada, Research Station, 6660 N.W. Marine Drive, Vancouver, B. C., Canada V4E 1T6.

Potato plants (cv. Russet Burbank) were tested at intervals to determine population changes of the ring rot bacterium during the latent phase of the disease. Bacterial populations were estimated on preparations stained by a monoclonal-immunofluorescence procedure using an automated microscope system. In a greenhouse experiment, the number of bacteria/g of tissue only increased in symptomless plants during the first 30 days although the total number of bacteria continued to increase. In the field, ring rot bacteria were detected in stems shortly after emergence at 10^3 cells/g. Populations increased logarithmically to 10^{11} cells/g of tissue by 91 days after planting when ring rot symptoms first appeared. (This research was supported in part by a grant from the Farming for the Future Council in Alberta).

741

BACTERIAL INVOLVMENT IN LATE PHYSIOLOGICAL DROP AND POST HARVEST COTYLEDON SPOT OF PECAN. C. C. Reilly and W. L. Tedders, USDA-ARS, P.O. Box 87, Byron, GA 31008

Pecan fruits affected by late (July-Aug) "physiological drop" were collected from commercial pecan orchards from widely separated areas in Georgia and Florida. Ten cultivars of pecan were sampled from 6 locations. Common symptoms on all fruits were black parallel lines associated with the vascular system of the shuck and black necrotic patches on the shuck surface, with cotyledons dark brown to black, shrunken and slimy to the touch. A bacterium, with distinctive colony color and morphology, was constantly isolated from affected shucks and cotyledons. Healthy adjacent fruits were also sampled but the bacterium was isolated infrequently. Late season infection of the fruits by inoculating with the bacterium resulted in cotyledon spot, characterized by dark brown to black patches on the seed coat; the area being confined to the vascular system but not entering the endosperm. The bacterium was readily isolated from these affected areas.

742

A NEW BACTERIAL DISEASE ON WATERMELON IN THE MARIANA ISLANDS. G. C. Wall and V. M. Santos, Agricultural Experiment Station, and Cooperative Extension Service, College of Agriculture & Life Sciences, University of Guam, Mangilao, GU, 96923.

A severe epidemic of a previously unreported disease occurred on watermelon fields on Guam and Tinian during the 1987 rainy season. The disease affected the rind of the fruit, causing a watersoaked lesion that eventually developed into fruit rot. A bacterium was isolated from infected rind tissue. Healthy fruit were inoculated with a dissecting needle, and by spraying a bacterial suspension over damaged (scraped) and undamaged fruit. Only damaged or injured tissue developed infection. The bacterium was re-isolated to complete Koch's postulates, and was identified as Pseudomonas pseudoalcaligenes. Descriptions of symptoms and the causal agent agree with those given for Fruit Blotch, reported from Australia, and caused by P. pseudoalcaligenes pv. citrulli.

743

CULTURAL CONTROL OF BACTERIAL DISEASES ON BELL PEPPER. G. C. Wall and E. R. Champaco, Agricultural Experiment Station, College of Agriculture & Life Sciences, University of Guam, Mangilao, GU, 96923.

Bacterial leaf spot, caused by Xanthomonas campestris pv. vesicatoria, and bacterial wilt, caused by Pseudomonas solanacearum are the most important diseases of bell peppers on Guam. Various cultural practices were included in a split-plot field test in an effort to find environmentally sound control measures for these diseases. The experiment was carried out during the rainy season in 1987. Bacterial wilt incidence was significantly reduced in raised beds (50 cm) with black plastic mulch; plant stand and yield were 110% and 265% higher, respectively, than in flat beds. Overhead polyethylene cover reduced leaf spot severity, and increased yield by 67.7%.

745

EFFECT OF WATER STRESS ON BOTRYOSPHAERIA DOTHIDEA INFECTION OF NONWOUNDED PEACH BARK. P. L. Pusey, USDA-ARS, Byron, GA 31008

Nonwounded stems of 1-year-old potted peach trees were inoculated with Botryosphaeria dothidea by applying conidial suspensions with a brush and wrapping with moist cheesecloth and parafilm. Test I: Inoculated trees had water withheld 0, 2, 4 or 6 da in 8-da cycles repeated from May to September. Test II: At varying times from April to October, water was withheld 2 da before inoculation and continued until leaf water potential (WP) was below -30 bars. Test III: Trees in various stages of wilt were inoculated and subjected to 94-98% relative humidity for 6 da to maintain WP levels. Test IV: April-inoculated and June-inoculated trees had water withheld at varying times from May to October until WP was below -30 bars. Only in Test I and IV did water stress increase disease severity. Stress imposed at the time of inoculation had no effect on disease, but stress 2-6 mo after B. dothidea introduction resulted in an increased ($P=0.05$) no. of necrotic lesions and gumming sites associated with lenticels.

746

CACAO SEEDLING BLIGHT CAUSED BY PHYTOPHTHORA PALMIVORA. J. Y. Uchida and M. Aragaki, Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822.

An outbreak of cacao seedling blight caused by Phytophthora palmivora in 1986, is the first record of Phytophthora on cacao in Hawaii. All diseased seedlings grew from seeds imported from Costa Rica or Belize. Cross inoculations have shown that P. palmivora (MF-1) strains from papaya, orchids, macadamia, and cacao are specialized. Chlamydospores of cacao isolates were slightly larger than those of papaya isolates, although sporangial dimensions were comparable. All isolates from infected cacao were of the A2 compatibility type while all papaya and macadamia isolates of P. palmivora were A1. With one exception, all isolates of P. palmivora from orchids in Hawaii were also A1. To prevent the establishment of a new P. palmivora strain, eradication of all infected seedling lots was recommended.

747

Comparison of plant and artificial media for pseudothecia and ascospore production of Venturia inaequalis. L. P. Berkett, A. R. Gotlieb, and J. A. Bergdahl. Department of Plant and Soil Science, University of Vermont, Burlington, Vermont 05405.

In a preliminary experiment, apple twig wood demonstrated a potential to produce high numbers of pseudothecia and ascospores. This work evaluates plant substrates and artificial media for their potential to produce Venturia inaequalis ascospores under aseptic laboratory conditions. Plant substrates included sterilized apple twigs and leaf disks. Artificial media consisted of filter paper disks saturated with water agar and the following additions: 0.5% malt extract; 0.5% malt extract + leaf decoction; 0.05% malt extract; 0.05% malt extract + leaf decoction; and leaf decoction. Each disk or twig was inoculated with an equal volume of conidial suspension of compatible mating types. Cultures were incubated for 14 days at 20°C and then placed at 8°C for 3, 4 and 5 months at which times cultures were evaluated. Data were collected on number and degree of maturation of pseudothecia and viability of ascospores.

748

ELECTROPHORETIC VARIATION AMONG ISOLATES OF PRUNUS NECROTIC RINGSPOT VIRUS. J.M. Crosslin, and G.I. Mink. Washington State University Irrigated Agriculture Research and Extension Center, Prosser, WA, 99350-0030.

The electrophoretic migration of partially purified nucleoproteins of several Prunus necrotic ringspot virus isolates were compared. Electrophoresis was conducted using 11.5 x 14.5 cm slab gels of 2% (w/v) low melting point agarose and 40 mM Tris-acetate buffer (pH 8.0) containing 1 mM EDTA at 100 V for 4 hr. Most isolates produced 3 distinct bands when stained with ethidium bromide and/or Coomasie blue. Other isolates produced 2-5 distinct to diffuse bands. There was no apparent relationship between migration pattern and serological characteristics of the isolate. A putative correlation was observed between migration pattern and symptomology on key indicator plants.

749

DETECTION AND ELIMINATION OF VIRUSES FROM PEAR GERMPLASM. J. D. Postman and B. J. Rebhuhn, USDA National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333.

Latent viruses were detected in USDA clonal Pyrus germplasm by inoculation to sensitive indicator plants. Of 778 pear cultivar accessions received by the National Clonal Germplasm Repository, 61% were found to be virus-infected. Viruses were eliminated from infected clones using alternating temperature thermotherapy and micropropagation. Shoot tips 1 mm or less from heat-treated plants were propagated in vitro. After several weeks growth in vitro, shoots were tip-grafted onto young seedling pear rootstocks in the greenhouse. Use of rootstocks with unique foliage characteristics facilitated differentiation between scions and rootstock sprouts. Virus elimination was more successful when meristems from heat-treated plants were propagated in vitro than when larger shoot tips were propagated by tip-grafting alone. Known viruses were successfully eliminated from over 300 Pyrus clones.

750

Sources of Variation in Interfruitlet Corking and Fruitlet Core Rot of Pineapple. J.E. Yuen, K.G. Rohrbach, and G.Y. Taniguchi, Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, Hawaii 96822.

Interfruitlet corking (IFC, caused by Penicillium funiculosum) and fruitlet core rot (FCR, caused by P. funiculosum and Fusarium moniliforme var. subglutinans) were studied in 8 sequential plantings of pineapple at 3 month intervals. Three different pineapple cultivars varying in disease susceptibility were used, and plots were inoculated with P. funiculosum and F. moniliforme at flowering. IFC and other related diseases induced by P. funiculosum occurred only in late September and December forcings, corresponding to fruit development during the cooler, wetter winter months. FCR developed during summer and fall forcings. More FCR developed due to F. moniliforme than P. funiculosum in only one forcing. Varietal differences and possible environmental factors influencing disease are discussed.

751

EFFECT OF FUNGAL "ELICITORS" ON PINUS ELLIOTTII CLONES DIFFERING IN RESISTANCE TO FUSIFORM RUST. Mark S. Lesney, Department of Forestry, University of Florida, Gainesville, FL 32611 USA

Suspension-cultured cells of Pinus elliottii Engelm. (Slash Pine) were initiated from cambial-derived callus of clones of resistant and susceptible slash pine trees. Incubation of cells with various "elicitors" (chitin, chitosan, Cronartium fusiforme mycelium and extracts) resulted in quantitative and qualitative changes in: peroxidase isozymes, various proteins, cell growth, browning and cell death (hypersensitivity). Although effects differed based on the "elicitor" used, preliminary results do not indicate specific disease correlations. Similar "elicitation" effects as described were seen for seedling-derived suspension cultures of slash, loblolly and longleaf pine as well.

752

LABORATORY STUDIES ON ANTAGONISM OF SCYTALIDIUM LIGNICOLA AGAINST WOOD DECAY FUNGI. T. L. Highley, Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53705-2398

The antagonistic ability of Scytalidium lignicola against white- and brown-rot wood decay fungi was evaluated. The decay fungi used were: Poria carbonica, P. placenta, Lentinus lepideus, Gloeophyllum trabeum, Coriolus versicolor, Irpex lacteus, Phlebia brevispora, and Phanerochaete chrysosporium. S. lignicola did not produce inhibition zones but overgrew the decay fungi on malt-agar and in most cases killed them. Pretreatment of Douglas-fir and pine blocks with S. lignicola prevented decay. Blocks that were heated to kill the antagonist were not decay resistant. Water extraction of S. lignicola treated blocks removed decay resistance. Wood blocks treated with filtrates of S. lignicola were not decay resistant, and filtrates were not inhibitory to growth of the decay fungi. S. lignicola was able to eradicate all the decay fungi in wood except for P. placenta and G. trabeum.

753

COMPANION PLANTING OF BLACK WALNUT WITH AUTUMN OLIVE TO CONTROL WALNUT ANTHRACNOSE. K. J. KESSLER, JR. USDA Forest Service, Forestry Sciences Lab., SIU, Carbondale, IL 62901.

Interplanting black walnut (BW) with autumn olive (AO) reduced lesion incidence and leaflet defoliation caused by Gnomonia leptostyla to less than 1/5 of that of monocultured BW. Interplanting reduced primary inoculum and interfered with dissemination of primary and secondary inoculum. Ground shading by AO and later leaf-shedding by AO than BW in the fall produced a ground microclimate that increased decomposition of infected fallen BW leaves and lowered perithecial production from them. The AO leaf layer overlying fallen BW leaves reduced dissemination of ascospore inoculum. AO foliage impeded conidial spread from infected foliage in BW plots to nearby companion-planted BW. Uninfected foliage on interplanted BW remained three or more weeks longer on the trees in late summer than on those grown in monoculture. Shading by AO induced earlier death of BW branches close to the ground zone where foliage was more susceptible to infection.

754

EFFECT OF TRIADIMEFON SEED TREATMENT PLUS FOLIAR SPRAYS ON CONTROL OF FUSIFORM RUST ON LOBLOLLY SEEDLINGS. W. D. Kelley and G. B. Runion, School of Forestry, Auburn Univ., AL 36849.

Results of tests at three nurseries over a three-year period demonstrated the importance of triadimefon seed treatment in fusiform rust control programs. Plots sown with treated and with nontreated seeds were subjected to the following foliar spray programs: 1) All sprays omitted; 2) first spray omitted; and 3) standard program (1st spray at 75% emergence; last spray mid June). Overall, incidence of fusiform rust was less ($p < 0.01$) in plots with treated seeds. With nontreated seed, rust incidence was higher when inoculum pressure was high in plots missing the 1st spray than in plots missing no sprays. Use of treated seed permitted missing the 1st spray without affecting rust incidence, regardless of inoculum pressure. These data demonstrate the importance of a triadimefon seed treatment in a fusiform rust control program.

755

EFFECT OF TRIADIMEFON AS A SEED TREATMENT ON EMERGENCE OF LOBLOLLY AND SLASH PINE SEEDLINGS. G. B. Runion and W. D. Kelley, School of Forestry, Auburn University, AL 36849.

Triadimefon is an effective seed treatment for protecting emerging pine seedlings from Cronartium quercuum f. sp. fusiforme; however, its effect on seedling emergence is not well documented. Loblolly (stratified) and slash pine seeds were subjected to a seed soak (SS) (800 mg ai triadimefon/L, 24 h soak) or a seed dressing (SD) (1.25 g ai triadimefon/kg seed) at rates of 1x (indicated), 2x, 3x, and 4x; controls received no fungicide treatment. Emergence counts of loblolly taken 20 days after sowing indicated that the SS at the 4x rate significantly ($p = .05$) decreased emergence; other rates and the other method had no effect. For slash, the SD had no effect on emergence regardless of rate; however, all rates of the SS significantly decreased emergence. Results indicate that slash seeds are more sensitive to triadimefon and that the SS is more damaging than the SD.

756

A NATURALLY OCCURRING FOREST DECLINE OF ABIOTIC ORIGIN AFFECTING *CHAMAECYPARIS NOOTKATENSIS* IN SOUTHEAST ALASKA. P. E. Hennon, USDA Forest Service, Juneau AK 99802; E. M. Hansen, Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; and C. G. Shaw III, USDA Forest Service, Fort Collins, CO 80524.

Alaska-yellow cedar (*Chamaecyparis nootkatensis*) is suffering from decline and

mortality of unknown cause on over 100,000 hectares of remote forest land in southeast Alaska. Since its nearly simultaneous onset at numerous sites around 1880, decline has apparently not spread to any new sites but has spread locally (up to 100m) along gradients from areas with wet, poorly drained soil to areas with better soil drainage. Dead fine and small diameter roots and necrotic lesions spreading from coarse roots up the bole are common on declining trees. Entire crowns of some affected trees die quickly; others thin gradually for ten or more years before death. VA mycorrhizae and *Mycelium radicis atrovirens* are common in cortical cells of fine roots on both healthy and declining trees. None of the organisms associated with declining cedar, including 50 taxa of fungi, nematodes, *Phloeosinus* bark beetles, and Alaska brown bears, appear to be the primary cause. Foliar and soil analyses indicate that nutrient deficiencies or toxicities do not cause decline. The date of onset, remoteness, and lack of human disturbance in declining forests suggest that this decline is not induced by human involvement.

757

COMPARATIVE PATHOLOGY OF FUSARIUM SUBGLUTINANS ISOLATED FROM MONTEREY PINE IN CALIFORNIA AND SOUTHERN PINES. L. D. Dwinell. USDA For. Serv., Southeast. For. Expt. Sta., Athens, GA 30602.

In a greenhouse study, new growth of 1-yr-old seedlings of Monterey, eastern white, Scots, Virginia, loblolly and slash pines was inoculated with 9 isolates of F. subglutinans taken from cankers on Monterey (7), loblolly (1), and slash (1) pines. There were 10 replicates of each treatment combination. Final data on shoot mortality and canker dimensions were taken after 8 wk. Experimental variation was attributable to pine host and not isolate or isolate x host interaction. Monterey and Virginia pines were the most susceptible species. Scots, slash, and loblolly pines were intermediate. Eastern white pine was moderately resistant. The pathotype of F. subglutinans causing pitch canker on Monterey pine in central coastal California appears to be the same as found on pines in the Southern U.S.

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FUSARIUM DISEASES OF CONTAINERIZED CONIFER SEEDLINGS IN NORTHERN ROCKY MOUNTAIN NURSERIES: SOURCES OF INOCULUM AND CONTROL TESTS. R. L. James, R. K. Dumroese and D. L. Wenny. USDA Forest Service TCFPM, P. O. Box 7669, Missoula, MT 59807 and University of Idaho Research Nursery, Moscow, ID 83843.

Sources of Fusarium inoculum causing diseases of containerized conifer seedlings in northern Rocky Mountain nurseries included infected seed, soilless media, greenhouse weeds, and styroblock or Leach ® pine cell containers which were reused for several crops. Fusarium spp. were isolated from seedcoats and endosperms at levels that differed widely among conifer species and seedlots. A treatment of Douglas-fir seed with 3% hydrogen peroxide after stratification was most effective in reducing seed contamination while maintaining high germination. Fusarium was not commonly isolated from peat vermiculite media. Weeds and other organic debris on the floors of greenhouses harbored several Fusaria. Containers used to grow seedlings were probably the most important inoculum source for new crops of seedlings. Standard cleaning treatments did not eliminate this inoculum.

759

SEIRIDIUM CANKER OF JUNIPERUS SPP. AND THUJA ORIENTALIS IN KANSAS. N. Tisserat and A. Nus, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A general decline and branch dieback has been observed in several eastern redcedar and oriental arborvitae windbreaks in northern Kansas. The decline has often been attributed to adverse environmental conditions or poor management practices. Nevertheless, close examination of declining trees often revealed flattened, resin-soaked cankers on the branches and main stem. A fungus, tentatively identified as Seiridium unicorne, was consistently isolated from and observed fruiting in cankers. Three trees each of one- to two-year-old Rocky Mountain juniper, eastern redcedar, and oriental arborvitae were inoculated by inserting mycelium of the fungus into fresh stem wounds. All trees developed sunken, bleeding cankers 1 mo after inoculation in the greenhouse. One tree each of eastern redcedar and Rocky Mountain juniper were killed after 3 mo. Results indicate that Seiridium canker may contribute to decline of these species in windbreaks.

760

ASSOCIATION OF PYTHIACEOUS FUNGI, ROOTLET MORTALITY, AND DIEOFF OF SHADSCALE IN THE GREAT BASIN OF UTAH. D. L. Nelson and D. J. Weber, USDA Forest Service, Intermountain Research Station, Shrub Sciences Lab., Provo, UT 84601, Brigham Young University, Provo, UT 84622.

Shadscale saltbush (Atriplex confertifolia), an endemic salt desert shrub of the western United States, forms vast genetically uniform polyploid populations on Pleistocene lake bottoms of the Great Basin. Toward the end of a historical record high precipitation period (1980-86), complete death of many populations occurred from an unknown cause on thousands of acres. Increasing fine rootlet mortality was correlated with dieoff symptom severity index. Pythiaceous fungi were isolated from rootlets and occurred at high soil population levels in soil assay tests. A complex of Fusarium spp. were consistently isolated from small rootlets, main tap roots, and basal stems of symptomatic plants. Alternaria was associated with shoot tip dieback.

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ENZYME ANALYSIS OF PERIDERMIUM HARKNESSII IN NEBRASKA PONDEROSA PINE PLANTINGS. J. A. Walla, Plant Pathology Dept, G. A. Tuskan, Horticulture & Forestry Dept, North Dakota State Univ, Fargo, 58105 and G. W. Peterson, Rocky Mountain Forest & Range Exp. Stn., Forestry Sciences Lab, Univ Nebr, Lincoln 68583.

Variation in P. harknessii in 2 Nebraska ponderosa pine plantings was evaluated using zymograms constructed from profiles of 14 isozymes resolved by horizontal starch gel electrophoresis. Infected seedlings planted in 1968 were later identified, recorded as epicenters of disease development and removed. In a planting where infection resulted from one gall, one homozygous zymogram (type A) in spores from each of 23 galls indicated stability of zymograms over time. In a planting where infection resulted from galls on an estimated 31 seedlings (the position of which caused 18 epicenters), spores from each of 4 galls around each of the 18 epicenters displayed one of two homozygous zymograms (type A,B). Type B was randomly distributed across the planting on 5 of 72 galls, and was possibly introduced from a later planting near this site.

762

FIELD PERFORMANCE OF SYMPTOMATIC SURVIVORS OF LOBLOLLY PINE FAMILIES INOCULATED WITH CRONARTIUM QUERCUUM F. SP. FUSIFORME. Thomas Miller and Fred R. Matthews, S.E. For. Exp. Sta., Dept. of For., Univ. of Florida, Gainesville, FL 32611 and For. Sciences Lab., Athens, GA 30602.

Progeny of four half-sib loblolly pine families and one seed source were inoculated with basidiospores of Cronartium quercuum f. sp. fusiforme to evaluate the field performance of seedlings developing stem symptoms other than galls. After 9 months, galled and asymptomatic seedlings were discarded. Symptomatic seedlings (SS) and uninoculated controls were outplanted in a high rust incidence area. Seedlings were examined at 1,2,4,6, and 12 years for rust and rust-associated mortality (RAM). Mean, cumulative rust (stem galls) for the SS families ranged from 17 to 56%, with 92% in the controls. Mean, cumulative RAM ranged from 2 to 22%, with 53% in the checks. The 12-year field performance of the SS families did not change the relative resistance rankings previously determined from inoculations at the seedling stage.

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ANATOMICAL RESPONSES OF SLASH PINES FOLLOWING GREENHOUSE INOCULATIONS WITH CRONARTIUM QUERCUUM F. SP. FUSIFORME. C. H. Walkinshaw, USDA, Forest Service, Southern Forest Experiment Station, Gulfport, MS 39505.

Twenty open-pollinated and 17 control-pollinated slash pine families were inoculated with 9 single gall field isolates of the rust fungus. Specimens for histology were fixed after 9 to 60 days in formalin acetic acid alcohol,dehydrated, embedded in paraffin, cut at 12 millimicrons, and stained according to a variety of schedules. Observations showed that the anatomy of initial lesions was determined by pine parents and by rust inocula. Variations in lesions included size, periderm formation, presence of viable rust hyphae, and accumulation of ergastic substances within affected cells. Rust resistance was indicated by a decrease in nuclear staining of cortical cells in the infection court. Susceptibility was accompanied by increased staining of cytoplasm in cortical cells beyond the penetration site.

764

PROTOPLASTS FROM CRONARTIUM QUERCUUM F. SP. FUSIFORME MYCELI AND BASIDIOSPORES. P.C. Spaine, O.M. De Vries, and W.

Otrosina. USDA, Forest Service, SEFES, Athens, GA 30602, University of Groningen, Haren, The Netherlands, USDA, Forest Service, PSW, Berkeley, CA 94704.

Mycelial cultures derived from basidiospores of Cronartium quercuum f. sp. fusiforme were initiated in a liquid Greshoff and Doy medium with 1 percent peptone and yeast extract. Mycelia were sampled approximately twice weekly and digested with a combination of lytic enzymes. Protoplasts were released in sufficient quantity from 3-week-old mycelium. Protoplasts were also obtained from freshly cast spores as well as those stored at 4° C for 4 days under sterile conditions. Novozyme 235 (10 mg/ml) plus chitinase (5 mg/ml) (1M $MgSO_4$) released protoplasts from the mycelium, while Novozyme 235 plus bovine serum albumin (10 mg/ml) released protoplasts from spores. Protoplasts of this pathogen will eventually be used to transfer genetic information and examine diversity within populations.

765

USE OF TISSUE CULTURE AND IN VITRO BIOASSAYS FOR THE DEVELOPMENT AND SELECTION OF DISEASE-RESISTANT TREES. M. E. Ostry and D. D. Skilling. USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Avenue, St. Paul, MN 55108.

Passage of plant cells through a tissue culture cycle can induce somaclonal variation (SCV). A potential benefit of SCV is the creation of useful genetic variability, such as disease resistance, without hybridization. Application of SCV technology, coupled with in vitro bioassays for early detection of disease resistance, can benefit tree breeding by reducing the time required for tree improvement. Tissue culture has been used to obtain Populus sp. and Larix sp. variants that have putative resistance to Septoria musiva and Gremmeniella abietina, respectively. These trees have been planted in the field and are being evaluated for growth characteristics and disease resistance.

766

ETHEPHON AND ECTOMYCORRHIZAL TREATMENTS ALTER ROOT GROWTH OF CONTAINERIZED BLACK SPRUCE SEEDLINGS. William H. Livingston, Dept. of Botany and Plant Path., Univ. Maine, Orono, ME 04469

Root diseases are associated with root deformities on planted conifers. Roots of containerized seedlings become deformed when deflected by the container's wall. Ethephon, an ethylene releasing agent, and ectomycorrhizal fungi were evaluated for their effectiveness in reducing elongation and deflection of black spruce (*Picea mariana*) roots in containers. Two-week old seedlings were inoculated with ectomycorrhizal fungi (*Laccaria* sp. and an unknown species) and treated weekly with 0, 75, or 150 ppm ethephon drenches for 14 weeks (30 seedlings/treatment). Ethephon treatments increased thickness of root growing tips and reduced elongation of first order lateral roots. Ectomycorrhizal inoculations were necessary to maintain similar shoot growth (length, wt) of ethephon treated seedlings compared to nontreated seedlings. Combined ethephon and ectomycorrhizal treatments have potential for reducing root deformities in containerized spruce seedlings.

767

TWO SOURCES OF PRIMARY INOCULUM IN ASH ANTHRACNOSE. S. C. Redlin and R. W. Stack, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105

The widely known and recently redescribed Discula anamorph of the ash anthracnose fungus produces acervuli on infected petioles that cling to branches of green ash (Fraxinus pennsylvanica Marsh.) over the winter. Conidia from these acervuli serve as primary inoculum. The clinging of the infected petioles, previously known only on velvet ash (Fraxinus velutina Marsh.) in California, was found on green ash in North Dakota, South Dakota and Minnesota. Perithecia of the previously unknown but recently described teleomorph, a new species of Gnomoniella, are commonly produced in overwintered leaflets on the ground and are a second potential source of primary inoculum. Leaf litter on the ground containing perithecia was associated with trees having overwintered infected petioles. Isolates of the fungus derived originally from conidia, ascospores or direct tissue isolation were all pathogenic.

768

RELATIVE SENSITIVITY OF FUSARIUM OXYSPORUM FROM CONIFER NURSERIES TO FUMIGATION WITH METHYL BROMIDE. R. W. Stack, North Dakota State Univ., Fargo and M. A. Palmer, USDA Forest Service, Corvallis, OR.

Root rot of conifer seedlings caused by Fusarium oxysporum is a problem in forest tree nurseries in the North Central states, including some nurseries where methyl bromide fumigation is regularly used. Isolates of F. oxysporum were collected from 5 nurseries having different histories of fumigation practice. Isolates were grown in cornmeal-sand mix and chlamydospore formation was induced by drying. Aliquots of the chlamydospore mix were dispensed in porous paper envelopes and fumigated in large glass chambers using methyl bromide at several concentrations. Dilution plating was used to determine survival. Sensitivity of individual isolates varied greatly. Mean sensitivities of isolates from given nurseries were not related to history of fumigation; isolates from the most heavily fumigated nursery were no less sensitive to methyl bromide than isolates from other locations.

769

TOXICITY OF FREE COPPER IONS AND INDUCTION OF COPPER RESISTANCE IN PSEUDOMONAS SYRINGAE IN CULTURE. O. Menkissoglu, G. Andersen, and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Organic compounds including citrate, glucose, fructose and culture media complex most added copper ions and the concentration of free cupric ions increases logarithmically with added $CuSO_4$. The LC_{50} of strains in culture media and in $CuSO_4$ solutions were similar only when expressed as free copper ions. The LC_{50} of copper sensitive and tolerant P. syringae and Xanthomonas campestris pv. vesicatoria strains grown in the absence of sub-lethal doses of copper was about 4 ppb and 20 ppb free copper ions respectively, while the LC_{50} of these strains grown in the presence of copper ions was about 4 ppb and 160 ppb respectively.

770

CHEMICAL FORMS OF COPPER ON LEAVES IN RELATION TO THE BACTERICIDAL ACTIVITY OF CUPRIC HYDROXIDE DEPOSITS. O. Menkissoglu and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Only free copper ions on plants were toxic to Pseudomonas syringae strains. The total amount of copper on navel orange and bean leaves decreased with time after spray treatment with $Cu(OH)_2$, while the amount of soluble but complexed copper increased with time for up to 20 days. The concentration of free cupric ions on wet leaves averaged about 85 ppb for about 20 days after treatment and was not greatly influenced by the quantity of $Cu(OH)_2$ applied. Copper tolerant and sensitive P. syringae strains placed on leaves survived only when the concentration of cupric ions was less than the LC50 for these strains determined in water (about 160 and 4 ppb, respectively).

771

OCCURRENCE AND CHARACTERIZATION OF A BACTERIAL PATHOGEN ON MILKWEED (ASCLEPIAS SPP.) IN NEBRASKA. P. J. Heise and A. K. Vidaver. Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE 68583-0722.

Properties of the latex and floss of the perennial plant milkweed (Asclepias spp.) have spurred interest in its development as an alternative commercial crop. A bacterial disease of milkweed was observed in field plots in central Nebraska. Foliar symptoms included watersoaking and necrotic areas surrounded by chlorosis. Soft black lesions occurred on stems. The pathogen has been identified as a xanthomonad using standard microbial methods and pigment analysis. The classification of the milkweed pathogen has been further investigated using SDS polyacrylamide gel electrophoresis of total cell proteins. These results further confirm the identify of the unknown pathogen as a xanthomonad and suggest that the pathogen is a pathovar of Xanthomonas campestris.

772

AN EXAMINATION OF THE POTENTIAL FOR SEED TRANSMISSION OF THE ENDOPHYTIC BACTERIUM CLAVIBACTER XYLI SUBSP. CYNODONTIS (CXC). S.M. Hakimi, J. Anders and J.W. Fahey, Crop Genetics International, 7249 National Drive, Hanover, MD 21076.

As part of an effort to develop techniques for the deliberate inoculation of the endophytic bacterium Cxc into seeds of 8

crop species, the potential of this bacterium to be seed transmitted was examined. Plants colonized by this endophyte were grown to physiological maturity and their seeds were harvested and assayed for evidence of bacterial transmission into the seeds. Plants were stem-inoculated with Cxc 2 weeks following seedling emergence in both greenhouse and field experiments. After seed set, seeds were harvested, some were planted, the rest surface-disinfested, homogenized and assayed for the presence of Cxc. Parental plants were also assayed for Cxc colonization. Cxc was recovered from all parental plants, but none of the seeds examined yielded Cxc in their homogenates or in plants grown from them.

773

CHARACTERIZATION OF ONE CULTIVAR OF PEA (PISUM SATIVUM) WITH REDUCED SUSCEPTIBILITY TO CROWN GALL. **S. L. Robbs and M. C. Hawes, Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721.**

Two weeks after inoculation with Agrobacterium tumefaciens strain B6, only 70% of inoculated 'Sweet Snap' pea seedlings developed tumors. In contrast, of 33 other cultivars tested, 90-100% of inoculated seedlings formed tumors.The mean weight of 'Sweet Snap' tumors was 22mg whereas the mean weights of tumors of the other cultivars ranged in weight from 30mg to 62mg. Preliminary experiments indicate that neither binding, chemotaxis nor induction of vir genes are limiting factors in 'Sweet Snap's' reduced susceptibility. The B-glucuronidase gene fusion assay is being utilized to determine if the efficiency of transformation is less than that of a more susceptible cultivar. In preliminary inheritance studies of the reduced susceptibility phenotype, F1 progeny from a 'Sweet Snap' X 'Wando'(a highly susceptible cultivar) cross formed tumors intermediate in size between the two parents.

774

IN SITU DETECTION OF INDOLEACETIC ACID PRODUCTION BY BACTERIA IMMOBILIZED ON A SOLID SUPPORT. J. M. Bric and R. M. Bostock, Department of Plant Pathology, University of California, Davis, CA 95616.

Distinct red haloes form around bacterial colonies producing indoleacetic acid (IAA) when grown on a membrane disc and subjected to biochemical assay. A membrane placed upon bacterial medium supplemented with 5 mM L-tryptophan is incubated at optimum temperature following inoculation until colonies reach 1-2 mm in diameter. The membrane is removed to a filter disc saturated with Salkowski reagent and incubated until color development. The colorimetric reaction to IAA is limited to a region immediately surrounding each colony, and is specific to strains producing IAA. The ability to differentiate between IAA-producing and non-producing strains from a colony plate lift provides a rapid and convenient method to screen large numbers of bacteria.

775

IDENTIFICATION OF OSMORESPONSIVE GENES IN PSEUDOMONAS FLUORESCENS: EVIDENCE OF PARTICIPATION OF CALCIUM AND PROLINE IN OSMOREGULATION. B. J. Schneider, R. A. Rupp, and C. S. Orser. Department of Bacteriology and Biochemistry, University of Idaho, Moscow ID 83843.

Pseudomonas fluorescens strain M4-80R was used as a model rhizosphere-associated bacterium to investigate the presence of genes involved in the osmotic response to high salt. M4-80R was grown to an optical density of 0.3 and exposed to NaCl with and without proline and proline plus calcium chloride. The remission of the toxic salt effects by proline required the presence of calcium. The same experiment substituting the organic nitrogen osmolyte glycine betaine for proline, did not indicate a requirement for calcium for enhanced osmoprotection. This would suggest the existence of separate uptake systems for proline and glycine betaine during osmotic stress, one calcium dependent and the other calcium independent, respectively. We have cloned and are characterizing a putative transport gene regulated by osmotic levels.

777

THE SYSTEMIC RESERVOIR OF AGROBACTERIUM TUMEFACIENS IN SUCKERS OF THE GRAPE CV. CHANCELLOR. R. N. Goodman, Department of Plant Pathology, University of Missouri, Columbia, MO 65211

Populations of Agrobacterium tumefaciens cells in Chancellor suckers (water sprouts) grown during the previous season were determined prior to bud swell (early April) by a low pressure water displacement procedure. During three successive seasons 10^1-10^3 cells/ml were detected in cane sections 8-10cm in length. The numbers of bacteria in suckers 60-120cm in length diminished with distance from the soil or crown of trunk. New water sprouts were sampled through the growing season and bacterial populations were determined. Significant levels of the pathogen were detected from July through November. However, suckers examined in January and February revealed few samples with any A. tumefaciens bacteria. Implications of a possible seasonal flush of the pathogen from reservoirs forced by root pressure prior to bud break will be discussed.

778

SELECTION OF MONOCLONAL ANTIBODIES FOR DETECTION OF ERWINIA AMYLOVORA BY ELISA. R. J. McLaughlin*, J. M. Wells*, and T. A. Chen**., *USDA-ARS-NAA-ERRC. Philadelphia, PA 19118 and **Dept. of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903.

Eight monoclonal antibodies (MA's), specific against antigens of E. amylovora, were evaluated for development of a mixture for use in ELISA for detection of the bacterium. Three of the antibodies (MA-12, MA-21, and MA-37) were observed to bind to different epitopes in an epitope specificity ELISA. Antibodies MA-27 and MA-34 were complemented by MA-8. Two MA's (MA-23 and MA-30) exhibited too low a reaction to determine epitope specificity. Three antibodies (MA-8, MA-12, and MA-21) were chosen, based on epitope specificity and strength of ELISA reaction at low cell dilutions, to determine whether a mixture would improve detection of the bacterium by ELISA. A significant increase in the sensitivity of the assay was observed with the mixture at the detection limits of ELISA (4.0 log cfu) and at cell concentrations up to 5.5 log cfu.

779

Isolation of the ***Azorhizobium caulinodans ntrA*** nitrogen regulatory gene and characterization of its role in ***Sesbania rostrata*** nodule symbiosis. G. R. Lazo, C. Paris, D. W. Nees, and R. A. Ludwig. Thimann Laboratories, University of California, Santa Cruz, CA 95064.

A. caulinodans (***Azr***), a symbiotic stem and root nodulating bacterium of the tropical legume ***S. rostrata***, is able to fix N_2 during free-living growth as well as during nodule symbiosis. ***Azr*** vector-insertion (***Vi***) mutants (Donald & Ludwig, J. Bacteriol. 162:317-323 [1985]) unable to utilize a broad spectrum of secondary-N sources were isolated. Wild-type DNA corresponding to the ***Vi*** mutagenized regions was isolated from a lambda phage genomic library of ***Azr***. Two mutants, 62004 and 62007, carried ***ntrA*** insertions. They mapped to the same locus and appeared related by DNA hybridization. Likewise related phage, with weak hybridization, were identified with a ***Salmonella typhimurium ntrA*** probe. DNA sequence data from this ***Azr*** locus exhibit homology with other bacterial ***ntrA*** sequences. The ***ntrA*** gene encodes a sigma factor which confers promoter specificity for the core RNA polymerase; when complexed as RNA polymerase holoenzyme, it allows activation of numerous genes responsible for acquisition of secondary-N sources. ***S. rostrata*** nodules incited with strains 62004 and 62007 do not fix N_2.

780 Withdrawn

781

SEQUENCE ANALYSIS OF THREE DISEASE RESISTANCE RESPONSE GENES OF PEAS (DRRGS). Chin C. Chiang, Dan Horovitz, Brian Fristensky and L. A. Hadwiger. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

The cloning and expression of pea DRRGs activities in temporal association with the cytologically observed disease resistance to F. solani f. sp. phaseoli, or following induction by chitosan, has been described previously [Physiol. Plant Path. 27:15 (1985)]. DNA sequence analysis of these genes shows close homology between pI49 and pI176, but not with pI206. Hydrophobicity plots of predicted protein sequence show the amino terminal end to be hydrophobic and the carboxyl end to be hydrophilic. The untranscribed region 5' of a genomic clone closely homol. with pI49 and pI176, has the typical consensus sequences, TATA, CCAAT, an SV40-like enhancer sequence, several direct repeats, two-zDNA sequences, a 26 bp dyad symmetry, and a cluster of topoisomerase cleavage sequences. The possible role of the latter sequences and the nuclear matrix in gene regulation will be described.

782

GENERATION AND CHARACTERIZATION OF TN5 INSERTION MUTATIONS IN PSEUDOMONAS SYRINGAE PV. SYRINGAE. L.K. Joe, J. Lindemann, T.V. Suslow. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA 94608.

Strains of *Pseudomonas syringae* pv. *syringae* cause 'holcus spot' of sorghum and 'bacterial canker' of fruit trees. For the purpose of developing biological control agents using *P. s.* pv *syringae* it is of interest to identify and characterize genes determining host range as distinct from basic pathogenicity. Tn5 insertion mutants were generated in *P. s.* pv *syringae*, 5D417, by mating the pathogen with an *E. coli* strain carrying pGS9, a suicide plasmid vector for Tn5. Using a heat shock mating technique, 1375 Km^r Tn5 insertion mutants were selected with frequencies ranging from 3×10^{-5} to 4×10^{-3}. Of those 1375 mutants 1.3% were also Cm^r, suggesting the presence of additional pGS9 sequences. Two independent inoculation methods were used on sorghum NK1580 for screening purposes. Seven of 1343 $Km^r Cm^r$ Tn5 mutants exibited no symptoms of pathogenicity. Southern blot analysis of the seven mutants revealed six single-site insertions and one double-site insertion into eight different sized EcoR1 fragments. Five of the seven mutants inserted into a 33 kb KpnI fragment. Two of those five mutants did not induce a hypersensitive response on tobacco.

783

EXTRACELLULAR CYTOKININ-LIKE ACTIVITY AND PRODUCTION OF IAA DETECTED IN FILTRATES OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI. M. Zapata, A.K. Vidaver and R. Dam. Dept. of Pl. Path., Univ. of Nebraska, Lincoln, NE, 68583-0722.

A bioassay was developed to differentiate strains of X. campestris pv. phaseoli (Xcp) by reactions in pods and cotyledons of the tepary bean (P. acutifolius). Some strains induce watersoaking plus callous tissue (CA^+) and others induce only watersoaking (CA^-). The relationship of CA^+ phenotype with the presence of hormones, plasmid relationships and conditions under which the reaction is induced were assessed. The CA^- strains had the highest IAA level and there were no differences between the CA^+ strains and a nonpathogenic mutant (Vir-). L-tryptophan (L-trp) was associated with IAA production and 2 plasmids in the CA^+ and 1 in the Vir^- strains. The CA^+ strains had plasmids of identical EcoRI digestion patterns; CA^- plasmids differed. Bioassays with pure IAA and kinetin together induced callous tissue, but not separately. The Vir^- and CA^- strains show that IAA production is insufficient to induce callous. Only CA^+ filtrates from minimal but not from L-trp media induced the CA^+ phenotype. These results confirm the production of IAA by Xcp and also show the production of an extracellular component with cytokinin-like activity that induces callous.

784

USE OF A POTATO TISSUE CULTURE SYSTEM FOR MOLECULAR ANALYSIS OF THE HYPERSENSITIVE RESPONSE (HR) INDUCED BY PSEUDOMONAS SOLANACEARUM. Y. Huang, P.L. Xu & L. Sequeira. Dept. of Plant Pathology, University of Wisconsin-Madison, WI 53706.

Two Tn5-insertion mutants of strains B1(BJA34, HR^-) and K60(KD688, Vir^-) of P. solanacearum were unable to cause rapid browning in calli or the HR in leaves of an incompatible clone(C-3) of Solanum phureja. In these two mutants, Tn5 is inserted in the same Eco R1 DNA fragment (7.0 Kb). The cloned fragment from BJA34, pT34, was transformed into K60. All the Km-resistant and Amp-sensitive transformants had the same phenotype as BJA34. Two randomly selected transformants carried the Tn5-containing fragment in place of the wild-type fragment and exhibited the same DNA hybridization pattern as BJA34. With pT34 as a probe, the corresponding wild-type fragment was identified in a K60 genomic library, subcloned, and conjugated into K60 transformants. All the transconjugants restored the phenotype of K60. The results suggest that virulence and HR genes are located in the same DNA region and may be closely linked.

785

HYDROXYPROLINE-RICH GLYCOPROTEIN ACCUMULATION IN CELL WALLS OF TOMATO INFECTED WITH FUSARIUM OXYSPORUM F. SP. LYCOPERSICI. J. C. Stutz and V. H. Bess. Arizona State University, Tempe, AZ 85287.

Hydroxyproline-rich glycoproteins (HRGP) have been shown to accumulate in cell walls of plants infected with plant pathogens. Two tomato cultivars, Improved Pearson and Improved Pearson VF-11, which differ in a single dominant gene for resistance to Fusarium oxysporum f. sp. lycopersici Race 1, were inoculated and cell wall hydroxyproline content was determined. Plants were cut transversely 2 cm below the cotyledon and inoculated with a spore suspension. Stems were harvested in consecutive segments of 2 cm lengths. By 48 hrs, hydroxyproline levels in lower segments of resistant plants were more than two times the levels found in susceptible plants. Differences were less apparent in segments further from the point of inoculation. The response of cell suspension cultures treated with an elicitor prepared from F. oxysporum was also investigated.

786

VARIATION IN AGGRESSIVENESS AMONG RACES OF PLASMOPARA HALSTEDII. T. J. Gulya and S. Masirevic, USDA-ARS, North Dakota State University, Fargo, ND 58105; and Institute of Field & Vegetable Crops, Novi Sad, Yugoslavia.

Thirty field isolates of sunflower downy mildew were evaluated in greenhouse experiments for aggressiveness. Ten isolates each of races 2, 3 and 4 were inoculated onto seedlings of Interstate 003, an oilseed hybrid with no mildew resistance genes. The inoculum concentration of 10,000 zoosporangia/ml was less than that required for 100% infection. Two weeks later when symptoms were evident, the plants were rated for the percentage of systemically infected plants, cotyledon-limited infection, damped-off seedlings, and dry weight of infected plants. Sunflower plants inoculated with race 2 downy mildew had significantly less total infection and less systemic infection, and more cotyledon-limited infection than plants infected with either race 3 or race 4. No differences were noted in the percentage of damped-off plants or in the dry weight of infected plants between races.

787

HOST-PARASITE RELATIONSHIPS OF ELM CALLI CHALLENGED WITH CERATOCYSTIS ULMI. C. R. Krause, J. M. Ichida, L. R. Schreiber and S. Domir, Nursery Crops Research Laboratory, USDA-ARS, 359 Main Rd., Delaware, Ohio 43015

Electron microscopy was used to visualize the response of elm calli to inoculation with the Dutch elm disease fungus, Ceratocystis ulmi. Calli were derived from an American elm seedling, a Siberian elm seedling, a C. ulmi-resistant American elm (8630) and from petunia. One mm filter paper squares, saturated with fungal spore suspensions, were placed onto the surfaces of calli and incubated at 22°C. Spores germinated within 8-12 hr. Calli were processed by conventional methods for examination with computer-controlled electron beam analysis. After 24 hr, hyphal penetration extended approximately 1 mm (<70 cell layers) into the calli of the American elm. Hyphal penetration into Siberian, 8630 and petunia calli was restricted to 10-20 cell layers.

788

IN VITRO SELECTION OF POTATO CULTIVARS RESISTANT/TOLERANT TO VERTICILLIUM DAHLIAE. J. R. Hess and W. M. Hess, Dept. of Bot. & R.S., Brigham Young Univ., Provo, UT. and W. B. Jones, NPI, Salt Lake City, UT.

Potato cultivars were tested for sensitive or non-sensitive responses to two forms of Verticillium dahliae toxin in vitro. The culture filtrate produced significant responses. The resistant cultivar A68113-4 was sensitive. Russet Burbank and NDA8694-3, susceptible cultivars, were also sensitive. Alpha, a resistant cultivar, was the most tolerant. These results indicate that V. dahliae culture filtrate may be useful for selection of some types of resistant/tolerant potato cultivars in vitro. Pieces of resistant Russet Burbank callus, grown on media containing V. dahliae culture filtrate, were selected. Surviving callus pieces produced shoots.

789

ULTRASTRUCTURAL INVESTIGATION OF THE ACREMONIUM COENOPHIALUM-ROOT ASSOCIATION IN TALL FESCUE. M. D. Azevedo, K. L. Cook, and R. E. Welty, Dept. of Botany and Plant Pathology, Oregon State University and USDA-ARS, Corvallis, Oregon 97331-7102.

Recent research demonstrated that A. coenophialum can infect, inter/intracellularly, roots of tall fescue grown on an aseptic culture medium. To investigate this relationship, seeds of endophyte-infected tall fescue were sterilized, cultured on PDA, and incubated in a growth chamber. After 4 and 8 wk, fungus-infected root tissue was examined by SEM, TEM, and light microscopy. Hyphae were densely packed between root epidermal cells and were often associated with root caps and meristems. Conidiophores and conidia formed on root hairs. Host cell walls adjacent to hyphae were occasionally ruptured, but no evidence of enzymatic degradation was observed. Electron dense deposits were found within and surrounding hyphal cell walls. The origin and composition of these deposits are being investigated. Infection of tall fescue roots by A. coenophialum in soil is currently being studied.

790

MORPHOLOGICAL AND BIOLOGICAL VARIATION AMONG POPULATIONS OF AULACORTHUM SOLANI, THE VECTOR OF SOYBEAN DWARF VIRUS. V. D. Damsteegt, USDA-ARS, Frederick, MD and D. J. Voegtlin, Illinois Natural History Survey, Champaign, IL 61820

Morphometric measurements of cauda length, length of ultimate rostral segment, and length of hind tarsal II clearly distinguished between Japan (green and yellow forms) vs CAlifornia and New Brunswick populations; New Zealand aphids were intermediate. The cultural host influenced body length but not cauda length. Cool temperatures (<10 C) favored longer body and cauda. Color variations were noted in all populations. All populations produced toxicogenic salivary secretions; J (green form) produced distinct yellow spots at feeding sites and all others produced veinal necrosis and/or plant stress. All populations transmit the three recognized strains of SDV; J forms are most efficient. J forms prefer soybeans as a food host if given a choice; CA, NB, and NZ forms favor leaf lettuce, clover, and curly dock. Natural (oriental) vectors can be readily distinguished from occidental populations.

791

SUPPRESSION OF PYRENOPHORA TRITICI-REPENTIS ASCOCARP PRODUCTION BY GLYPHOSATE-CONTAINING HERBICIDE. U. Sharma, E. A. Adee, and W. F. Pfender. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Nonsterile wheat straws infested with Pyrenophora tritici-repentis were dipped in solutions of glyphosate herbicide (Roundup, Monsanto Agric. Co.) prepared at labelled rates (1%, 1.7%). These straws were incubated in the greenhouse under conditions conducive for ascocarp formation. Herbicide treatments were applied before, or at various times during, the 3-wk incubation period. The herbicide completely inhibited ascocarp formation when applied before the ascocarp-conducive environment was imposed. This inhibition was diminished as the time delay increased, and was not significant if the herbicide was applied to straws after 10 days of continuously favorable conditions. Experiments conducted on autoclaved, inoculated straw indicated that the herbicide did not greatly reduce mycelial growth rate of P. tritici-repentis.

792

SOURCES OF RESISTANCE TO SEPTORIA NODORUM BLOTCH IN WINTER BARLEY. B. M. Cunfer, J. W. Johnson, and P. L. Raymer, Dept. of Plant Pathology and Dept. of Agronomy, Georgia Station, University of Georgia, Griffin, GA 30223-1797

Septoria nodorum blotch of barley is part of a complex of foliar diseases in the southern U. S. which includes spot blotch and scald. The foliar disease caused by Leptosphaeria nodorum has been overlooked in selection of lines for disease resistance. Therefore, 155 barley cultivars and lines were tested in replicated field tests during 1983-87. Naturally-occurring inoculum was supplemented in some tests with a mixture of virulent isolates. Other foliar diseases were present only at low levels in all tests. Lines with low disease severity in preliminary trials were retested in subsequent seasons. In these tests 5, 13, and 9 lines were consistently rated very resistant, resistant, and moderately resistant, respectively. Therefore, a number of breeding lines with resistance to septoria nodorum blotch are now available for cultivar development.

793

THE INFLUENCE OF TWO ENVIRONMENTAL PARAMETERS ON SEVERITY OF CEPHALOSPORIUM GRAMINEUM ON WINTER WHEAT. R. H. Johnston and D. E. Mathre. Dept of Plant Pathology, Montana State University Bozeman, MT 59717.

The interaction between soil temperature at 10 cm expressed as degree days, seasonal precipitation and disease severity for the growing seasons 1982 through 1988 was examined to explain the seasonal variation which occurs under uniform inoculation regimes (yield loss of 0 to 82%). Increased disease severity could be explained by high numbers of soil degree days (>1000) from the period following seeding until the soil profile froze in Nov of each year, followed by cool spring (Mar-April) temperatures (degree days <400) with high numbers of freeze-thaw cycles (>10) as recorded in the upper 10 cm of the soil profile. Precipitation recorded from Sept-Nov and Feb-April of each year, though varied did not appear to be an important factor in determining disease severity.

794

CULTURAL INSTABILITY OF PSEUDOCERCOSPORELLA HERPOTRICHOIDES. A.M. Julian, M.J. Hocart, and J.A. Lucas, Department of Botany, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

Field isolates of Pseudocercosporella herpotrichoides (Fron. Deighton), causal agent of eyespot disease of cereals, can be categorized into two pathotypes; BW and BWR, based on a differential ability to infect rye. Usually these differences in pathogenicity are correlated with differences in growth rate and colony morphology. These criteria are used in field surveys of the pathogen population. In many cases, however, BWR type isolates are unstable and regularly sector to produce fast-growing, even-edged colonies characteristic of the BW pathotype. By introducing genetic markers into unstable isolates and their progeny the nature of this instability has been studied. Pathogenicity trials, isozyme analysis and tests on in vitro response to fungicides confirm that the two pathotypes are biologically distinct, but suggest that morphological criteria are unreliable. Genetic evidence, so far, indicates that morphological instability is not a cytoplasmically inherited trait, and is not based on heterokaryosis.

795

COMBINED EFFECT OF POWDERY MILDEW (ERYSIPHE GRAMINIS F.SP. HORDEI) AND DROUGHT ON BARLEY. F. Zine Elabidine, M. Reinhold, and A. L. Scharen, USDA, ARS, Plant Pathology Dept., Montana State University, Bozeman, MT 59717-0002.

Seedlings of a susceptible barley variety (Manchuria) were inoculated with an isolate of powdery mildew (Erysiphe graminis). Dry weight of shoots and roots of both inoculated and control plants were taken, in order to determine the effect of the fungus on plant growth. Roots of diseased plants weighed significantly less (P=0.05) than roots of control plants. Four levels of drought (no water, 1/4, 1/2, and 3/4 of normal watering volume) were simulated to determine the effect of both moisture stress and disease on the plants. Inoculated plants wilted significantly sooner than non-inoculated plants under water stress. Seedling infection by powdery mildew permanently impairs the barley plant's ability to absorb and utilize water when drought occurs later in the growth cycle.

796

BIOLOGICAL CHARACTERISTICS OF LAGENA RADICICOLA. CULTURE CONDITIONS AND LIGHT MICROSCOPE APPEARANCE. L. Zhang and W. G. Langenberg. USDA/ARS, Department of Plant Pathology, University of Nebraska, Lincoln, 68583.

Lagena radicicola Vanterpool Ledingham is an obligate fungal parasite of cereal roots belonging to the Lagenidiales, Oomycetes. The fungus is of interest because its pathogenicity to roots is little known while it could serve as a vector of virus diseases by means of its zoospores. L. radicicola grew well at 5°C. Oospores in soil debris

germinated after as little as 12 hours. An exposure of wheat seedling roots for 10 min to a zoospore suspension was sufficient for infection of the roots. The zoospores penetrated and formed unicellular mycelia in root epidermal cells including root hairs. The individual mycelia were generally limited to one to a cell in a configuration resembling a coiled nematode. However, some penetrated to neighboring cells and then were long and linear in shape. Zoospores were formed and liberated from vesicles.

797

COMPARISON OF EAR AND STALK INOCULATION METHODS WITH FUSARIUM MONILIFORME ON MAIZE. W J. Drepper, B. L. Renfro. CIMMYT, Lisboa 27, 06600 Mexico, D. F.

Six different ear and stalk inoculation methods were evaluated for their ability to cause ear or stalk rot. The experiments were performed in 1987 during two seasons at three locations in Mexico (tropical, subtropical and high-altitude). Disease severity for ear or stalk inoculations was highest when a wounding type inoculation was used. Highly significant positive correlations were found between inoculation tool diameter and ear or stalk rot severity. Location had very little effect on rank order of disease severity of the different methods. Location, however, had a significant effect on the general level of disease severity observed.

798

THE SEXUAL STAGE OF PYRENOPHORA TERES IN CALIFORNIA BARLEY FIELDS. R. D. Cartwright, R. K. Webster, and B. J. Steffenson, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.

Barley straw was examined during the winter and spring for fertile pseudothecia of P. teres, the cause of barley net blotch. Dark, setose pseudothecia (460 x 635 µm) containing bitunicate asci (32 x 228 µm) and multiseptate ascospores were found on straw at several locations. Mature ascospores (22 x 54 µm) were present as early as January 11 but were more common in April collections. Monoascosporic isolations on V-8 agar were incubated 14 days at room temperature with supplemental light. Resulting colonies and conidia were typical of those previously described for P. teres. Conidia of each isolate were harvested in sterile water and spray inoculated on seedlings of 24 barley genotypes. Inoculated seedlings were incubated 48 hrs in a mist chamber at 22 C, then transferred to the greenhouse. Net blotch lesions were observed in 4-7 days on susceptible genotypes and the fungus was repeatedly recovered. We believe this is the first report of the sexual stage of P. teres in California. Studies on its epidemiological role are continuing.

799

INCREASED RICE SHEATH BLIGHT DUE TO NITROGEN FERTILIZATION AND PLANTING RATE. D.E. Groth, Rice Research Station, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P.O. Box 1429, Crowley, LA 70527-1429.

Rice (Oryza sativa L. cv. Lemont) plots were drill seeded at rates of 28 to 224 kg/ha. Plots were preflood fertilized at rates of 0 to 224 kg/ha N with ammonium sulfate. After permanent flood plots were inoculated with Rhizoctonia solani grown on a moist rice hull:rice grain mixture (2:1). Stand establishment, tiller production, and disease development were monitored through the season. Plots were combined harvested at maturity. Disease severity as measured on a 0-9 scale was increased four to five disease units between the low and high planting and fertilization rates. Sheath blight severity increased with increasing plant stands and fertility levels. The increased disease was apparently due to crop canopy differences rather than N induced physiological changes. Management for agronomic factors had more effect on yield than management for disease control.

800

DETERMINATION OF OPTIMAL SAMPLING MONTHS TO EVALUATE WINTER WHEAT WITH ELISA FOR RESISTANCE TO WHEAT SOILBORNE MOSAIC VIRUS (WSBMV). C. R. Armitage, R. M. Hunger, and J. L. Sherwood. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

ELISA is used in Oklahoma to evaluate the reactions of winter wheat to WSBMV. To facilitate this evaluation, the optimal time to collect foliar samples for ELISA was determined by developing virus antigen (VA) titer curves. Two resistant (Hawk and Newton) and two susceptible (Sage and Vona) cultivars of winter wheat were analyzed for three growing seasons. Titer curves varied from year to year and between cultivars with the same disease reaction. Resistant cultivars occasionally became infected by WSBMV resulting in VA titers approaching those of the susceptible cultivars in March or April. During February, however, VA titers were consistently higher in the susceptible cultivars than the resistant cultivars. Thus, we recommend sampling field plots in Oklahoma during February to evaluate WSBMV reaction by ELISA.

801

FOLIAGE ROT OF DELPHINIUM CONSOLIDA CAUSED BY ERWINIA CAROTOVORA PV. CAROTOVORA. L. E. Pierce and A. H. McCain, Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, CA 94720.

In the spring of 1986 we received specimens of field-grown Delphinium consolida L. with a foliar soft rot beginning at the growing point and extending down the stem. Erwinia carotovora pv. carotovora was isolated from the diseased tissues. Younger plants were inoculated by spraying inoculum on wounded or unwounded foliage. A wound was necessary for infection. Disease was minimal at 15 C, moderate at 21 C and severe at 26 or 32 C. Older, less succulent plants that were flowering and going to seed were not susceptible to the disease. Streptomycin sprayed on wounded plants at concentrations of 100 or 200 µg/ml was effective in controlling the disease when applied prior to inoculation.

802

EVALUATION OF PYTHIUM BLIGHT PREDICTION MODELS USING AN ANTIBODY-AIDED DETECTION TECHNIQUE. W. W. Shane, Department of Plant Pathology, Ohio State University, Columbus, OH 43210.

Evaluations were made of two models (Nutter, et al. (N) and Hall, et al. (H)) for prediction of Pythium blight on turfgrass. Pythium-specific antibodies (Agri-Diagnostics, Cinnaminson, NJ) were used to monitor fungal populations on bentgrass/annual bluegrass fairways not treated with Pythium-specific fungicides. Replicated 10 gm samples of fresh grass clippings were homogenized in 100 ml and aliquots were tested for Pythium in conjunction with standard isolation procedures. Pythium activity could occasionally be detected with the antibody-technique when no symptoms were obvious. Over two years, Pythium activity was predicted too frequently by the H model and too infrequently by the N model. The antibody-aided technique allows refinement of models by providing data on Pythium population beyond that given by symptoms alone.

803

TIME COURSE OF PYTHIUM BLIGHT DEVELOPMENT ON TURFGRASS UNDER CONTROLLED ENVIRONMENTAL CONDITIONS. J. C. Stier and W. W. Shane, Department of Plant Pathology, Ohio State University, Columbus, OH 43210.

Mycelia, sporangia, and blight development were monitored on leaves of Poa pratensis 'Baron' inoculated with 3 strains of Pythium aphanidermatum, the primary cause of warm season Pythium blight in Ohio. Inoculated plants were incubated at constant temperatures of 30.6, 26.7, or 21.1 C. Differences in aggressiveness among the 3 strains were noted, but in general, first symptoms, primarily cottony blight, were detected at 48, 48, and 72 h following incubation at 30.6, 26.7, or 21.1 C, respectively. Sporangia production followed cottony blight and was generally more abundant at higher temperatures. Sporangia persisted longer than mycelia at the later stages of disease development. Our results show that development of significant Pythium blight symptoms requires more than 24 h, especially at lower temperatures--a fact not incorporated into current models for Pythium blight.

804

THE RELATEDNESS OF PATCH CAUSING FUNGI IN THE MIDWEST. H.T. Wilkinson and R.T. Kane, Department of Plant Pathology, Univ. of Illinois, Urbana-Champaign, IL, 61801.

Summer patch, poa patch, zoysia patch and take-all patch, appear to be caused by at least two different genera, three species, and several biotypes. Diseased turf samples were collected from eight Midwest states and isolation of ecototrophs was done. Cultural characteristics, pathogenicity on six grass species, anatomosis groups, temperature optima,

isoenzymes, and DNA:DNA hybridization were used to characterize the isolates and determine relatedness. Isolates collected from P. pratensis and P. annua appear to be genetically related and belong to the genus Magnaporthe. Both mating types were isolated from either species of bluegrass, but biotypes from P. annua differed in pathogenicity and temperature optima. Not all isolates from Poa species were of the genus Magnaporthe, nor were they all pathogens. The pathogen from Agrostis palustris was a Geaumannomyces sp. The species from Z. japonica displays characteristics different than either this genus or Magnaporthe.

805

ETIOLOGY AND EPIDEMIOLOGY OF ZOYSIA PATCH IN ZOYSIA japonica. H.T. Wilkinson, Department of Plant Pathology, University of Illinois,Urbana-Champaign, Illinois, 61801.

A highly destructive disease of Z. japonica was observed in turfs in the climatic-transitional zone of the U.S.A. Zoysia patch has not been reported in other regions. Zoysia patch is perennial and disease severity is difficult to predict. Symptoms may appear in the spring and/or fall when soil temperatures are 15-20 C. Ectotrophic root colonization is followed by root destruction, premature death of lower leaves and finally the entire shoot. Rhizomes appears more resistant, but are colonized. Penetration of the root is direct and colonization is mainly in the cortical region, with occasional penetration of the stele. Root colonization and disease severity are enhanced by periodic wetting and draining of the root zone. The fungus is capable of colonizing turf 1 m radially in nine months and forms abundant phialospores on infected turf and in culture. A teliomorphic state has not been observed. The anamorphic state is morphologically similar to the summer patch pathogen.

806

OPHIOSPHAERELLA HERPOTRICHA ASSOCIATED WITH SPRING DEAD SPOT OF BERMUDAGRASS IN KANSAS. N. Tisserat, J. Pair, and A. Nus, Departments of Plant Pathology and Horticulture, Kansas State University, Manhattan, KS 66506.

Ophiosphaerella herpotricha was consistently isolated from dead patches of bermudagrass afflicted with spring dead spot (SDS) in Kansas. Pathogenicities of O. herpotricha and Leptosphaeria korrae, an incitant of SDS in other areas of the U.S. and in Australia, were compared in the greenhouse on common bermudagrass. Both fungi significantly increased root discoloration and decreased root dry weight ($p < .05$) 4 mo after inoculation. O. herpotricha-inoculated plants had significantly ($p < .05$) lower root weights than those inoculated with L. korrae. In Sep 1987, two 10-cm-diam. plugs were removed from each of three replicate plots of 15 bermudagrass cultivars in the field. Ten g of oats either infested with O. herpotricha or sterile (control) were added to the bottom of the hole on each plot; the plugs were then replaced. In May 1988, 71% of all inoculated plugs were dead; no control plugs died.

807

DIFFERENTIATION OF OPHIOSPHAERELLA HERPOTRICHA AND LEPTOSPHAERIA KORRAE BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS. N. Tisserat. Department of Plant Pathology, Kansas State University., Manhattan KS 66506.

Restriction fragment length polymorphisms (RFLP) were used to distinguish Ophiosphaerella herpotricha (Oh) and Leptosphaeria korrae (Lk); these fungi are associated with spring dead spot of bermudagrass. Total DNA was extracted from isolates and digested with EcoRI or HindIII. DNA fragments were separated by agarose gel electrophoresis and stained with ethidium bromide. Six of seven Lk isolates had an identical satellite DNA fragment pattern. None of the Oh isolates showed a similar banding pattern. A rDNA genomic clone from Armillaria mellea is being used in Southern blot analysis of RFLP gels to differentiate Lk and Oh. This technique may be useful in identifying fungi associated with patch diseases of turfgrass which do not readily fruit in culture.

808

FACTORS AFFECTING PRIMARY INOCULUM PRODUCTION AND INFECTION OF DOUGLAS-FIR BY MELAMPSORA OCCIDENTALIS. G. A Chastagner and J. M. Staley, Washington State University, Puyallup, WA 98371.

The effects of light (UV, fluorescent and dark), temperature (5,10,15,20,25,30C) and time period (May-June) on the germination of M. occidentalis teliospores were studied. Light did not affect germination. Although overall percent germination was similar at 10-20C, a greater percent of teliospores produced moderate or abundant levels of basidiospores at 15C. Limited sporulation occurred at 5 and 25C; none at 30C. Teliospores on Populus leaf discs from the field sporulated through 8 June 1985 and 23 June 1986. The percent of teliospore germination and the level of inoculum produced was greatest in early May. To identify infection periods, field grown Douglas-fir trees were exposed to infected Populus leaves for 1-wk periods between 10 May and 28 June 1985, and 12 May and 14 July 1986. The only trees with significant levels of rust were those exposed between 24 May and 7 June 1985 and 12 May and 2 June 1986.

810

ALTERNARIA LEAF SPOT OF CANDYTUFT IN CALIFORNIA. Robert D. Raabe, Dept. of Plant Pathology, University of California, Berkeley, 94720.

A serious leaf, stem and flower spot of Iberis umbellata in the Half Moon Bay area of California was found to result from infection by a large-spored, short-chained Alternaria. Inoculations of I. umbellata, I. amara, I. gibralatarica, I. jordani, I. prostii, I. pruitii, I. saxatilis, I. semperflorens, I. sempervirens, and I. tenoreana were successful, as were inoculations of radish, cabbage, cauliflower, Brussels sprouts and broccoli. Because of spore shape and sizes plus the host range, the fungus is identified as A. brassicae. Although I. amara was listed as being naturally infected by A. brassicae in Denmark, this is believed to be the first report of the fungus on Iberis spp. in the United States. In Europe, the fungus also has been reported to infect Matthiola incana. Inoculations of this plant produced small necrotic flecks but the fungus was not recovered from them.

811

DOWNY MILDEW OF PANSY IN CALIFORNIA. R. D. Raabe and T. E. Tidwell, Department of Plant Pathology, University of California, Berkeley, CA 94720 and Calif. Dept. of Food and Agriculture, Sacramento, CA 95814.

In the winter of 1987-1988, downy mildew on pansy was found in the San Francisco Bay Area. Plants infected included pansy (Viola x wittrockiana), viola (V. cornuta) and Johnny-jump-up (V. tricolor). Successful inoculations were made on pansy and Johnny-jump-up. Although a Peronospora sp. was reported on Viola spp. from Europe and the U.S. and a Bremiella has been reported as occurring on Viola in the U.S., the fungus in California does not belong to either of these genera. Sporangiophores are not completely dichotomously branched and the portion bearing the sporangium is usually straight rather than curved. The sporangia are poroid and germinate to produce zoospores. For these reasons, the fungus is put in the genus Pseudoperonospora. Biweekly sprays of metalaxyl, fosetyl or mancozeb and combinations of mancozeb with metalaxyl or fosetyl were tried. Control resulted only when metalaxyl was used.

812

ECTOTROPHIC FUNGAL COMPARISONS BETWEEN PATCH DISEASE-AFFECTED AND SYMPTOMLESS KENTUCKY BLUEGRASS TURF IN WISCONSIN. Jana Stewart and G. L. Worf, Dept. of Plant Pathology, University of Wisconsin-Madison, WI 53706.

Identically appearing dark hyphae were observed associated with roots of both naturalized, symptomless (NBS) and cultivated, diseased Kentucky (Ky) bluegrass (Poa pratensis) (CBD). Direct and trap plant isolations yielded distinct differences. Phialophora sp. was isolated from plants collected at all 17 NBS sites, and two of 15 CBD sites. By contrast, Leptosphaeria korrae was obtained from plants from 14 of 15 CBD sites, but no NBS sites. In greenhouse seedling inoculations, both organisms developed ectotrophic patterns. All L. korrae but no Phialophora isolates tested were pathogenic on 'Georgetown' and/or wheat. Phialophora may be ubiquitous as a turf colonizer but L. korrae appears to be the primary Ky bluegrass patch disease agent in Wisconsin.

813

HELICONIA ROOT ROT CAUSED BY CYLINDROCLADIUM SPATHIPHYLLI F. SP. HELICONIAE. M. Aragaki, P. S. Yahata and J. Y. Uchida, Dept. of Plant Pathology, U. of Hawaii, Honolulu, Hawaii 96822.

A Cylindrocladium sp. closely resembling C. spathiphylli was isolated from diseased roots of declining heliconia plants with yellow, drying leaves. The same fungus was associated with tan, papery spots of leaves, petioles, and sheaths. Rhizomes of declining plants with severe root rot were virtually unaffected, except for blackened root traces. Isolations were made from leaf spots and root rots from several cultivars representing Heliconia psittacorum, H. angusta, H. stricta, H. wagneriana, H. caribaea, and H. bihai. The heliconia Cylindrocladium and C. spathiphylli were not cross-pathogenic. Conidia of the heliconia Cylindrocladium were 79.8 ± 7.4 x 7.6 ± 0.6 um, mostly uniseptate, but two or three septa were common. Vesicles were mostly globose, 13.6 ± 1.4 um diameter, but ellipsoid types were common. We propose the designation C. spathiphylli f. sp. heliconiae based on morphological similarities to C. spathiphylli and pathogenic specialization.

814

LAVANDULA ANGUSTIFOLIA, A NEW HOST FOR PHYTOPHTHORA PARASITICA. M.L. Putnam, MD Dep. Agric., Annapolis 21401

Several hundred 4 in. pots of lavender at a Maryland nursery were observed in various stages of decline or were dead in the fall of 1987. Symptoms included discoloration (silvering) and death of the foliage from the soil line upwards, discoloration of vascular tissue and watersoaking of stems, branches, and roots. Isolations from discolored stem tissue yielded a fungus with characters similiar to Phytophthora parasitica. Positive identification was based on cultural characteristics; caudicity, size, and shape of the papillate sporangia; oogonial and antheridial characters; and pairing with known A^1 and A^2 mating types of P. parasitica. Sexual structures were formed only with the A^1 mating type. Koch's postulates were fulfilled by inoculating 2-year old plants and rooted cuttings with mycelial mats and zoospores, respectively. Symptoms were identical to those originally observed, and re-isolation yielded only P. parasitica. This is the first time Lavandula has been reported as a host for P. parasitica.

816 Withdrawn

817

ANALYZING SPATIAL AND TEMPORAL FLUCTUATIONS IN TWO PATHOGEN POPULATIONS. D. Marshall, Texas A&M University Research and Extension Center, 17360 Coit Rd., Dallas, TX 75252.

Over a three year period, uredinial isolates of Puccinia recondita, and pycnidial isolates of Septoria tritici were each analyzed for virulence on a seperate set of differentials. For each isolate, the virulence data were identified by the cultivar from which it originated, the severity and incidence of the disease, and the date and location of the collection. The virulence and identification data of each isolate was put into a vector and analyzed for relatedness by several cluster-seeking algorithms including minimum distance to the mean and nearest-neighbor analysis. Probability density functions indicated that more variability was detected in the P. recondita population during cooler months, regardless of location. However, location and cultivar had significant effects on variability in the S. tritici population, whereas time of collection did not.

818

RELATIONSHIP BETWEEN PRIMARY INOCULUM OF PYRENOPHORA TRITICI-REPENTIS AND DISEASE PROGRESS CURVES. E. A. Adee and W. F. Pfender, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Epidemics of wheat tan spot, caused by Pyrenophora tritici-repentis, are initiated by ascospores produced on residue from previously infected wheat. Because secondary cycles follow the initial infection, it was not clear whether lowering primary inoculum would significantly change epidemic development. We quantified inoculum by counting the ascocarps per gram of culm tissue used to infest field plots. Three levels of inoculum, comprising approximately a 100-fold range in ascocarp numbers, were applied to the plots in the fall. Following the initial infections, the tan spot severity was rated every 5-8 days until leaf senescense. Disease progress curves were constructed. The areas under the curves were positively correlated with the number of ascocarps per plot.

819

WHEAT LEAF RUST AND AEGILOPS CYLINDRICA. D. L. Long, A. P. Roelfs, and J.F. Schafer, Cereal Rust Laboratory, USDA-ARS, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

In December of 1987, TAM 101 wheat in the southern Great Plains was heavily infected with an avirulent leaf rust population, demonstrated by the widespread presence of resistant lesions. Gene postulation showed that TAM 101 probably possesses Lr10. Inoculum to develop such an infection on a resistant cultivar is believed to have been generated locally as no source of an Lr10 avirulent leaf rust population occurred in the northern spring wheat area. Avirulence to Lr10 is rare in the United States, making up less than 10% of the population in 1986 and 1987 and less than 5% in the Great Plains. Collections of leaf rust were made from Aegilops (Triticum) cylindrica Host.(goatgrass) in or near wheat fields in the southern Great Plains in 1986 and 1987. All isolates were avirulent to Lr10. Inoculum from a widely distributed host such as goatgrass could provide adequate inoculum to generate the observed infection levels.

820

WEATHER CONDITIONS ASSOCIATED WITH THE DEVELOPMENT OF SOUTHWESTERN RUST ON COTTON.
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Weather conditions leading to the development of southwestern rust (Puccinia cacabata) on cotton were evaluated at 3 locations in southeastern Arizona. Rust appeared following an extended period of wet, humid weather. In 1987, more than 16 hours of wet canopy/high humidity conditions were observed on two consecutive days between 5 and 7days prior to the appearance of rust pustules. Temperatures during the wet canopy/high humidity periods were moderate, ranging from 65F to 76F. Afternoon rain showers initiated these extended periods of wet canopy/high humidity conditions.

821

CULTIVAR AND ENVIRONMENTAL EFFECTS ON SPREAD OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA AND SOYBEAN STEM CANKER DEVELOPMENT. J.P. Damicone, G.T. Berggren, and J.P. Snow, Dept. of Plant Path. & Crop Physiol., La. Agric. Exp. Sta., La. State Univ. Agric. Center, Baton Rouge, LA 70803.

Development of soybean stem canker arising from inoculum point sources was quantified on cultivars Bay (resistant), W550 (intermediate), and Bedford (susceptible) in the field in 1987. Stem canker progress was fit to the Weibull model and had a shape parameter equal to the idealized for a monocyclic disease for Bedford. Disease gradients were fit to the exponential model and had increasing rates of decline (b) with increasing stem canker resistance (Bedford b=0.2, W550 b=0.25, Bay b= 0.58). Predicted yield loss (derived through linear regression) at 100% disease incidence was 52% for Bedford, 58% for W550, and 0% for Bay. Stem canker incidence was greater in W550 in 1987 (56.7%) compared to 1986 (23.2%). Rainfall levelsincreased 103% in June, 49% in July, and 177% in August of 1987 compared to 1986.

822

STABILITY OF GENERAL RESISTANCE IN THE POTATO LATE BLIGHT PATHOSYSTEM. J. M. Parker, H. D. Thurston, and W. E. Fry, Plant Pathology Department, Cornell University, Ithaca, NY 14853.

The resistance ranking of four potato cultivars was determined in different environments, in different years, and with different isolates of *Phytophthora infestans*. In the field in 1986 and 1987, three of the four cultivars had the same resistance ranking in Toluca, Mexico as in Freeville, NY. In both locations, cultivar ranking did not change regardless of the isolate used. One cultivar, Alpha, had a different resistance ranking in Toluca relative to its ranking in Freeville. Under controlled conditions, cultivar ranking did not change in response to changes in photoperiod and light intensity. For those interested in new or introduced potato cultivars, the general resistance they express may therefore be stable, but exceptions should be expected.

823

FACTORS AFFECTING LEAF RUST EPIDEMICS ON WINTER WHEAT IN LOUISIANA. K.V. Subba Rao, J.P. Snow, and G.T. Berggren. Dept. of Plant Path. and Crop Phys., La. Ag. Expt. Sta., LSU Ag. Center, Baton Rouge, LA 70803.

Four epidemics were generated by sequential inoculation of plots of a leaf rust-susceptible cultivar, McNair 1003, at 15-day intervals from Feb 1 to Mar 18, 1987, at Baton Rouge, and weekly progress of the epidemics was monitored. The dates of inoculation Feb 1, 15, Mar 1, and 18, had a significant effect on the average apparent infection rates of the epidemics which were 0.09, 0.08, 0.15, and 0.21, respectively. The incubation period for the four epidemics varied from 8 to 18 days. Although the weekly apparent infection rates for all the epidemics were similar from April 12-May 2, the AUDPC for for epidemics initiated Feb 1 and Feb 15 were significantly different from those begun Mar 1 and Mar 18. Disease severity was highly correlated with minimum temperature and hours of leaf wetness, indicating the importance of these variables in the leaf rust progress in Louisiana. Multiple regression analysis of the natural log transformed disease severity with minimum temperature and hours of leaf wetness also indicated the importance of these two variables in disease development.

824

THE SPREAD OF LETTUCE ANTHRACNOSE IN SPACE AND TIME. Parman and T.V. Price, School of Agriculture, La Trobe University, Bundoora, Victoria 3083, Australia.

In Spring (Oct.) 1986 lettuce anthracnose appeared 7 days after pots of infected plants or soil were placed within lettuce plots. The first infections were up to 7 m distant from the source. Infections spread slowly; 35 days after introduction of inoculum 39% of plants in channel irrigated and 49% of plants in overhead irrigated plots were infected. The distribution of infected plants was not always aggregated. In Winter (July) 1987 lettuce anthracnose appeared 13 days after planting (DAP); 32% and 45% of plants were infected 53 DAP in channel and overhead irrigated plots respectively. In Spring (Oct.) 1987 infected plants were aggregated around pots of infested soil irrespective of irrigation treatment. The results indicate that sources of inoculum, wind and water splash influence the spread of lettuce anthracnose.

825

PREDICTION OF SOYBEAN STEM CANKER CAUSED BY SOUTHERN STRAINS OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA. P. A. Backman and Elisa F. Smith. Dept. Plant Pathology, Auburn University AL.

A model has been developed that allows prediction of stem canker disease severity and losses. A threat index is generated based on the cultivar planted and previous disease history. Growers are advised to check for perithecia on soybean debris, when rainfall frequency reaches a threshold value during a 7-day period. If mature perithecia are found, a susceptible cultivar has been planted, and infection conditions occur, then disease severity is predicted based on the growth stage at the time of infection. Growers are advised of disease severity and losses both with and without the use of a fungicide. The stem canker model has been linked into a comprehensive IPM program (AUSIMM) for southern soybeans.

826

WEEDS AS ALTERNATE HOSTS OF COLLETOTRICHUM CAPSICI. K. S. McLean and K. W. Roy. Department of Plant Pathology and Weed Science, Mississippi State University, Miss. State, MS 39762.

Colletotrichum capsici (Syd.) Butler & Bisby, a pathogen of cotton and tomato, was isolated from asymptomatic leaves and stems of several weeds sampled from different locations in Mississippi. Frequency of isolation of C. capsici was: cocklebur (0-5%), johnsongrass (0-4%), pitted morningglory (0-8%), pigweed (0-9%), prickly sida (0-32%), smallflower morningglory (0-8%), spotted spurge (0-4%), and sicklepod (0-6%). All isolates caused cotyledonary, leaf and stem lesions on inoculated cotton seedlings. Isolates from pitted morningglory, prickly sida, pigweed, and smallflower morningglory were more virulent than the others. When tomato fruits were inoculated with infested broom straw sections, all isolates were pathogenic and caused dark lesions. Johnsongrass isolates produced significantly smaller lesions than the others. The weeds species investigated may serve as alternate hosts of C. capsici.

827

CALIBRATION OF A CERCOSPORA LEAFSPOT DISEASE PROGRESSION MODEL COUPLED TO PNUTGRO, A PEANUT GROWTH SIMULATOR. G. Bourgeois, R.D. Berger, and K.J. Boote, Departments of Agronomy and Plant Pathology, University of Florida, Gainesville, Fl 32611.

The major foliar diseases affecting peanut are early and late leafspots. These diseases cause necrotic lesions on leaflets, reduce their photosynthetic rate, and induce early senescence. A model of the progression of leafspot diseases has been developed and coupled to PNUTGRO in order to predict disease-induced reductions in dry matter production. Propagation and infection of the pathogen are driven as functions of temperature and relative humidity. The leaf area is divided into non-infected, latently infected, pre-infectious, infectious, and post-infectious states. Parameters such as the incubation, latent, and infectious periods, the infection efficiency of the conidia, and the conidial production per unit infectious leaf area, were estimated from the literature. The model reasonably predicted observations made in 1986, which included disease severity and dry matter yield of major plant parts.

828 Withdrawn

829

TECHNIQUE FOR THE IN VITRO INOCULATION OF POTATO BY ERWINIA. C. H. Skroch, D. J. Gallenberg, and P. L. Spinski. Plant Science Department, South Dakota State University, Brookings, SD 57007.

An in vitro inoculation technique was designed to evaluate the development of soft rot/black leg caused by Erwinia carotovora subspecies carotovora and atroseptica on potato tissue culture plantlets. The technique was used to study the combined effect of cultivar resistance and calcium level on 4 week old plantlets inoculated with Erwinia. Cultivars possessing different levels of resistance to blackleg varied in their response when grown on MS media modified to contain different levels of calcium. This evaluation of the disease reaction involving Erwinia provides a basis for future study on the feasibility of using in vitro techniques to screen for disease resistance in potato.

830

OPTIMIZATION OF AN ELECTRIC-PULSE PROCEDURE FOR TRANSFORMATION OF FUSARIUM AND ASPERGILLUS PROTOPLASTS. M. G. Richey, E. T. Marek, C. L. Schardl, and D. A. Smith. University of Kentucky, Lexington, KY 40546-0091.

Parameters were determined for the successful introduction of foreign DNA into Fusarium solani (FB1-S4) and Aspergillus nidulans (UCD1) protoplasts by electric-pulse treatment using

geneticin resistance in FB1-S4 and tryptophan prototrophy in UCD1 as the selection markers. Voltage, capacitance, medium, protoplast concentration and transforming DNA concentration were varied in order to determine the optimum conditions for transformation of the two fungi. The conditions were established using a Bio-Rad Gene Pulser with a 5 ohm resistor. Transformation frequencies were six transformants per µg of DNA for FB1-S4 and nine transformants per µg of DNA for UCD1. The electric pulse system will be used to study the transient expression of chloramphenicol acetyltransferase in electric-pulse treated cells of both fungi.

831

A SEEDLING TEST FOR RESISTANCE TO FOOT ROT IN WINTER WHEAT CAUSED BY PSEUDOCERCOSPORELLA HERPOTRICHOIDES. C. A. Strausbaugh and T. D. Murray. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

A rating system based on epidermal cell responses (papilla formation, cell wall thickening, hypersensitive response, and successful penetration) was used to evaluate seedlings of cultivars Sel 101 and Daws (both susceptible), Cappelle-Desprez (moderately resistant), and VPM-1 (resistant) for resistance to foot rot. Four weeks after inoculation, 6% of the first-leaf-sheaths had 20 or more infection sites at 5 C, 74% at 10 C, 83% at 15 C, and 28% at 20 C when averaged across cultivars; no differences were found among the cultivars. Infection occurred 1-2 cm from the crown where the coleoptile contacted the first-leaf-sheath. Disease ratings based on fungal penetrations stopped at the cell surface by papillae or within a epidermal cell by thickened cell walls provided the best separation of resistant and susceptible plants.

832

A NEW, EFFICIENT SYSTEM FOR STUDYING SNOW MOLD DISEASES. J. H. MCBeath and M. Adelman. Agricultural and Forestry Experiment Station, Universtiy of Alaska, Fairbanks, AK 99775-0080

A simple, space-saving, snow mold chamber and a new method for inoculating snow mold fungi were developed. Forty winter wheat seeds were sown singly in a rack of "conetainer" containing sterile soil. At the three-leave stage, twenty seedlings were inoculated with sclerotial low temperature basidiomycete (SLTB) impregnated rye kernals (3 gms/plant). A sterile filter paper cylinder was used to keep the inoculum moist and in close proximity to the plant. Inoculated plants and controls were then placed in a box, covered with a canopy of cheesecloth (8 layers), and incubated in the dark at 4 C. A wick of the cheesecloth dipping in a reservoir of sterile distilled water helped maintain 100% RH in the snow mold chamber. Plants were harvested at three day intervals. Penetration and sclerotia development were examined by light and scanning electron microscopy.

833

A FIELD MOISTURE GENERATOR FOR STUDYING SHOT HOLE OF ALMOND CAUSED BY *STIGMINA CARPOPHILA*. **D.A. Shaw, Coop. Ext., Univ. of CA, San Diego, CA 92123, J.E. Adaskaveg, and J.M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616.**

A portable, self-contained, moisture generator controlled by a micrologger was constructed for the study of shot hole disease of almond in the field. The system produced localized conditions of free water on selected plant tissues and recorded environmental parameters during infection periods of *Stigmina carpophila*. Programmed misting at 2.5 or 5 min intervals with a 4 sec duration resulted in conditions conducive for infection. The greatest number of lesions per leaf occurred with intermittent misting for a 16-hr period compared to 0, 6, 8, 10, 12, and 14-hr treatments after inoculation with 300-400 conidia/leaf. Fewer lesions developed when intervals between misting were increased to 10, 20, 30, or 60 min. Misting durations longer than 5 sec caused excessive water runoff from leaves which reduced inoculum. Infections also were obtained when two different types of electrical conductance leaf wetness sensors were used to monitor and govern moisture regimes.

834

A LABORATORY METHOD FOR EVALUATION OF WILD RICE (Zizania palustris) GERMPLASM FOR RESISTANCE TO FUNGAL BROWN SPOT CAUSED BY Bipolaris oryzae. D. R. Johnson and J. A. Percich, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Embryos were extracted from mature wild rice (Zizania palustris) seeds, washed in 70% methanol for 30 min., rinsed twice in sterile distilled water and placed on a modified Murishige-Skoog solid culture medium in 100 x 25 mm petri plates. The embryos were incubated for 21 days at 25 C with a 12 hr light/dark period. Developed plantlets were inoculated with B. oryzae by streaking a conidial suspension on the leaves with a sterile cotton swab and incubated in the culture plates at 25 C and 100% RH. Disease ratings were made 8-10 days after inoculation. Selected plantlets were transplanted to 20 cm flooded pots filled with a peat and mineral soil (1:1) mix and maintained at 25 C and 16/8 hr light/dark until maturity. This is a reliable method of germinating dormant wild rice germplasm and screening for resistance to fungal brown spot.

835

QUANTIFICATION OF INFECTIOUS UNITS (IFU'S) OF PYTHIUM SPP. PER GRAM OF WHEAT ROOTS USING A MOST PROBABLE NUMBER (MPN) APPROACH. L. L. Singleton and W. Schuh, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285.

Individual assay units (AU) (cylinders, 1.5cm I.D. X 3.5cm; PVC [polyvinyl chloride]) were used for root:sand (R:S) (fresh:dry weight) dilution mixtures. Field roots were collected, washed and cut into 5mm lengths. R:S dilution mixtures were prepared (10 replications) and added to the AU's. Three germinated wheat seeds were placed in each. After 7 days (24C±2, 12/12h light/dark), seminal roots were removed from the AU's, washed, placed onto 0.1X cornmeal agar, incubated (15C±1, dark), and scored for Pythium growth (24-48h). One g of fresh roots was equivalent to about 522 cm of root. Therefore gram quantities of root tissue could be assayed efficiently. IFU's of Pythium populations from roots were compared with at several locations. A higher percentage of pathogenic strains was recovered compared with standard soil plating and dilution techniques.

836

EFFECTS OF STORAGE TEMPERATURE AND TIME ON MODIFIED NASH AND SNYDER'S MEDIUM. P. M. Kinney, J. C. Rupe, and M. L. Courtney, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Selective media dispensed into petri dishes are often stored until use, but the effects of storage conditions on media efficacy are unknown. Plates of Nash and Snyder's medium were stored at 4 or 25 C for 9 wk. Each week, plates were used to determine the number of propagules of Fusarium solani in soil from a field with a history of soybean sudden death syndrome. During the course of the experiment, the ability to detect F. solani propagules was significantly reduced in plates stored at 25 C but not in plates stored at 4 C. After 2 wk, bacterial contamination was present only in plates stored at 25 C. The results indicate that refrigeration may be of value in extending shelf life of selective media.

837 Withdrawn

838

POTATO VIRUS ELIMINATION BY HEAT AND RIBAVIRIN. H.M. Griffiths & S.A. Slack, Dept. Plant Pathology, University of Wisconsin-Madison, WI 53706

Potato plantlets (18 genotypes) with single or multiple virus infections were established in vitro on Murashige and Skoog (MS) medium with or without ribavirin. One or more of potato viruses X (PVX), S (PVS), Y (PVY), M (PVM), and leafroll (PLRV) were present in parent plantlets. Quantitative virus data were obtained using ELISA. Plantlets were grown at 23C or under a 35C/31C, 4-hr alternating light/dark regime. After 4 weeks, nodal cuttings were established on MS. Plantlets testing virus-free were container-grown until fully developed to confirm virus freedom. Ribavirin, with or without heat therapy, eliminated PVX, PVS and PVM without using meristem culture. Virus titer was reduced for PVY and PLRV, but elimination was not obtained. Heat therapy alone did not eliminate viruses. These results demonstrate that ribavirin is an effective viricide and that in vitro potato plantlets can be used to screen chemicals for viricidal activity.

839

FUSION OF PELLUCID BODIES BEFORE OOSPORE GERMINATION IN PHYTOPHTHORA. J. Jiang and D. C. Erwin. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Microscopic observation showed that pellucid bodies (PB) in oospores migrated toward each other and fused prior to oospore germination. Oospores were categorized into three types based on no. PB per oospore: (i) without PB (nonviable), (ii) with ≥ 2 PB, and (iii) with only one PB (fused). In young oospores (< 19 days) the % with ≥ 2 PB was greater than in older oospores (> 25 days) for both (*Phytophthora megasperma* f.sp. *medicaginis* [*Pmm*] and *P. cactorum* [*Pc*]). Conversely, the % germination was much lower in young oospores than in old oospores. Fusion of PB occurred much later in *P. megasperma* f.sp. *glycinea* (*Pmg*) than in *Pmm* and *Pc*. This was correlated with a lower rate of oospore germination of *Pmg* than of *Pmm* and *Pc*. Use of the nuclear stain, aceto-orcein, on 40-day-old oospores of *Pmg* showed that the no. of nuclei/oospore varied from 0 to 8, which corresponded to the no. of PB. The evidence supports the contention that pellucid bodies contain nuclei.

840

DIFFERENTIAL REACTIONS OF FOUR AGARICUS BISPORUS (LANGE) IMBACH CULTIVARS TO VERTICILLIUM DISEASE. P. J. Wuest and L. H. North, Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Mushroom compost was spawned with an A. bisporus cultivar, MGA 615, 816, 901 (Mushroom Growers Cooperative Assoc., Kennett Square, Pennsylvania) or Amycel 191 (Amycel, San Juan Bautista, CA). Verticillium fungicola spores from infected mushrooms served as inoculum. Healthy mushrooms (g) and disease incidence were assessed. Inoculation and cultivar treatments affected yield. Significant loss (g), in inoculated 191 and 615 strains, both smooth white cultivars was contrasted with no yield affect with either brown cultivar, 816 or 901. Disease incidence data corroborates this response. Verticillium disease can be present at relatively high levels without causing a significant yield decrease.

841

STUDIES ON THE GENETIC VARIATION AT THE MITOCHONDRIAL DNA LEVEL WITHIN SPECIES OF PHYTOPHTHORA. Helga Förster and M. D. Coffey. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Due to a high degree of morphological variability, taxonomy of the fungal genus *Phytophthora* is frequently difficult. Improved knowledge concerning the extent of genetic variation at the DNA level might help to provide a better understanding of what comprises a species within this genus and could be useful for determining relatedness between species. As an initial step in the study of phylogenetic relationships within this genus we have characterized the degree of intraspecific variation in mtDNA in isolates of *P. citrophthora*, *P. citricola*, *P. palmivora*, *P. capsici*, and *P. megakarya*. Genetic distance values were calculated from the restriction fragment patterns of the mtDNA for each species. Our data show that there is a high degree of diversity within these five species. Many isolates within a single species can be placed into distinct subgroups, the interrelationships of which will be investigated in future studies.

842

APPRESSORIUM DIFFERENTIATION BY PERONOSPORA TRIFOLIORUM AS A RESPONSE TO THIGMOTROPIC STIMULUS. D. M. Trigo-Stockli and D. L. Stuteville. Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

The host stimulus responsible for inducing appressorium formation by Peronospora trifoliorum d By., the causal fungus of downy mildew of alfalfa (Medicago sativa L.), was studied. To determine if appressorium formation was a response to the physical topography of the leaf surface and not to substances associated with the host, conidia in water were sprayed onto smooth and frosted glass slides, water agar, scratched and unscratched polyethylene and polystyrene membranes, and leaves and polystyrene leaf replicas of host and nonhost (mung bean and wheat) plants. Conidial germination and appressorial formation were microscopically determined 12 hr after inoculation. Conidia germinated readily on all smooth and rough surfaces. However, appressoria formed only on rough surfaces, indicating that contact stimulus was involved. On leaves and leaf replicas of host and nonhost plants, nearly all appressoria formed in grooves between epidermal cells.

843

INHIBITION OF AFLATOXIN BIOSYNTHESIS BY ASPERGILLUS FLAVUS AND A. PARASITICUS WITH CHLOBENTHIAZONE. Wheeler, M. H., D. Bhatnager and J. W. Bennett. USDA, ARS, Cotton Pathology Research Unit, P. O. Drawer JF, College Station, TX 77841; USDA, ARS, New Orleans, LA 70179; and Tulane University, New Orleans, LA 70118.

Studies were made to determine if the melanin pathway reductase inhibitor, chlobenthiazone, inhibits aflatoxin synthesis in Aspergillus spp.. A. flavus and A. parasiticus were grown in shake cultures containing up to 8 μg/ml chlobenthiazone. This compound had a strong inhibitory effect on the accumulation of aflatoxins B_1 (AFB_1) and B_2 in cultures of both fungi. In a typical study at 8 μg/ml, it caused a 24% decrease in the mycelial dry weight of both fungi. Controls of A. flavus and A. parasiticus contained 221 ± 32 and 205 ± 79 μg of AFB_1 per gm mycelial dry weight, respectively. Quantities of AFB_1 in cultures of A. flavus were decreased by 90 and 99% at 1 and 4 μg/ml chlobenthiazone, respectively. Quantities of AFB_1 in cultures of A. parasiticus were decreased by 64, 81, and 86% at 1, 4, and 8 μg/ml chlobenthiazone, respectively.

844

AFLATOXIN AND SCLEROTIA PRODUCTION BY ASPERGILLUS FLAVUS: INFLUENCE OF PH. P. J. Cotty, USDA-ARS, Southern Regional Research Center, P. O. Box 19687, New Orleans, LA 70179

Sodium nitrate and ammonium sulfate have different influences on aflatoxin and sclerotial production when used as sole nitrogen sources in solid media. Our results indicate the effects are attributable, at least in part, to pH. Ammonium-based media became acidified during culture while aflatoxin production increased and sclerotial production was totally inhibited. Sclerotial production occurred on buffered ammonium media and increased with pH while aflatoxin production declined; aflatoxin was not produced after sclerotial maturation (5 days incubation). Nitrate-based media pH increased with fungal growth and production of large quantities of sclerotia and low concentrations of aflatoxin. Lowering nitrate media pH during culture with exogenous HCl resulted in total inhibition of sclerotial production and tenfold increases in aflatoxin production. Results suggest interrelated regulation of sclerotial morphogenesis and aflatoxin biosynthesis.

845

POSTHARVEST CONTROL OF BOTRYTIS CINEREA ON TABLE GRAPES BY REDUCED DOSAGES OF SULFUR DIOXIDE. Joseph L. Smilanick, J. M. Harvey, P. L. Hartsell, C. M. Harris, and D. C. Fouse. USDA-ARS, Fresno, CA 93727.

The EPA recently established a 10 ppm tolerance for SO_2 residues in table grapes. To determine if SO_2 rates could be reduced without efficacy loss, dosages less than those commercially used were applied for 30 min to Emperor, Thompson, and Black Ribier grapes stored at 0 C. Initial dosages were 625, 1250, 2500, and 5000 ppm followed by weekly applications of half these rates. The two lowest dosages left residues below the tolerance, while those from the higher rates occasionally exceeded 10 ppm. Percent decay of all cultivars after 0, 625, 1250, 2500, and 5000 ppm initial and half rate weekly fumigations for 8 weeks was 21.7, 2.5, 2.0, 1.9, and 1.0, respectively. Acceptable efficacy and lower residues probably could be obtained with less than the 5000 ppm initial/2500 ppm weekly industry dosage.

846

INFLUENCE OF MUSKMELON CELL WALL EXTRACTS ON RORIDIN E PRODUCTION BY A PATHOGENIC STRAIN OF MYROTHECIUM RORIDUM. J.O. Kuti, G.A. Bean, W.A. Mackay, and T.J. Ng. Departments of Botany and Horticulture, University of Maryland, College Park, Maryland, 20742.

Addition of fruit cell wall extracts from two muskmelon cultivars into liquid media significantly affected roridin E mycotoxin production by a strain of Myrothecium roridum pathogenic to muskmelon. Cell wall extracts from a susceptible cultivar('Iroquois') stimulated toxin production while cell wall extracts from a resistant cultivar('Hales Best') inhibited toxin production. Media containing 0.1 or 1.0 mg ml^{-1} stimulated toxin production better than media containing 10 or 100 mg ml^{-1} of the cell wall extracts. Previous studies in our laboratory suggest that roridin E may be involved in virulence or pathogenicity of M. roridum. The present study indicates that host cell wall polysaccharides may act as a regulator of roridin E production during the host-pathogen interactions.

847

MOISTURE VARIABILITY IN SOYBEANS. Flavio A. Lazzari and Richard

A. Meronuck, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Moisture content (MC) of samples of soybeans was determined by: 1) Drying single seeds at 103°C for 72 hr; 2) Drying 10-gram samples at 103°C for 72 hr; 3) Motomco meter. The average and range in MC as determined by these three methods were: Single seeds, avg. 11.06%, range 9.95-12.06%; 10-gram samples, avg. 9.64, range 9.29 - 10.12%; Motomco meter, avg. 9.64%, range 9.56 - 9.79%. In all samples the MC of the single seeds was higher than the MC determined by the other methods. The range in MC was greater with the 10-gram samples than with the Motomco meter.

848

DRY MATTER LOSS CAUSED BY STORAGE FUNGI GROWING IN STORED YELLOW DENT CORN. C. M. Christensen, R. A. Meronuck and F. A. Lazzari, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Replicate 200-g samples of yellow dent corn were stored at moisture contents (MC) of 14.5, 15.5, 16.5, 17.5, 18.5 and 19.5%, tested every 30 days for 180 days for dry matter loss (DML) and periodically for storage fungi. At 60, 90, and 120 days, respectively, the DML at the various MC was: 14.5% MC, 0,0,0; 15.5% MC, 0.18, 0.46, and 0.55%; 16.5% MC, 1.66, 1.76, and 2.17%; 17.5% MC, 2.03, 2.86 and 3.69%; 18.5% MC, 2.66, 3.69, and 4.80%; 19.5% MC, 3.58, 4.55, and 5.37%. By the time DML had reached 0.5 - 1.0%, the germs of most kernels were heavily invaded by fungi, especially Aspergillus glaucus, and it seems probable that corn in farm or commercial storage that had suffered that amount of DML from invasion by storage fungi probably would be at risk of developing grade-reducing damage during subsequent storage or shipment.

849

OCCURRENCE OF FUNGI AND MYCOTOXINS ASSOCIATED WITH FIELD MOLD DAMAGED SOYBEANS IN ILLINOIS. B. J. Jacobsen, K. S. Harlin, S. P. Swanson, and J. B. Sinclair. University of Illinois, Urbana

Soybeans that were refused or discounted by elevators in the fall of 1986 were analyzed for damage, germination, fungal infection, and for mycotoxins. Seeds from 24 lots were found to be infected with Alternaria alternata, Fusarium graminearum and Phomopsis sp. Zearalenone, was detected from whole soybeans, soybean hulls, meal and oil (0 - 11 ppm) fraction. Zearalenol was found only in the hull and meal (0 - 1.2 ppm) fractions. Deoxynivalenol was detected in whole soybeans, soybean hulls, meal, and oil (0 - 0.3 ppm). Diacetoxyscirpenol was detected in whole soybeans hulls and meal (0 - 0.02ppm). T-2 (primarily HT-2) was found in whole soybeans, hulls, meal and oil. Aflatoxin B was found in hulls (0 - 5.8 ppb).

850

DECAY CONTROL AND QUALITY MAINTENANCE AFTER MOIST AIR HEAT TREATMENT OF PLASTIC WRAPPED NECTARINES. B. R. Anthony, D. J. Phillips, Y. Aharoni, U.S. Department of Agriculture, Fresno, California 93727.

Heat treatment for 15 min with 52°C moist air controlled decay in plastic wrapped or naked-nectarine fruit puncture-inoculated with Monilinia fructicola. Heat treatment for 5 or 10 min slowed decay development. In treatments where decay occurred, the wrap increased decay. Separate tests of wrapped and naked fruit treated at 52°C for 15, 30, or 45 minutes showed that the heat treatment slowed softening of fruit. When the fruit was held at 20°C after treatment, the wrap lowered ethylene production 75% and respiration 12%, but did not significantly influence softening. The wrap reduced or eliminated undesirable skin browning associated with heat treatments when fruit was evaluated after no cold storage or after 2 week storage at 1°C. Thus, the moist air heat treatment of plastic wrapped nectarines, in addition to decay control, slowed softening, lowered respiration and maintained good fruit appearance.

851

FORMATION OF MYCOTOXINS BY FUSARIUM CROOKWELLENSE KF 748. R. F. Vesonder and P. Golinski. Northern Regional Research Center, ARS/USDA, 1815 N. University St., Peoria, IL 61604

Fusarium crookwellense KF 748 (NRRL A-28100), isolated from potato tubers in Central Poland, produced six mycotoxins on solid substrates at 25 C. The yield of F. crookwellense metabolites depended on fermentation medium and was estimated to be fusarenone-X 15 and 25 µg, nivalenol 2 and 5 µg, zearalenone 20 and 100 µg, fusarin C 10 and 15 µg per gram of corn and rice, respectively. Also, α-trans-zearalenol and β-trans-zearalenol were produced at levels of 50 and 100 µg per gram of rice, respectively. This is the first report of formation of α-trans- and β-trans-zearalenol, fusarenone-X, and nivalenol by F. crookwellense. A rapid method is also presented for zearalenone and zearalenol extraction from corn substrate. Since F. crookwellense is found on a wide variety of host plants and debris from soil in the U.S., its ability to produce zearalenol may be important since it is more estrogenically active than zearalenone.

852

FREQUENCY AND TOXICITY OF FUNGI ISOLATED FROM WEATHER DAMAGED SOYBEANS. D. M. Wilson, D. V. Phillips and R. W. Beaver. UGA Coastal Plain Experiment Station, Tifton, GA 31793 and UGA Georgia Experiment Station, Experiment, GA 30212.

Phompsis and Fusarium spp. were isolated more frequently from seed from weather damaged soybeans in Georgia and South Carolina than from sound soybeans. These fungi were apparently the primary causes of the seed deterioration during the extended wetting period which delayed harvest. The quality of damaged soybeans as measured by oil, FFA, PV, P and fatty acid composition was variable, but never as high overall as undamaged soybeans. Many of the Phompsis spp., Fusarium spp. and Collectotrichum spp. isolates were toxic to day-old chicks, and these fungi may produce toxic metabolites that contaminate damaged soybeans.

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FACTORS AFFECTING HOST-PATHOGEN INTERACTIONS BETWEEN ELM CALLUS CULTURES AND CERATOCYSTIS ULMI. L. Schreiber, S. Domir, J. Ichida and C. Krause, Nursery Crops Research Laboratory, USDA-ARS, 359 Main Rd., Delaware, OH 43015.

We determined the effects of temperature, inoculum concentrations and plant source on the growth of the Dutch elm disease fungus, Ceratocystis ulmi, on elm calli. Calli were derived from an American and Siberian elm seedling and a C. ulmi-resistant American elm (8630). Calli were incubated at 16, 22, and 28°C for 3 da, inoculated with $3x10^5$, $2x10^6$ and $1.5x10^7$ C. ulmi conidia/ml, and returned to treatment temperatures. After 48 hr, most fungal growth had occurred on 8630 calli followed by American then Siberian elm. Most growth occurred at 22°C and was porportional to the inoculum concentration. By 96 hr, all calli at 22°C and all 8630 calli were colonized equally. While growth was more rapid over 8630, it was more dense on American elm calli. Inoculated calli became lighter in color.

708

NUCLEOTIDE SEQUENCE AND GENE EXPRESSION OF A TOMBUSVIRUS. D.M. Rochon and J.C. Johnston. Agriculture Canada Research Station, Vancouver, B. C. V6T 1X2 and Plant Science Dept., University of British Columbia, Vancouver, B. C. V6T 2A2.

The complete nucleotide sequence of the genome of cucumber necrosis virus, a Tombusvirus, has been determined. The genome contains five long open reading frames (ORF) with the potential to encode proteins of MW 33K, 92K, 41K, 21K and 20K. The 33K protein terminates with an amber codon which, if read through, would produce a 92K protein. The ORF for the 20K protein is nested within the ORF for the 21K protein. Amino acid sequence comparisons with other viruses indicate that the 92K protein is the replicase and the 41K protein the coat protein. Translation of CNV virion RNA in rabbit reticulocyte lysates gives rise to a single 32,000 M_r protein whereas translation in wheat germ extracts gives rise to 45,000, 32,000, 21,000 and 17,000 M_r proteins. Translation products of synthetic CNV transcripts, currently being investigated, will also be presented.