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Abstracts

1

APHID FEEDING DETERRENCE ASSOCIATED WITH PYRROLIZIDINE ALKALOIDS PRESENT IN ENDOPHYTE-INFECTED TALL FESCUE. M. C. Johnson*, L. P. Bush**, and M. R. Siegel***, Dept. of Biology, Georgetown College, Georgetown, KY 40324*; Depts. of Agronomy** and Plant Pathology***, University of Kentucky, Lexington, KY

Feeding deterrents and toxic factors to Rhopalosiphum padi, oat bird cherry aphid, were primarily associated with methanol extracts obtained when seed or leaf sheaths of endophyte-infected tall fescue were serially extracted with hexane, ethyl acetate, and methanol. A silica gel column was used to obtain further separation of aphid feeding deterrent activity in a methanol extract prepared from endophyte-infected seed. The fraction containing the highest concentration of pyrrolizidine (loline) alkaloids deterred aphid feeding to the greatest extent. In contrast, no feeding deterrence was evident in the fraction which contained the lowest concentration of loline alkaloids. Vegetative tall fescue stems dipped in methanol solvent were preferred by R. padi over stems dipped in methanol containing N-formyl loline at concentrations of N-formyl loline as low as 78 ug/ml.

2

Myriosclerotinia Snow Scald control in high mountain bluegrass in the Colorado Rocky Mountains. C.H. Rasmussen-Dykes and W.M. Brown, Jr. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Myriosclerotinia borealis (Bub. & Vleug.) Kohn (syn. Sclerotinia borealis Bub. & Vleug.) was found attacking Kentucky Bluegrass in Breckenridge, CO (alt. 2,745 m) in June 1983. Subsequent field evaluation of numerous fungicides in 1983, 1984, and 1985 have consistently shown PCNB (both granular and WP formulations) and triadimephon to give best control when used at label rates. Noram Prochloraz and SN84364 (114 g a.i. and 71 g a.i. per 305 sq m respectively) used in combination gave better control than either material used alone. Benomyl and chlorothalonil, contrary to previous reports by other authors, gave no detectible control when used at label rates.

3

ASSOCIATION OF Phialophora radicicola CAIN WITH DECLINING BERMUDAGRASS IN FLORIDA. T. E. Freeman and B. J. Augustin, University of Florida, Gainesville 32611.

An unusual decline of bermudagrass (Cynodon dactylon L.) has occurred on Florida golf courses in recent years. Initially, patches of chlorotic grass ca 20-60 cm in diameter appear. Grass in the patch thins and a bare spot in the turf frequently develops. Greenshoots of grass commonly occur next to chlorotic ones at the edge of the patch. Affected plants have short brown roots without feeder roots and root hairs. Rhizomes are usually absent and very few stolons are present. Roots are consistently colonized by a fungus that forms coarse brown hyphal runners on the surface. Smaller, lighter-colored connecting hyphae penetrate the root. This fungus has been isolated and identified as Phialophora radicicola Cain, a species previously reported on wheat and corn but not on C. dactylon. The fungus appears to be primarily a pathogen of stressed plants. It is not controlled by commonly used turfgrass fungicides, but damage is reduced by cultural practices that increase grass vigor.

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THE ETIOLOGY OF POA PATCH. H.T. WILKINSON AND R.E. WAGNER DEPT. OF PLANT PATHOLOGY, UNIV. OF ILLINOIS, URBANA, IL, 61801

Swards of Poa annua in the central U.S. commonly display symptoms of yellowing and necrosis when daily air temperatures average 28-32 C for 10-14 days or longer. Water stress is not judged to be responsible for this decline of P. annua. In 1983, frog-eye patterns of yellowed P. annua appeared after 14 days when temperatures averaged 31 C and ranged from 25-37 C. Examination of afflicted crowns and roots revealed black lesions (ca. 2 mm), dark runner hyphae, and a general reduction of root mass. A Gaeumannomyces-like fungus was isolated from the diseased roots and crowns. The roots and crowns of P. annua, seeded in soil infested with oat grains colonized with the fungus and incubated at six different temperatures for 28 days, were rated for disease severity. The fungus was pathogenic at 25-33 C (optimum = 31 C) , but not pathogenic at 23 or 35 C. At 30 C, P. annua and Triticum aestivum were very susceptible, P. pratensis was mildly susceptible and Agrostis palustris, Lolium perenne, and Avena sativa were not susceptible. We suggest the name Poa Patch for this disease of P. annua.

5

DETECTION OF PYTHIUM BLIGHT IN TURFGRASS USING A MONOCLONAL ANTIBODY-BASED DIAGNOSTIC TEST. S. A. Miller, G. D. Grothaus, F. P. Petersen, and S. L. Papa. Agri-Diagnostics Associates, 2611 Branch Pike, Cinnaminson, NJ 08077.

A double antibody enzyme-linked immunosorbent assay (ELISA) has been developed to detect Pythium Blight in turfgrass. The test is carried out in 96-well microtiter plates and utilizes a monoclonal antibody directed against Pythium species. The monoclonal antibody is the product of hybridoma cell line PA5IIIF11, which was produced by standard cell fusion procedures, utilizing Pythium aphanidermatum as the immunogen. The antibody binds P. aphanidermatum, P. graminicola, P. myriotylum, and P. ultimum, all of which can be involved in warm season Pythium Blight. Pythium spp. were detected in inoculated and naturally infected turfgrass samples, and the color intensity of the final reaction product of the immunoassay was directly related to the level of disease present. No cross-reactivity was observed with other common turfgrass pathogens.

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MANAGEMENT OF TAKE-ALL PATCH OF CREEPING BENTGRASS WITH NITROGEN, SULFUR AND PMA. P.H. Dernoeden, Dept. of Agronomy, University of Maryland, College Park, MD 20742

Ammonium chloride alone and phenyl mercury acetate (PMA) plus ammonium sulfate effectively controlled take-all patch (incitant Gaeumannomyces graminis var avenae) of Agrostis palustris in both years of a field study. Ammonium sulfate and urea reduced disease severity to an acceptable level only in the second year. Sulfur was ineffective in controlling take-all patch. Reduction in disease severity was weakly correlated (R=0.35) with thatch pH measured in distilled water. However, when 0.01 M $CaCl_2$ was used to suspend thatch or soil samples for pH measurement, no correlation was found between disease injury and thatch or soil pH. Failure to find a strong correlation between disease and pH was attributed to the highly buffered nature of thatch. Treat-

ments that effectively reduced disease, increased thatch biomass. This was attributed to stimulation of plant growth accorded by N, and either a direct or indirect effect of the beneficial treatments on the ability of the pathogen to colonize bentgrass roots in thatch.

7

INTERACTION AMONG ELK, FUNGI AND ASPEN IN ROCKY MOUNTAIN ECOSYSTEMS. J. H. Hart, Botany and Plant Pathology, Michigan State Univ., East Lansing, MI 48824.

The separate and joint effects of herbivory and disease on aspen ecology were measured in Rocky Mountain National Park, Colorado, and in the Jackson Hole area of Wyoming. Re-examination of plots in Wyoming which in 1970 had an annual mortality rate of 3.8 percent and hence a predicted 15-year decrease of 44 percent, had 39 percent fewer stems than in 1970. Larger stems (>15 cm) had lower mortality rates than smaller stems. Mortality caused by pathogenic fungi (primarily Cenangium singulare and Cytospora chrysosperma) was correlated with the amount of prior cervid injury to stem boles. Aspen sprouts exposed to cervids rarely exceeded 1 m in height. The major difference between stands within exclosures and outside was the nearly complete lack of 2.5 - 15 cm diameter stems on the outside. These observations indicate that herbivory and disease, superimposed on successional events, are exerting strong negative effects on the distribution of aspen in these ecosystems.

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DROUGHT AND GROWTH DECLINE OF LOBLOLLY PINE ON HIGH RISK LITTLE-LEAF SITES. F.H. Tainter, J. Jacobi; and S.W. Oak, Department of Forestry, Clemson University, Clemson SC 29634-1003; and USDA Forest Service Southern Region, Forest Pest Management, Asheville, NC 28803.

Loblolly pine is recommended as a replacement for shortleaf pine on high risk littleleaf sites. This research explores drought as a contributing factor to the severe decline of loblolly observed on these sites. Based on data collected from 17 loblolly pine stands near Union, SC, radial growth at DBH correlated relatively well with total annual ppt. (0.508), total Mar.-Oct. ppt. (0.592), and calculated Mar.-Oct. soil moisture deficits (-0.661) for the period 1950-73. Growth from 1974-83 was less well correlated with these variables due to decreasing root efficiency caused by littleleaf disease. Severe droughts occurred in 1951, 1954, 1956, 1970, and 1980. A less severe but prolonged drought from 1962-64 resulted in a noticeable effect on growth. Although the 1980 drought affected growth of all trees, the growth decline characteristic of littleleaf symptomatic trees was evident starting in about 1974.

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DISEASE AND SPORE DISPERSAL GRADIENTS OF WESTERN GALL RUST. Kan-Fa Chang and Peter Blenis, Department of Plant Science, University of Alberta, Edmonton, Canada T6G 2P5.

Two parallel rows of Rotorod spore samplers, 5 m apart, were placed in an open field to collect spores at distances of 2, 5, 10, 20, 40 and 80 m perpendicular to the edge of a stand of heavily infected lodgepole pine trees. The samplers were operated from 9:30 to 11:30 a.m. for 16 days during 1985. Wind speed and direction were recorded by a CR-21 micrologger. Spore dispersal gradients fit Gregory's model ($r^2 > 0.7$) for 9 of the 16 days. The slope values obtained for these 9 days were not correlated with average wind speed perpendicular to the stand edge. Incidence of infection of 2 to 6 year-old seedlings was determined on 75 m^2 plots surrounding the rotorods. No seedlings occurred in plots less than 40 m from the stand. Infection frequencies at distances of 40, 60, 80, 100 and 120 m were 13.2, 25.7, 16.2, 20.0, and 11.2%, respectively.

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RELEASE AND SURVIVAL OF PERIDERMIOID TELIOSPORES OF ENDOCRONARTIUM HARKNESSII PRODUCED ON LODGEPOLE PINE. Kan-Fa Chang and Peter Blenis, Department of Plant Science, University of Alberta, Edmonton, Canada T6G 2P5.

Two spore traps were placed beside each of two sporulating galls near Hinton, Alberta during the summer of 1985. A CR-21 micrologger was placed nearby to record meteorological data. On rainless days, most spores were released after 10 a.m. with very few spores being trapped at night. This period corresponded to the times of maximum temperature, wind speed, and sunlight. Spores were deposited on microthreads wound on rectangular frames and were exposed to sunlight, at a height of 100 cm above ground. Germinability of the spores was reduced by 2, 24, 33, 37, and 48% after 2, 4, 6, 8 and 10 hr exposure, respectively. Spores were incubated on microthreads at 6, 15 and 24°C and 40, and 98% relative humidity in a three by two factorial design. Initial results suggested that spore survival decreased with increasing relative humidity and temperature.

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STAND AND SITE CONDITIONS ASSOCIATED WITH MORTALITY BY VERTICICLADIELLA WAGENERI IN MENDOCINO COUNTY, CALIFORNIA. T. T. Lawson and F. W. Cobb, Jr., Department of Plant Pathology, University of California, Berkeley, CA 94720.

A survey of 35,820 ha of forest land in western Mendocino County CA in 1984 identified 281 areas containing dead and dying Douglas-fir (Pseudotsuga menziesii). Plots were established in all mortality areas, and 17 stand and site conditions were measured. A total of 183 areas, or 65 percent of all mortality areas, were attributed to infection by V. wageneri. Stand and site conditions associated with incidence of mortality by this root pathogen were aspect, elevation, position on the slope, disturbance by forest management activities, and stocking levels of Douglas-fir and redwood (Sequoia sempervirens).

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AN ECOLOGICALLY BASED APPROACH TO CONTROL BLACK WALNUT MYCOSPHAERELLA LEAFSPOT. Kenneth J. Kessler, Jr., USDA Forest Service, North Central Forest Experiment Station, Carbondale, IL 62901.

Interplanting walnut (W) with the leguminous shrub, autumn olive (AO), produced the following effects: (1) Perithecial production within infected fallen leaves was reduced by increased microarthropod feeding and increased colonization by fungal saprophytes. (2) Dissemination of ascospores from the leaf litter was reduced by a mechanical overbarrier of AO leaves, which are shed later than W leaves. (3) Live AO leaves (which are fully formed in the spring before W leaves develop) served as a barrier to the movement of ascospore and conidial inoculum. (4) Companion planting, which increased the normal planting distance between W trees, created a discontinuous foliar canopy less suited to secondary spread of M. juglandis between adjacent W trees during early plantation years. (5) Shading by AO induced earlier death of lower W branches in the microclimatic zone near the ground most conducive to disease establishment. (6) Association with AO, a nitrogen-fixing legume, increased W foliar nitrogen, which increased natural resistance to leaf infection. During 3 years of measurement, maximum Mycosphaerella lesions per leaflet in August averaged 9 the first year, 16 the second year, and 4 the third year in the companion planting. Corresponding lesion numbers in W monoculture plots were 84, 102, and 95. Each September complete defoliation occurred on monoculture plots; no defoliation occurred in companion plots.

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CONVERSION OF VIRULENT CHESTNUT BLIGHT CANKERS INITIATED AFTER AN APPLICATION OF HYPOVIRULENT CONIDIA. K. L. Scibilia and L. Shain, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Dormant American chestnut stem segments with cankers induced by a virulent (V) strain of Endothia parasitica (EP 155) were sprayed with conidia from a cytoplasmic hypovirulent (CH) convert (EP 780) derived from EP 155. Additional cankers were initiated 0, 1, 2, and 3 weeks after the conidial spray dried. Stems were incubated in plastic bags at room temperature. Cultures from isolations that were made 5 weeks after spraying were judged as V or CH by their morphology. V to CH conversion was observed in 75% of the cankers established before the spraying and 75%, 38%, 12%, and 25% of the cankers initiated at 0, 1, 2, and 3 weeks after spraying, respectively. Conversion to CH was not observed in unsprayed check cankers. Additional tests are planned to determine if CH conidia can protect American chestnut from lethal V cankers under field conditions.

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MORTALITY OF SCOTS PINE FOLLOWING INOCULATION WITH Bursaphelenchus xylophilus. P.J. Bedker and R.A. Blanchette. Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

On 25 June 1985 40, 13-yr-old Scots pine trees in Lee County, IA were inoculated with the pine wood nematode using two techniques. Nematodes were used either to inoculate wounds produced on four branches in a single whorl in the midcrown of the trees or wounds drilled into the bole on opposite sides of the trees at 1.5 m above the ground. An equal number of trees, which served as controls, were wounded but not inoculated with nematodes. Four mo after treatment 50% of the trees inoculated with B. xylophilus were dead or dying. Nematodes were recovered from all twenty inoculated trees harvested at 4 mo. An average of 393.1 nematodes/g of wood were extracted from inoculated

branches and 64.3 nematodes/g of wood from the main stems of branch-inoculated trees. An average of 329.9 nematodes/g were extracted from bole-inoculated trees. In general, higher numbers of nematodes were extracted from trees with symptoms than from those without symptoms.

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CHARACTERIZATION AND COMPARATIVE PATHOGENICITY OF FOUR NECTRIA GALLIGENA ISOLATES FROM FLORIDA. E. L. Barnard, S. P. Gilly, and N. E. El-Gholl. Florida Department of Agriculture & Consumer Services, P.O. Box 1269, Gainesville, FL 32602.

Perithecia of N. galligena were observed in the field in association with stem galls of Cercis canadensis, crotch cankers of Swietenia mahagoni, and stem cankers of Quercus laurifolia and Acer rubrum. Canker symptoms developed under greenhouse conditions on all four hosts in response to wound inoculations with isolates from each host. However, symptoms on Swietenia inoculated with the Swietenia isolate were notably mild. Morphological comparisons of ascospores and conidia of the Cylindrocarpon heteronema anamorph revealed no distinct differences among isolates. All isolates were homothallic and produced perithecia in culture on carnation leaf pieces on water agar. C. canadensis and S. mahagoni represent new host records for N. galligena.

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EFFECT OF TEMPERATURE AND SNOW COVER ON SCLERODERRIS SHOOT BLIGHT. M. Marosy and R. F. Patton, Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706

We examined the effect of winter temperature and snow cover on Scleroderris shoot blight by artificially inoculating red pine seedlings with the NA serotype of Gremmeniella abietina. In nine locations in Wisconsin and Upper Michigan, symptoms developed on seedlings which for at least 45 days between 1 November and 28 February were exposed to ambient temperatures between -6 and +5 C for the entire day, or were covered by at least 20 cm of snow which maintained a similar temperature in the canopy. The incidence of disease in plots that had a snow cover throughout the winter was 70%, whereas there were no symptoms on seedlings on which snow accumulation was prevented. Seedlings maintained at 4 C developed typical symptoms after 8 wk; those maintained at -30, 16, 20, or 24 C or at 26 C day/21 C night were symptomless. These results suggest that a relatively mild canopy temperature during the winter favors disease development and may explain the restriction of this disease to latitudes that receive sustained snow cover.

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GROWTH OF NECTRIA FUCKELIANA CANKERS IN WHITE FIR (ABIES CONCOLOR) IN CA AND POSSIBLE RESISTANCE MECHANISMS. M. Schultz and J. R. Parmeter. Plant Pathology Department, University of California, Berkeley, CA 94720.

A total of 40 trees were inoculated on each of 6 different dates with 6 single-ascospore isolates of Nectria fuckeliana. Inoculation of each isolate was systematically varied relative to other inoculations on the stem. Trees were inoculated in Nov. 1983, and in Jan., Apr., May, Aug. and Oct. 1984 (approximately 2 mo. intervals). Cankers from inoculations in Oct., Nov., and Dec. grew faster, became twice as large and remained active longer (23 mo.) than did cankers from Apr., May, and Aug. inoculations. The latter healed within 19 mo. The physiological slowing of the host during the winter months while the pathogen was still able to grow may explain these differences. The rate of longitudinal growth in Jan. and May is about the same for five of the isolates but for Oct. it was variable. The isolate with the fastest in vitro growth was not affected by date of inoculation.

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ORIGIN, DISTRIBUTION, AND TAXONOMY OF CRONARTIUM RIBICOLA AND RELATED SPECIES. Y. Hiratsuka, Northern Forestry Centre, Canadian Forestry Service, Edmonton, Alberta, Canada T6H 3S5

Morphological similarities and overlapping alternate hosts between white pine blister rust on five-needle pines, Cronartium ribicola, and resin top blister rust on two-needle pines, C. flaccidum, are discussed in relation to several interesting blister rust forms found in Japan, the Republic of Korea, and the People's Republic of China. Considering the distribution of such pine species as Pinus pumila, P. koraiensis, P. sylvestris, P. sibrica, and P. densiflora, speculation is offered on the possible origin of white pine blister rust in North America. The need for critical studies of blister rust forms on Pinus pumila and several other species in northern China and the Siberian region of the USSR is pointed out.

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MODELLING CONTROL STRATEGIES FOR PHELLINUS WEIRII ROOT ROT IN MANAGED STANDS: MODEL DEVELOPMENT. W.J. Bloomberg, Canadian Forestry Service, Pacific Forestry Centre, 506 W. Burnside Rd, Victoria, B.C., V8Z,1M5.

A biologically realistic model of *Phellinus weirii* root rot was developed to assess potential control strategies in managed Douglas-fir stands. The model comprised key processes in disease initiation and development quantified as functions of time and space. These were horizontal and vertical tree root distribution, root contact with inoculum and among root systems, spread of mycelium through root systems, reduction of growth in infected trees, tree mortality and persistence of inoculum in roots of stumps and killed trees. The functions were incorporated in a computer program to calculate spread of the disease and stand growth loss and mortality. Data for quantification of functions were obtained by experiments and from the literature on the disease. Simulated control practices included infected stump removal, sanitation fellings, planting mixtures of Douglas-fir and resistant species. Accuracy of the model was tested by comparing calculated disease spread and mortality 1) with that measured in four 60-year old, 1-ha stands in Oregon, 2) with results from an existing statistical spread and damage model which has performed satisfactorily and 3) with observed spread and damage behavior in stands of different ages and growth rates. Results from the model compared favorably with all of the above situations.

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ESTIMATION OF SEASONAL ASCOSPORE PRODUCTIVITY OF MYCOSPHAERELLA POPULORUM USING DEGREE DAYS. C. J. Luley and H. S. McNabb, Jr. Plant Pathology, Seed and Weed Sciences, Iowa State University, Ames, Iowa 50011.

Ascospores of Mycosphaerella populorum are produced in pseudothecia that mature in overwintered Populus leaves. Spore levels peak in May and decline to extinction later in the summer. Cumulative ascospore productivity was shown to be directly related to degree day accumulation. A model utilizing degree day accumulation (base 0 C) from the time ascospores first matured was developed to describe the relationship. Spore productivity was quantified in controlled temperature chambers and within a Populus plantation during 1984 and 1985. A Gompertz transformation linearized the ascospore productivity-degree day curve. The model estimated ascospore productivity closely in 1984, when adequate moisture was available. Dry spring weather in 1985 slowed spore production and resulted in significant deviations. Historic degree day data enables the model to predict ascospore productivity and subsequent infection of Populus stems and leaves.

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EFFECTS OF WHEAT GENOTYPE ON ROOT COLONIZATION BY A TAKE-ALL SUPPRESSIVE STRAIN OF PSEUDOMONAS FLUORESCENS. D. M. Weller, USDA-ARS, Pullman, WA 99164

Studies were conducted to determine the differences among 11 wheat cultivars in their ability to support root colonization by take-all suppressive P. fluorescens 2-79RN$_{10}$. Individual seeds containing 10^8 CFU were sown in plastic tubes containing 100 g of Palouse silt loam at -0.5 bars soil matric potential and incubated at 15 C for 15 days; no water was added during that time. Populations of 2-79RN$_{10}$ were determined on sections of seminal roots 3-5 and 5-7 cm below the seed; populations were generally greater on the upper than on the lower section. There were significant differences in the population of 2-79RN$_{10}$ on roots of the different cultivars. The $\log_{10}$ populations of 2-79RN$_{10}$ on the 3-5 cm root section of the most supportive cultivars, Wampum and Daws, and least supportive cultivars, Omar and Brevor, were 5.4, 5.3, 3.9, and 3.4, respectively. Using cultivars that support the highest population of antagonists may improve biocontrol of take-all.

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Use of mobile nurseries to assess the Erysiphe graminis f. sp. tritici population in North Carolina. S. Leath, USDA-ARS, and J. P. Murphy, Dept. of Crop Science, North Carolina State University, Raleigh, NC 27695-7616.

Twice in spring 1985 and 1986 and in December 1985 thirty-two mobile wheat nurseries were placed across North Carolina. Two nurseries were placed in fallow fields and in fields planted to Coker 747, Coker 983, Saluda and FL 302

for 12-24 hr. Nurseries consisted of 55x38x8 cm flats with four three-week old seedling of Chancellor and 11 near-isogenic lines each with a different gene for resistance to the wheat powdery mildew fungus, Erysiphe graminis f. sp. tritici. Virulence was detected for all genes tested: Pm1, Pm2, Pm2+, Pm3a, Pm3b, Pm3c, Pm4, Pm6, Pm7, Pm8 and a gene considered to be from Michigan Amber. Virulence was detected least often (p=0.05) for genes Pm1, Pm3b, Pm4, Pm7 and Pm8. Results varied across sampling times and locations. Frequency of virulence to Pm3a was greater than had been reported elsewhere. This survey covered an aerial distance of 650 km and an elevation change of 1762 m.

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NUMBER OF LOCATIONS AND YEARS NEEDED FOR EFFECTIVE SCREENING FOR RESISTANCE TO KERNEL DISCOLORATION. M. R. Miles, and R. D. Wilcoxson, Dept. of Plant Path., Univ. of Minn., St. Paul, MN 55108.

Thirty barley cultivars and lines representing the range of kernel discoloration were grown in 1982 and 1983 at Crookston, Morris, Rosemount and St. Paul, MN under irrigation at Rosemount and St. Paul. Black stain and carameling were evaluated on 1 to 5 scale. Genotype effects were significant in each test but the genotype by year and genotype by location interactions were also significant. Examination of variances and allocation of number of years and locations needed to reduce the genotype variance indicated that selection for black stain resistance could be effective at a single location for 2 years but two locations 2 years would be needed to evaluate carameling resistance. Irrigation significantly increased the severity of black stain but not carameling. Screening in an irrigated nursery at one location for 1 year was as effective as 2 years without irrigation.

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CONTROL OF LEAF RUST, TAN SPOT, AND SEPTORIA LEAF BLOTCH OF WINTER WHEAT WITH STEROL-INHIBITING FUNGICIDES. J.E. Watkins, B. Doupnik, Jr., and L.V. Coziahr, Dept. of Plant Pathology, 448 Plant Sciences Hall, Univ. of NE, Lincoln, NE 68583-0722.

Five triazole fungicides, evaluated in small plot field trials, show good potential for control of leaf rust and fair potential for control of tan spot and Septoria leaf blotch. When data were averaged over three years, the experimental fungicides HWG 1608, XE 779, and RH-3866 reduced rust severity to 10 percent or less on flag leaves. Bayleton and Tilt held rust severities to less than 30 percent, but were not as effective as mancozeb. Effective leaf rust control was obtained with single applications of HWG 1608, XE 779, and Tilt and dual applications of RH-3866, Bayleton, and mancozeb. None of the fungicides were as effective against tan spot and Septoria leaf blotch as they were against leaf rust. Bayleton was ineffective against these leaf-spotting diseases, whereas the others showed a moderate reduction in leaf spot severity of the flag leaf. Grain yields were 3-7 bu/A higher in treated plots than in untreated plots.

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TANK MIXES OF MANCOZEB WITH TRIADIMEFON OR PROPICONAZOLE FOR FOLIAR DISEASE CONTROL ON WHEAT. A. K. Hagan and R. T. Gudauskas. Auburn University, AL 36849.

Triadimefon (69 and 140 g a.i./ha) and propiconazole (126 and 252 g a.i./ha) alone or tank mixed with mancozeb (1794 g a.i./ha) were evaluated for control of septoria glume blotch and leaf rust on 'Blueboy' wheat during 1983 thru 1985. Mancozeb (1794 g a.i./ha) and an unsprayed control were also included. Fungicides were applied to plots in a randomized complete block design at growth stage 9 and 10.1 (Feeke's scale) in 85 l/ha water. Disease severity was assessed on the flag leaf and seed head at growth stage 11.1. All treatments except mancozeb consistently reduced septoria and leaf rust severity. Disease control with triadimefon clearly was superior to mancozeb, but often inferior to that with propiconazole. Addition of mancozeb did not increase efficacy or yield response with triadimefon or propiconazole except in 1985. Yields from all plots treated with triadimefon and propiconazole were significantly higher than those treated with mancozeb.

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CONTROL OF RICE DISEASES WITH FOLIAR APPLICATIONS OF BENOMYL, IPRODINE, AND PROPICONAZOLE. D.E. Groth, Rice Research Station, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P. O. Box 1429, Crowley, LA 70527-1429.

Labelle rice (Oryza sativa) plots were inoculated with Rhizoctonia solani (sheath blight) grown on a moist rice grain:rice hull mixture (1:2) 45 days after planting. Fungicides were applied with a Solo backpack sprayer delivering 190 l/Ha using a low drift fan nozzle at 1-2 cm panicle and 10% heading growth stages. Rates were 0.56 kg A.I./Ha for Benomyl and Iprodine and 0.19 kg A.I./Ha for Propiconazole. Plots were rated for disease development at harvest maturity. Both Propiconazole and Iprodine effectively controlled sheath blight and brown spot (Bipolaris oryzae). Propiconazole eliminated narrow brown spot (Cercospora oryzae), and leaf smut (Entyloma oryzae), however, Iprodine had little effect on these diseases. Benomyl reduced all four diseases but was not as effective as Propiconazole.

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EFFECT OF SOIL FUMIGATION WITH METHYL BROMIDE, METHAM, AND FORMALDEHYDE ON GERMINATION OF TELIOSPORES OF Tilletia indica. J. L. Smilanick and J. M. Prescott. USDA-ARS, Logan, UT 84322 and CIMMYT, Londres 40, 06600 Mexico, D. F.

Teliospores of the Karnal bunt fungus, Tilletia indica, contained in nylon mesh bags, were placed at the surface, 5-cm, and 10-cm deep in pre-irrigated (wet) or dry soil plots prior to 48 h fumigation with methyl bromide (Meth-O-Gas; 570 kg/h), metham (Vapam; 1100 l/h), or formaldehyde (37% a.i.; 1900 l/h). Two days after fumigation and tarp removal, the teliospores were recovered and plated on water agar. Germination was assessed after 3 wk incubation at 15 C. Teliospores from untreated soil germinated 28.0% to 41.3%. In wet soil, methyl bromide fumgation reduced germination of teliospores at all soil depths more than 98%, whereas metham and formaldehyde were effective only on the surface. In dry soil, metham reduced teliospore germination at the surface, 5-cm, and 10-cm depths 99%, 57%, and 96%, respectively, whereas methyl bromide and formaldehyde were less effective.

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GENOTYPE EFFECTS ON THE EXPRESSION OF A MITOCHONDRIAL GENE ASSOCIATED WITH T-TOXIN SENSITIVITY. John C. Kennell (1), Roger P. Wise (1), Daryl R. Pring (2) and Burle G. Gengenbach (3); (1) Plant Pathology Department and (2) USDA-ARS, Univ. of Fla., Gainesville, 32611, and (3) Department of Agronomy and Plant Genetics, Univ. of Minn., St. Paul, MN 55108.

Maize plants having the Texas male sterile cytoplasm (Tcms) are susceptible to toxins produced by Helminthosporium maydis race T and Phyllosticta maydis. A 345 bp open reading frame (T ORF13), found in Tcms but not N-cytoplasm mitochondrial genomes, is associated with toxin sensitivity and male sterility. The expression of this gene was affected by nuclear genotype, as transcripts of varying size were found in different non-restoring nuclear backgrounds. The level of expression of transcripts in a 5 kb DNA region 5' to T ORF13 also seems to be affected by nuclear genotype. This 5 kb region also is 5' to the atp 6 gene in Tcms mitochondria.

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MUTATION TO MALE FERTILITY AND TOXIN INSENSITIVITY IN T-CYTOPLASM MAIZE IS ASSOCIATED WITH A FRAMESHIFT IN A MITOCHONDRIAL OPEN READING FRAME. **R. P. Wise (1), D. R. Pring (2), and B. G. Gengenbach (3); (1) Plant Path. Dept., and (2) USDA-ARS, Univ. of Fla., Gainesville, 32611, and (3) Dept. of Agronomy and Plant Genetics, Univ. of Minn., St. Paul, 55108.**

Male-fertile plants resistant to Helminthosporium maydis race T and to Phyllosticta maydis have been regenerated from tissue culture of susceptible, male-sterile T-cytoplasm maize. These traits are maternally inherited and are associated with a 6.7 kb XhoI mitochondrial DNA restriction fragment found in T-cytoplasm maize. A 345 bp open reading frame (T ORF13) is located within the 6.7 kb XhoI fragment. N-cytoplasm maize and all T mutants except one (T-4) lack T ORF13. T-4 has a 5 bp insertion centrally located within T ORF13. Transcription in this region appears identical between T and T-4. The predicted amino acid sequence of T ORF13 would code for a polypeptide of 12,961 Mr. The 5 bp insertion in T-4 causes a shift in the reading frame resulting in a premature stop codon that would truncate this polypeptide at 8,305 Mr.

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CORNSTALKS AS RESERVOIRS OF Fusarium SPECIES PATHOGENIC TO CEREALS IN NORTHWEST MINNESOTA. Carol E. Windels, Northwest Experiment Station, Univ. of Minn., Crookston, MN 56716, T. Kommedahl and W. C. Stienstra, Univ. of Minn., St. Paul, MN.

Short-season corn hybrids are increasing in acreage in NW Minnesota--an area producing 61% of wheat and 83% of barley in the state. In 27 of 28 cornfields, in early October 1985, stalk rot incidence was <1%. One or more of 17 Fusarium spp. were isolated from 72% of 1390 cornstalks. Stalk rot fungi in the Liseola Section included: F. subglutinans, F. proliferatum and F. moniliforme at 30, 5 and 1%, respectively. F. graminearum Group 2, the cause of corn stalk rot and scab of cereals, was in 20% of 1390 stalks (0-40%). Scab fungi F. avenaceum and F. culmorum were isolated from 3 and 1% of the stalks, respectively. In 8 of 28 fields, 28% of old corn debris on the soil surface yielded F. graminearum Group 2, 6% F. culmorum and 5% F. avenaceum. In a region where small grains predominate, symptomless stalks and their residues are reservoirs of Fusarium spp. pathogenic to cereals.

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INFECTION OF WHEAT EMBRYOS BY PYTHIUM AND SEEDLING RESPONSE AS INFLUENCED BY AGE OF THE SEED. R. James Cook, USDA, ARS, T. F. Hering, 367 Johnson Hall, WSU, Pullman, WA 99164

Pythium species (mainly P. irregulare and P. ultimum) infect the embryos of wheat within the first 48 hr after planting in soil at -0.3 bar or wetter. Field surveys revealed up to 70% of wheat seedlings emerged following such infections. Some 1985-vintage seedlots of Nugaines, Stephens, and Daws planted in Pythium-infested soil in the greenhouse produced seedlings nearly as large as those from seed sown in Pythium-free (pasteurized) soil. In contrast, only 5-25% of 1981-vintage seed of the same cultivars emerged in Pythium-infested soil and the seedlings were severely stunted, yet the seedlots graded 95-98% "strong germ" and 95-98% emerged when sown in Pythium-free soil. The response of 1984 and 1983 seed was intermediate between the 1985 and 1981 seedlots. Emergence of old seed was improved by treatment with captan, thiram, or metalaxyl but seedlings were still inferior to those from new seed. Some Pythium control may be possible by planting new seed.

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THE EFFECTS OF MIXTURES OF MYCOSPHAERELLA GRAMINICOLA ISOLATES ON THE EXPRESSION OF SYMPTOMS ON WHEAT SEEDLING LEAVES. N. Zelikovitch, E. Levy and Z. Eyal, Dept. of Botany, Tel Aviv University, Tel Aviv 69978, Israel.

Virulent Mycosphaerella graminicola (anamorph, Septoria tritici) isolates grown in liquid shake cultures were used to inoculate seedlings of susceptible and resistant wheat cultivars. Inoculations with certain combinations of isolates grown together in mixtures or grown separately and mixed prior to inoculation resulted in a marked reduction in level of symptoms compared to the level on plants inoculated separately with the individual components of the mixture. Symptom suppression was dependent on the ratio of each of the isolates in the mixture. Challenging inoculation with an appropriate isolate several days following an initial inoculation also resulted in significant symptom reduction. The reduction of symptoms was assessed utilizing isolates possessing specific virulence on differential cultivars. In addition, resistance to fungicides of some M. graminicola isolates in the mixture was used as markers in verifying their resultant position in the pycnidia produced on leaves.

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ZEARALENONE DECOMPOSITION IN THE PROCESS OF MAKING TORTILLAS FROM CORN. H. K. Abbas*, C. J. Mirocha*, R. Rosiles**, and M. Carvajal**. Department of Plant Pathology*, University of Minnesota, St. Paul, MN 55108, and Departmento de Botanica**, Instituto de Biologia, UNAM, Delegacion Coyoacan, 04510 Mexico, D.F.

Tortilla dough (masa) was prepared from corn (Zea mays) samples naturally contaminated with zearalenone (0.23 ppm and 4.23 ppm) which were divided into 300 g portions and treated with Ca(OH)2 (boiled for 5 min and soaked for 12 hrs). The tortillas were baked on a laboratory flat plate at 110-120 C for 7 or 8 minutes on each side and later analyzed for zearalenone residue. The lime water used to boil the corn contained trans-zearalenone and the tortillas made from corn heavily contaminated with zearalenone (4.23 ppm) contained both cis and trans-zearalenone. Tortillas made from corn amended with zearalenone at 5 ppm also contained cis and trans-zearalenone, but those amended at 1 ppm did not.

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EFFECT OF ISOLATES OF PYRENOPHORA TRITICI-REPENTIS FROM BROMUS INERMIS ON WHEAT. J.M. Krupinsky, USDA, ARS, Northern Great Plains Research Lab, P.O. Box 459, Mandan, ND 58554.

Wheat (Triticum aestivum) and smooth bromegrass (Bromus inermis) were inoculated with 31 isolates of Pyrenophora tritici-repentis obtained from smooth bromegrass. Detached seedling leaves of wheat were inoculated to compare five bromegrass isolates to one wheat isolate in each inoculation. Leaves were visually assessed for percent necrosis and lesion length. While all isolates caused symptoms on wheat and smooth bromegrass, the bromegrass isolates varied in their ability to cause disease symptoms on wheat. Most bromegrass isolates caused disease symptoms on wheat similar to those caused by wheat isolates, but some caused less and some more disease symptoms than the wheat isolates. In a related study, greenhouse inoculations were conducted to compare isolates which caused different levels of disease symptoms. The greenhouse inoculations with whole plants confirmed the results obtained with the detached seedling leaves.

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IMMUNOFLUORESCENCE EVIDENCE FOR THE ORIGIN OF NUCLEAR INCLUSION AND CYTOPLASMIC CRYSTALS INDUCED BY BEAN YELLOW MOSAIC VIRUS. C. A. CHANG, D. E. PURCIFULL, E. HIEBERT and J. R. EDWARDSON. Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611.

Epidermal strips of bean yellow mosaic virus(BYMV-PV-2) inoculated pea leaves were taken daily, from 24 hr after inoculation, and processed for immunofluorescence tests using homologous antisera to 54k nuclear inclusion(NI) protein, 49k NI protein, cylindrical inclusion protein, and capsid protein. The plasmalemma of some locally infected cells showed specific immunofluorescence with all four antisera 48 hr after inoculation. Small NI were detected in nuclei 72 hr after inoculation. With time, the size of the NI and the number of NI in nuclei gradually increased. About 7 days after inoculation most of the nuclei were packed with NI and some nuclear membranes were consequently ruptured so that crystals could be seen in the cytoplasm. These cytoplasmic crystals immunofluoresed specifically with 54k and 49k NI protein antisera.

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DIFFERENTIATION OF CLOVER YELLOW VEIN VIRUS FROM BEAN YELLOW MOSAIC VIRUS BASED ON ANTIGENIC DISTINCTIONS BETWEEN NUCLEAR INCLUSIONS. C. A. CHANG, D. E. PURCIFULL, and E. HIEBERT. Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611.

Nuclear inclusions(NI) induced by the PV-2 isolate of bean yellow mosaic virus(BYMV-PV-2) and an isolate of clover yellow vein virus(CYVV-P) were purified from infected pea tissue. BYMV-PV-2 NI contained 54k and 49k protein monomers, while CYVV-P NI contained 60k and 49k monomers. Antiserum against BYMV-PV-2 54k NI protein consistently detected differences between CYVV-P and several BYMV strains but gave identical reactions among BYMV strains in SDS-immunodiffusion tests. Antiserum to CYVV-P 60k NI protein also consistently detected differences between CYVV-P and several BYMV strains. The 49k NI proteins, cylindrical inclusion proteins, and capsid proteins induced by BYMV were serologically different from those of CYVV-P, but these proteins induced by different BYMV strains were also serologically variable. Among BYMV strains, antigenic characteristics of the 54k NI protein was the most conserved of the four viral-specified proteins tested.

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A COMPARATIVE STUDY OF ALFALFA MOSAIC VIRUS DETECTION IN ALFALFA POLLEN BY DOT HYBRIDIZATION AND ELISA. Z. Pesic and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Sensitivity of dot hybridization using a randomly ^{32}P-labeled complementary DNA probe prepared by reverse transcribing alfalfa mosaic virus (AMV) total RNA, and direct double sandwich ELISA were compared for AMV detection in alfalfa pollen. Ten-fold dilutions of pollen extracts from infected plants, purified AMV, and purified AMV in virus-free extracts were assayed. AMV was detected in as little as 1.5 µg of pollen by dot hybridization and 30 µg by ELISA. Minimum detection level was 25 pg for purified AMV added to virus-free extracts and 2.5 pg for purified AMV and AMV-RNA by dot hybridization. AMV was detected by ELISA up to 10 ng/ml in a mixture of AMV and virus-free extracts, and up to 1 ng/ml in a purified virus preparation. No AMV was detected in pollen from uninoculated plants. This study shows that dot hybridization is at least ten times more sensitive than ELISA for AMV detection in alfalfa pollen.

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PRODUCTION OF AN ANTI-IDIOTYPIC ANTIBODY TO AN ANTI-BARLEY YELLOW DWARF VIRUS MONOCLONAL ANTIBODY. J. S. Hu and W. F. Rochow, Dept. of Plant Pathology, Cornell University, and ARS, U.S. Dept. of Agriculture, Ithaca, NY 14853; and S. A. Slack, University of Wisconsin-Madison 53706.

High titer antisera were produced in rabbits against purified anti-barley yellow dwarf monoclonal antibody (mAB-RPV1, from mouse), which neutralizes RPV transmission by Rhopalosiphum padi. Active antibody (AB) was purified from the rabbit antisera with a Protein A Sepharose column. After immunoprecipitation with both mouse serum and mouse IgG, the AB reacted with mAB-RPV1, but not with several other anti-RPV mABs of the same subclass. The AB inhibited reaction between RPV and mAB-RPV1; the reaction between mAB-RPV1 and the AB was also inhibited by RPV in competitive enzyme immunosorbent assays. Based on these results, we conclude that an anti-idiotypic AB against mAB-RPV1 was produced. We are using the anti-idiotypic AB to study epitope specificity of our anti-RPV mABs and to probe salivary gland membranes for virus receptors.

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CHARACTERIZATION OF EIGHTY-EIGHT MONOCLONAL ANTIBODIES TO TOMATO RINGSPOT VIRUS. Charles A. Powell, PA Dept. of Agric., 2301 N. Cameron St., Harrisburg, PA 17110.

Some of the properties of the immunoglobulin (Ig) secreted by eighty-eight different cloned hybridoma cell lines, which had been previously shown to react with tomato ringspot virus (TmRSV), were determined. The supernatant from 62 of the cell lines reacted specifically with rabbit anti-mouse IgM, 14 reacted with anti-IgG_{2a}, 6 reacted with anti-IgG_{2b}, and 6 reacted with anti-IgG_1. Using competitive enzyme-linked assays, the monoclonal antibodies identified two distinct epitopes in a solution of purified TmRSV similar to that used for immunization. Individual cell lines varied greatly in both their rate of synthesis and/or secretion of antibody and the affinity of that antibody for the homologous epitope. None of the monoclonal antibodies was as sensitive in detecting TmRSV as an equal concentration of polyclonal Ig. Mixing some of the monoclonal antibodies resulted in an Ig preparation which equaled the polyclonal Ig in sensitivity.

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A DOT IMMUNODETECTION PROCEDURE FOR PLANT VIRUSES. M.K. Nakhla, S. Kartaatmadja, and O.P. Sehgal, Univ. of Missouri, Columbia, Missouri 65211.

An immunobinding assay was standardized for detecting selected isometric and rod-shaped viruses. Viral antigens immobilized on a nitrocellulose membrane were reacted with rabbit antibodies (first antibody), exposed to goat anti-rabbit IgG (second antibody) conjugated with alkaline phosphatase (AP), and then treated with a histochemical substrate for the enzyme. Appearance of a distinctive purple spot indicated a positive reaction. The minimum detection levels for the various viruses were: southern bean mosaic virus (SBMV) virions = 50 pg or leaf extract dilution > 1:50,000; brome mosaic virus virions = 200 pg or leaf extract dilution > 1:10,000; purified bean pod mottle virus = 200 pg; purified tobacco mosaic virus = 400 pg. Extracts from the comparable healthy plants at 1:500 dilution gave negative reaction. The detection level for SBMV was not improved if a highly purified IgG fraction of the first antibody was used. Similarly, horseradish peroxidase-conjugated second antibody, avidin-horseradish peroxidase conjugate, or protein A-gold proved less sensitive than AP-conjugated second antibody for SBMV detection. This dot immunoassay permits a rapid identification of viruses and is considerably more sensitive than the other serologic testing procedures.

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HISTOPATHOLOGY, SEROLOGY AND TRANSMISSION ASSAY OF AY AND OBDV CAUSING INTERFERENCE IN DUALLY INFECTED ASTER LEAFHOPPERS. T. Atcham and E.E. Banttari. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Dual acquisition of the aster yellows mycoplasmalike organism (AY) and the oat blue dwarf virus (OBDV) reduced transmission of these agents by the aster leafhopper. Interference occurred when leafhoppers sequentially acquired both pathogens as age-synchronized adults or as nymphs or when AY or OBDV-infective aster leafhoppers were injected with extracts of the opposite pathogen. Reduced OBDV titers were detected by ELISA in salivary glands and bodies of individual insects which acquired the virus as the challenge agent. Mycoplasmalike bodies or OBDV-like particles were seen in thin sections of salivary glands of leafhoppers in which interference was detected to either AY or OBDV. When healthy leafhoppers were injected with triturates of salivary glands from non-viruliferous leafhoppers in which there was interference due to AY, it was shown that non-viruliferous leafhoppers contained infective OBDV.

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DOUBLE-STRANDED RNAS FOR IDENTIFICATION AND CLASSIFICATION OF LUTEOVIRUSES. A. D. Hewings[1], J. F. Murphy[2], C. J. D'Arcy[2], and V. D. Damsteegt[1]. [1]USDA-ARS, Ft. Detrick, Frederick, MD 21701 and [2]Dept. of Plant Path., Univ. of Illinois, Urbana, IL 61801

DsRNA patterns were determined for two strains of soybean dwarf virus (SDV-D, SDV-Y), a strain of subterranean clover red leaf virus (SCRLV), two strains of beet western yellows virus (BWYV-CA, BWYU-IL), four strains of barley yellow dwarf virus (BYDV-RPV-IL, BYDV-RPV-NY, BYDV-PAV-IL, BYDV-PAV-NY) and a strain of bean leaf roll virus (BLRV-OR). One dsRNA approximately double the molecular weight of the genomic ssRNA and a second dsRNA, most likely a subgenomic species, were observed. Depending upon the virus or the strain, the number of additional dsRNAs varied from none to three. Preliminary data suggest that dsRNA analysis is a useful tool for classification of luteoviruses but may be of limited value for identification because of the small differences in relative mobility, and the difficulty in accurately determining the number of subgenomic dsRNA species.

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IMPROVED PURIFICATION OF ROSE MOSAIC VIRUS BY REDUCTION OF HOST PROTEINS WITH POLYETHYLENE GLYCOL PRECIPITATION. Wong, S. -M. and Horst, R. K. Dept of Plant Pathology, Cornell University, Ithaca, NY 14853.

Rose mosaic virus was isolated from young leaves of rose cultivar 'Sweet Surrender' in New York. Considerable host proteins were present in partially purified virus prepared by the procedure described by Fulton (Phytopathology 57:1197-1201). Host proteins can be visualized in tubes containing 10-40% linear-log sucrose density gradient solution after 4 h of centrifugation at 25,000 rpm in a SW 28 rotor. The use of 10% polyethylene glycol (PEG, M.W. approx. 8,000) and 1% sodium chloride to precipitate viruses from supernatant after treatment with hydrated calcium phosphate reduced virus yield 30-40%, however, host proteins were reduced by 8-10 fold. Spectrophotometric absorbance of 260/280 was consistently higher, averaging 1.60 with the PEG method, as compared to 1.48 with the 2-cycle differential centrifugation method. Only 1 cycle of differential centrfugation is required with the PEG method before the sucrose density gradient centrifugation.

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CHARACTERIZATION OF INTERCELLULAR WASH FLUID (IWF) FROM TOBACCO MOSAIC VIRUS (TMV) INFECTED PINTO BEAN PLANTS. S.M. Geske and M. Chessin, Botany Dept., Univ. of MT, Missoula, MT 59812.

Previous studies have found that the intercellular fluid isolated from virus infected tobacco leaves is inhibitory to the infecting virus. In this study, eleven day old primary leaves of Phaseolus vulgaris var. Pinto were inoculated with TMV. IWF extracts, isolated by infiltration with phosphate buffer in vacuo followed by centrifugation, were taken at selected days after inoculation. Inhibitory activity against virus establishment was assayed by mixing the extracts with TMV, applying the mixture onto healthy bean plants, and counting the resulting lesions. IWF taken 3 and 7 days after inoculation reduced lesion numbers. Protein contents of IWF followed a trend, with peak concentrations occurring in 4 and 7 day extracts. Protein concentrations of these 2 extracts were 26% to 48% greater than the controls. Ongoing studies of IWF include: determination of inhibitory activity against virus replication, Western Blot analysis, pH stability, and thermal inactivation.

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Temporal accumulation of virus specific proteins in differential temperature treated whole plants. Gray, S.M. and Moyer, J.W. Dept. Plant Pathology, North Carolina State University, Raleigh, NC 27695.

A differential temperature treatment (DTT) of whole plants was investigated for its potential use in studying the effects of plant mediated virus resistance on the temporal accumulation of virus proteins. Watermelon mosaic virus 2 (WMV 2) protein accumulation in systemically infected muskmelon leaves was enhanced by the use of the DTT procedure. Plants were maintained at differential temperatures (20-30 C and 7 C) for 5 days following inoculation and then transferred to a permissive temperature (25 C). Virus proteins were assayed in 4.5 mg tissue disks which allowed repeated sampling from a single, systemically infected leaf. Capsid protein (CP) was detected, using an indirect immunobinding assay, after 24 hrs at 25 C. Levels of CP and the rate of accumulation of CP in the DTT tissue were higher than in plants maintained at 25 C for similar incubation periods. Preliminary results indicated that WMV-2 cylindrical inclusion protein accumulation was similar to CP.

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EVIDENCE THAT THE POTYVIRUS HELPER COMPONENT IS A GLYCOPROTEIN. P. H. Berger and T. P. Pirone, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The potyvirus aphid transmission helper component protein (HC) may be a glycoprotein. HC stains in SDS-PAGE with the PAS and silver periodate methods. Using various sugars that commonly occur in glycoproteins as potential 'competitors' with HC only N-acetylgalactosamine reduced HC activity in aphid transmission bioassays. Other analyses suggested that HC may contain terminal N-acetylgalactosamine or galactose.

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A POORLY APHID TRANSMISSIBLE VARIANT OF ZUCCHINI YELLOW MOSAIC VIRUS. Hervé Lecoq, INRA, Station de Pathologie Végétale B.P. 94, 84140 Montfavet France

A Zucchini Yellow Mosaic Virus (ZYMV) variant poorly aphid transmissible (E15 PAT) was obtained from normally aphid transmissible strain E15. In comparison tests, probabilities of transmission by a single Myzus persicae were estimated 0.446 and 0.004 for E15 and E15 PAT respectively. Both strains had similar symptomatology, host range and serological properties. Loss of aphid transmissibility could not be attributed to the nature of the host or of the vector. When purified E15 or E15 PAT viruses were added to Helper Component (HC) containing extracts from E 15 infected plants both strains were readily acquired through membrane and transmitted by M. persicae ; in contrast no transmission occurred when similar extracts from E15 PAT infected plants were used. This suggest that loss of aphid transmissibility of ZYMV E15 PAT is rather related to a modification of HC properties than to changes in the coat protein.

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COMPARISON OF THE DETECTION OF SQUASH LEAF CURL VIRUS NUCLEIC ACID IN ITS INSECT VECTOR WITH THE ABILITY OF THE VECTOR TO TRANSMIT. Jane E. Polston, J. Allan Dodds, Dept. of Plant Pathology; T. M. Perring, Dept. of Entomology, University of California-Riverside, Riverside, CA 92521; and S. Lazarowitz, Carnegie Inst. of Washington, Baltimore, MD 21210.

Nucleic acid hybridization has been used successfully to detect squash leaf curl virus (SLCV) nucleic acid in its vector, Bemisia tabaci, using the dot spot assay and cloned DNA of SLCV. The detection of SLCV nucleic acid in individual insects was compared to the ability of each insect to transmit virus 24 and 48 hr after a 48 hr acquisition access period. There did not appear to be a direct correlation between the detection of nucleic acid and the ability of a whitefly to transmit since SlCV nucleic acid was detected in whiteflies which did not transmit. The detection of nucleic acid by dot spot assay was compared to the detection of virus antigen by ELISA in individual insects. The detection rate of viral antigen by ELISA was lower than that of viral nucleic acid when equal numbers of individual whiteflies were tested.

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PRODUCTION OF IMMUNOSUPPRESSIVE COMPOUNDS IN PEANUTS BY THERMOPHILIC AND THERMOTOLERANT FUNGI. J. P. Stack[1], E. C. Shepherd[2], D. N. McMurray[3], T. D. Philips[2], and R. E. Pettit[1], [1]Dept. Plant Pathology & Microbiology, [2]Dept. of Vet. & Public Health, and [3]Dept. of Medical Microbiology & Immunology, Texas A&M University, College Station, TX 77843.

Fungi isolated from peanut seed were tested (lymphocyte blastogenesis assay) for their ability to produce immunosuppressive compounds in synthetic medium and in peanuts colonized by the fungi. Thermotolerant strains of Aspergillus nidulans and Penicillium sp. and a thermophilic strain of Paecilomyces varioti produced compounds which inhibited (100%) the incorporation of 3H-thymidine into lymphocytes in the presence of the mitogen concanavalin A. There was no effect upon lymphocyte viability even in extracts capable of 100% suppression. The compounds were nonmutagenic (Maron and Ames assay), soluble in butyl acetate, and remained stable after boiling for 45 min at pH 2, 7, or 12. This has significant potential ramifications.

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EFFECTS OF ASPERGILLUS PARASITICUS INOCULATION ON PEANUTS WITH DIFFERENT IRRIGATION AND GYPSUM APPLICATIONS. D. M. Wilson and M. E. Walker, Coastal Plain Station, Tifton, GA 31793.

The effects of gypsum on preharvest aflatoxin contamination of peanuts have been variable in Georgia, sometimes gypsum applications have significantly reduced aflatoxin contamination. The relationship between calcium nutrition, moisture availability and infectivity of the A. flavus group may be a factor in aflatoxin contamination. Tests in 1984 and 1985 assessed effects of irrigation, gypsum and inoculation. Two rows in each split-plot were inoculated at early bloom, there were two irrigation regimes and four gypsum rates. Soil samples were collected three times during the growing season and at harvest to monitor fungal populations. A. parasiticus populations decreased with time in the soil samples from inoculated plots. Inoculation decreased yield in 1984. No aflatoxins were found in peanut pods collected at harvest but more kernels were infected by A. parasiticus or A. flavus from plots with no gypsum in 1984 and no irrigation in 1985.

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PRODUCTION OF ZEARALENONE BY GROUP 1 AND GROUP 2 OF Fusarium graminearum AND ITS CORRELATION WITH PERITHECIAL PRODUCTION. C.E. Windels, Northwest Experiment Station, Univ. of Minn., Crookston, MN 56716; C.J. Mirocha, H.K. Abbas, and W. Xie, Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

Fusarium graminearum Group 1 (soilborne, causes cereal root rot and rarely produce perithecia) is contrasted with Group 2 which is airborne, causes above ground diseases and readily forms perithecia. The cultures were grown on a solid rice medium and analyzed for zearalenone (ZEA) as well as deoxynivalenol (DON) and its derivatives. In Group 1 (10 isolates), 70% produced an average of 1,079 ppm ZEA whereas Group 2 (78 isolates) produced an average of 1,144 ppm. Both groups produced ZEA in significant amounts and thus no clear correlation was established between production of ZEA and perithecial formation. Both groups produced DON, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol. The latter are not involved in perithecial production.

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PHYTOTOXIN PRODUCTION BY DIAPORTHE PHASEOLORUM VAR CAULIVORA AND ITS ROLE IN THE STEM CANKER DISEASE OF SOYBEAN. Lalitha Rao Burra, J.P.Snow and G.T.Berggren,Jr. Department of Plant Pathology and Crop Physiology, 302 Life Sciences Building, Louisiana State University, Baton Rouge, LA 70803.

Symptoms of Stem Canker as expressed in susceptible cultivars in the field were produced by cell free culture filtrates of Diaporthe phaseolorum var caulivora (Dpc). Characteristic symptoms were expressed in 24-48 hours. Of the four isolates tested, the Opelousas isolate produced the most pronounced symptoms. Cultivars grown under dark conditions produced greater amounts of toxin than those grown under light. Trifoliates from plants at anthesis were more sensitive to the toxin extract than those from earlier stages of growth. Differences in response to toxin extract were observed between varieties of soybean. The toxin was partially purified by chloroform extraction.

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LEAFSPOT PATHOGENS OF ALFALFA IN NORTH CAROLINA. K. Von Chong, C. L. Campbell, and E. Echandi, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Alfalfa leafspot is caused by a complex of fungal pathogens including Leptosphaerulina briosiana, Stemphylium botryosum, and Phoma spp. Occurrence of these pathogens was monitored at 2-wk intervals from March to October 1985 at two sites by arbitrarily collecting 25 stems of 'Arc' alfalfa and isolating from one diseased leaflet per stem. Correlation between frequency of occurrence of each fungus and mean maximum (MAXT) and minimum (MINT) daily temperature, mean rainfall, and rain frequency during the preceding 2 wk was determined. Frequency of occurrence of S. botryosum was negatively correlated (P=0.01) with MAXT and MINT at both sites. Occurrence of L. briosiana at one location was correlated positively with MINT (P=0.01) and frequency of rain (P=0.10) and negatively (P=0.02) with occurrence of the other two pathogens. A positive correlation (P=0.15) was found between occurrence of S. botryosum and Phoma spp. L. briosiana was detected at every date and was, overall, the dominant leafspot pathogen.

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EFFECTS OF ALUMINUM ON SPORE GERMINATION OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI AND HYPHAL PENETRATION OF BEAN ROOTS. A.

W. Barkdoll and N. C. Schenck, Department of Plant Pathology, University of Florida, Gainesville, FL 32611

The effects of low to phytotoxic aluminum concentrations in five natural soils or a limed soil (3-82 meq Al/100 g soil) and four nutrient solutions (0-10 ppm Al) on 10 fungal isolates representing four species (Glomus mossea, G. manihotis, Gigaspora pellucida and Acaulospora longula) were evaluated. Percent spore germination and number of fungal penetration points in the host were determined. There was extreme variability in responses of the fungi to aluminum levels; differences among isolates within a species were as great as those between species. Root penetration was more affected by soil treatments than was germination. In nutrient solution-sand culture where [Al^{3+}] varied independently of principle plant nutrients, germination and penetration were lower at the lowest concentration (0.5 ppm Al) than at 0 ppm.

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EFFECTS OF TILLAGE ON SPATIAL PATTERN OF MACROPHOMINA PHASEOLINA. O. M. Olanya and C. L. Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

The effects of tillage on spatial pattern of Macrophomina phaseolina were investigated at eight sites in 8x8 m plots containing 64 1x1 m quadrats. A soil core was removed from the center of each quadrat before tillage, and after one and two diskings. Inoculum density (ID) was measured from a 10 g subsample of air dry soil using a selective assay. Mean ID and values of Morisita's index were compared by analysis of variance and overall variance about the mean ID was compared among tillage operations at each location. Mean ID was similar among tillage treatments within a site. Low to moderate aggregation of inoculum was detected consistently. Aggregation decreased with each tillage and variance about the mean ID within rows and columns of quadrats generally decreased after one tillage operation. Physical redistribution of inoculum was most evident in plots with high inoculum density. Thus, soil tillage operations can affect spatial pattern of sclerotial fungi.

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SCREENING PROCEDURE AND VIRULENCE OF ISOLATES OF Macrophomina phaseolina TO BEANS. G.S.Abawi and M.A. Pastor-Corrales, Cornell University, Geneva, N.Y. 14456 and CIAT, Cali, Colombia, S.A.

In greenhouse tests, dried sclerotia of Macrophomina phaseolina (Mp) were highly affective for determining the reaction of bean accessions and the virulence of isolates of Mp. Sclerotia were produced abundantly in liquid medium containing 10 g peptone, 15 g dextrose, 0.25 g MgSO4, 7H20 and 0.5 g K2HPO4 in 1 L of water. After 2 wk at 30°C, the mycelial mats with sclerotia were homogenized in a mixer with distilled water, centrifuged, washed once and dried for 48 hr. Sclerotia were mixed with pasturized soil (2 g/kg) and bean seeds were covered with 2 to 3-cm layer of the infested soil. Most seedlings of susceptible lines such as A 464 failed to emerge, and those which did so, exhibited disease symptoms, often dying within 2 wk. Seedlings of resistant lines such as G 5059 exhibited very slight or no symptoms. Isolates of Mp differed significantly in their virulence to beans when evaluated at 0.5, 1,2 or 4 g dry sclerotia/kg soil. The most virulent isolate -Mp 34- was obtained from the bean cv. Chileno grown near Palmira, Colombia, S.A.

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SEED TRANSMISSION AND EFFECT OF FUNGICIDE SEED TREATMENTS AGAINST Macrophomina phaseolina IN BEANS. G.S. Abawi and M. A. Pastor-Corrales, Cornell University, Geneva, NY, 14456 and CIAT Cali, Colombia.

Surface-and internally-borne Macrophomina phaseolina (Mp) were detected in seeds of many bean accessions grown at Palmira and Quilichao, Colombia, S.A. THe seedborne nature of Mp was confirmed by plating seeds on potato-dextrose agar and planting seeds in pasturized soil or sand. Seeds obtained from infected pods exhibited symptoms of Mp infection including discoloration, presence of pycnidia and sclerotia, and generally failed to germinate and/or emerge (e.g. 1/296 and 0/80). Seeds obtained from symptomless pods on infected plants showed as high as 28% infection. Surface disinfestation for 2 min in 0.6% NaOCl reduced the latter infection level to 3 to 5%. Six out of 62 lines grown in sand exhibited Mp incidence ranging from 5 to 30%. Six fungicides were evaluated as slurry seed treatments at 2.5 g formulation/kg seeds in Mp-infested soil in the greenhouse. Benlate 50 wp was the most effective with Vitavax 75 Wp a close second.

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ACTIVITY OF DINICONAZOLE ON SCLEROTIUM ROLFSII AND RHIZOCTONIA LIMB ROT OF PEANUT. A. S. Csinos, C. S. Kvien, and R. H. Littrell. Coastal Plain Experiment Station, P. O. Box 748, Tifton, GA. 31793.

In vitro evaluation of diniconazole (Ortho XE-779) for radial growth inhibition of Sclerotium rolfsii and Rhizoctonia solani isolated from peanut (Arachis hypogaea, L.) was conducted. Concentrations of diniconazole at 100, 10, 5, 1, 0.1, and 0.01 µg/ml were compared with PCNB at the same concentrations. Diniconazole was more active than PCNB on S. rolfsii at all concentrations and on R. solani at most concentrations. In the field diniconazole at 0.54, 0.67 and 0.97 kg ai/ha reduced damage from S. rolfsii and R. solani limb rot in two tests in Georgia. Diniconzaole controls peanut leaf spots caused by Cercosporidium personatum and Cercospora arachidicola and is a growth regulator. This broad spectrum activity may be important in peanut production.

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FUNGAL COLONIZATION PATTERNS IN THE COTTON, TOMATO, AND BARLEY RHIZOSPHERES. G. A. Fisher and O. C. Huisman, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

The fungi colonizing feeder roots of cotton, tomato, and barley in Verticillium dahliae infested soil were studied using a root-plating technique. The same fungi dominated the mature fungal microflora of all three plants, although some differences in colony frequencies were observed. In addition to V. dahliae, these fungi included species of Fusarium, Penicillium, Gliocladium, Aspergillus, and Trichoderma. Several other fungi were found only in association with tissue near the root apex. The biomass dynamics of V. dahliae, Fusarium spp., and Penicillium spp. were also studied, using antisera made against soluble proteins of these fungi. Use of the antisera, both for modified ELISA and specific staining, showed biomass increases with distances from the root tip.

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CHARACTERIZATION OF AN UNDESCRIBED ANASTOMOSIS GROUP OF RHIZOCTONIA SOLANI. D.E. Carling, R.H. Leiner and K.M. Kebler. University of Alaska Agricultural and Forestry Experiment Station, 533 E. Fireweed, Palmer, AK. 99645.

Isolates of Rhizoctonia solani Kuhn representing an undescribed anastomosis group have been recovered from plant and soil samples collected in southcentral and interior Alaska. These isolates do not anastomose with tester isolates representing anastomosis groups (AG) -1, -2-1, -2-2, -3, -4, -5, -6, -7, -8 and -BI. The designation AG-9 is proposed for this group. These isolates have been observed to produce hymenia on the stems of field grown potato plants and lesions on stems and stolons of potato plants grown in field and growth chamber. They also induce hypocotyl necrosis on germinating cauliflower seedlings. The perfect state, Thanatephorus cucumeris (Frank) Donk, forms in vitro on V-8 juice agar. Metabasidia average 16.2 x 8.6 µm in size. One to six sterigmata per basidium are produced, with an average length of 28.7 µm. Basidiospores average 9.1 µm long and 5.8 µm wide.

61

CONTROL OF BOTRYTIS BUNCH ROT OF GRAPE THROUGH CANOPY MANAGEMENT. W. D. Gubler, J. J. Marois, and L. Bettiga, Department of Plant Pathology, University of California, Davis, CA 95616.

Botrytis bunch rot of grapes (var. Chenin blanc) was significantly reduced in canopy management field trials established in 1984 and 1985 in Monterey Co., CA. Treatments consisted of hedging, shoot removal, leaf removal, a moveable trellis system and a non-managed control. An additional trial in 1985 in the Napa Valley compared leafed vines with non-managed control vines. All plots were established in a split plot design, with and without fungicides. Control of bunch rot was greatest in the leaf removal treatments. Trial in 1984 showed that leaf removal reduced disease incidence from 11.9% to 1.8%. Fungicides further reduced disease only by 0.2% to 1.6% disease. In 1985 leaf removal alone reduced disease incidence from 55% to 22.9%. Application of fungicide further reduced disease 16.9%. Disease in the Napa trial also was reduced by leaf removal, from 30.5% to 6.2%. One application of fungicide at preclose further reduced disease to 4.0%.

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DEVELOPMENT OF TISSUE CULTURE PROCEDURES IN PECAN (CARYA ILLINOENSIS). D.W. Laird, C.H. Graves, and P.A. Hedin, Dept. of Plant Pathology and Weed Science, Miss. State, MS 39762, and Crop Science Res. Lab., ARS USDA, Miss. State, MS 39762

Tissue culture as a breeding tool for developing disease resistance in pecans (Carya illinoensis) holds promise. McCown and Lloyd's woody plant medium (WPM) supplemented with 64 combinations of two auxins (NAA and 2,4-D) and two cytokinins (kinetin and BAP) at four rates each, was tested for callus induction from pecan leaf disc explants. Explant browning, initial response, callus color, size, dry weight, and organogenesis were monitored for 90 days. Explant browning and callus color were influenced by the auxin/cytokinin treatment; kinetin, with either auxin, generally promoted browning. Calli with desirable growth rate, color and friability were produced in 13 treatments. Root morphogenesis occurred in callus cultures containing 4.0, 20.0 or 40.0 M NAA in combination with 2.0 M BAP. Partial somatic embryogenesis appeared to have occurred in 90 day old cultures in some treatments.

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FLEXUOUS ROD PARTICLES ASSOCIATED WITH SHEEP PEN HILL DISEASE OF HIGHBUSH BLUEBERRIES. E. V. Podleckis, R. F. Davis, Dept. Plant Pathol., Rutgers Univ., New Brunswick, NJ 08903; A. W. Stretch, USDA/ARS, Rutgers Blueberry/Cranberry Research Center, Chatsworth, NJ 08019 and C. P. Schulze, NJ Dept. Agriculture, Trenton, NJ 08625.

Sheep Pen Hill Disease (SPHD) of New Jersey highbush blueberries is characterized by a blighting of both flowers and new vegetative growth at or near full bloom. Some plants also develop a necrotic line pattern just prior to leaf drop. The causal agent of SPHD is unknown but line pattern symptoms were reproduced by graft-transmission of an agent(s) from diseased scions to symptomless rootstocks. Electron microscopy of negatively-stained extracts of flowers and leaves of blighted blueberries revealed flexuous rod virus-like particles about 12x750 nm. Various fibrous virus-like inclusions were detected in diseased flower, leaf and bark tissues. When viewed in cross section, individual particles within bundles appeared as tetrads of four particles aligned along their long axis.

65

EFFECTS OF BRANCH AGE AND WOUNDING ON INVASION OF PEACH TREES BY BOTRYOSPHAERIA SPP. Kerry O. Britton and Floyd F. Hendrix. Dept. of Plant Pathology, Univ. of Ga., Athens, GA 30602.

Six-mo-old (green) and 18-mo-old (hardened) peach branches were inoculated with mycelial plugs of B. dothidea, B. obtusa, or B. rhodina. Half were wounded with a scalpel, and all were wrapped with adhesive tape. Disease was visually assessed after 2 yr and branch segments were plated on acidified PDA. All three fungi formed cankers more frequently and caused more extensive vascular discoloration on wounded than on non-wounded tissue. B. rhodina was more aggressive on green versus hardened wood; whereas B. obtusa and B. dothidea were more aggressive on hardened than on green tissue. This may explain the predominance of the latter two species in natural cankers, since these are found only on hardened growth. B. dothidea was not reisolated from non-wounded green wood, but B. obtusa and B. rhodina reisolation percentages were not affected by wounding or branch age. Reisolation distances were 2-4 times greater distal than proximal to the inoculation point with an average colonization rate of less than 25 mm/yr.

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ENVIRONMENTAL REQUIREMENTS FOR INFECTION OF SOUR CHERRY BLOSSOMS BY MONILINIA FRUCTICOLA. W.F. Wilcox, Plant Pathology Dept. N.Y.S. Agr. Expt. Sta., Cornell Univ., Geneva 14456.

When potted Montmorency sour cherry trees were inoculated with 5,000 conidia/ml of Monilinia fructicola, placed in mist chambers at 8, 12, 16 and 20 C for 0-24 hr, then incubated for 7 days at 20 C/60% r.h., the incidence of blossom blight was directly proportional to the duration of wetness and temp. Blight incidence was nil without wetting and minimal with only 3 hr wetting, regardless of temp. However, blight incidence was 5, 7, 28 and 72% with a 5 hr wetting period at 8, 12, 16 and 20 C, respectively. Disease incidence increased to 7, 40, 83 and 92% after 8 hr wetting; to 21, 78, 90 and 98% after 10 hr wetting; and to 85, 93, 96 and 97% after 24 hr wetting, at the same respective temps. Percent conidium germination was strongly correlated with percent blossom infection at the various wetness duration x temp combinations. A 10-fold increase or decrease in inoculum dose significantly affected disease incidence relative to the 5,000 conidia/ml standard.

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BIOLOGICAL AND INTEGRATED CONTROL OF GRAY MOULD OF GRAPE IN ITALY. M.L.Gullino and A.Garibaldi, Istituto di Patologia vegetale, Via Giuria 15, 10126 Torino, Italy.

Mutants of Trichoderma spp. resistant to the fungicides commonly sprayed against several grape pathogens were selected by means of UV radiations and used as biocontrol agents against gray mould incited by Botrytis cinerea. During field experiments carried out throughout the years 1978-1985 Trichoderma spp. gave a partial control of B.cinerea. Better results were obtained by alternating one chemical spray, carried out with a dicarboximide or a benzimidazole (at changing of colour of berries) with 2-3 Trichoderma treatments. Trichoderma showed interesting results in 1984 and 1985 in vineyards where benzimidazole and dicarboximide resistance was consistent.

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SEASONAL FLUCTUATIONS IN SUSCEPTIBILITY OF SIX CITRUS ROOTSTOCKS TO PHYTOPHTHORA PARASITICA AND P. CITROPHTHORA. M. E. Matheron and J. C. Matejka. Department of Plant Pathology, University of Arizona, Yuma Mesa Agric. Center, Yuma, AZ 85364

Phytophthora parasitica and P. citrophthora are routinely recovered from diseased citrus groves in Arizona. Excised stem sections were collected monthly from Citrus macrophylla, C. jambhiri (Rough lemon), C. aurantium (Sour orange), C. reshni (Cleopatra mandarin), Troyer citrange (C. sinensis x Poncirus trifoliata) and C. volkameriana. Stem sections were wounded, inoculated with mycelium of P. parasitica or P. citrophthora, then incubated for 7 days at 21 C in moist chambers. For all tested rootstocks, the smallest cankers were produced on tissue collected in December, January and February, the winter dormancy period for citrus in Arizona. The period of highest susceptibility ranged between April through August, dependent upon the rootstocks tested. Apparently, these six citrus rootstocks possess seasonal differential susceptibility to P. parasitica and P. citrophthora.

69

ADDITION OF THE SURFACTANT AG98 TO CHLORINE TO IMPROVE CONTROL OF DECAY OF PEAR FRUIT. R.A. Spotts and L.A. Cervantes, OSU Mid-Columbia Agric. Res. and Ext. Center, Hood River, OR 97031

The effect of the nonionic surfactant Ag98 (Rohm and Haas Co.) on germination and growth of several decay fungi was studied. Also, efficacy of Ag98, alone and in chlorine solutions, was evaluated for control of postharvest decay of pear fruit. Ag98-amended potato dextrose agar inhibited spore germination, germ tube growth, and mycelial growth of Botrytis cinerea, Mucor piriformis, and Penicillium expansum. However, germination of conidia suspended in aqueous solutions of Ag98 was not affected after conidia were removed from the Ag98, washed and transferred to PDA. Control of decay in laboratory and packinghouse studies was greater when Ag98 was combined with chlorine than when chlorine was used alone. The fungistatic and antifoaming properties of Ag98 make it more suitable for commercial use than previously tested surfactants for addition to chlorine to control decay of pear fruit.

70

POSTHARVEST DECAY CONTROL IN MANGOS WITH COMBINATIONS OF IMAZALIL, HOT WATER AND GAMMA RADIATION. D. H. Spalding and W. F. Reeder, USDA, ARS, Subtropical Horticulture Research Station, Miami, Florida 33158.

Combination treatments gave more effective control of anthracnose caused by Colletotrichum gloeosporioides, and stem-end rot, caused by Diplodia natalensis or Phomopsis citri, of Tommy Atkins mangos than single treatments. The combination of hot water (53 C for 3 minutes) with imazalil (0.1% a.i.) was more effective than either treatment alone. Decay incidence was least in mangos treated with heated imazalil, hot water plus irradiation (200 or 750 Gy) or heated imazalil plus irradiation. Irradiation in combination with hot water or heated imazalil gave more effective decay control at 750 than at 200 Gy. Irradiation at 750 Gy inhibited development of ripe skin color and caused some browning and pitting of the skin. Effects of irradiation on skin color and injury were partially offset when a heat treatment preceded irradiation.

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POPULATION DYNAMICS OF MUCOR PIRIFORMIS IN PEAR ORCHARD SOILS OF HOOD RIVER, OREGON. R. Dobson, T.Michailides, L. Cervantes, and R.A. Spotts, OSU Mid-Columbia Agri. Res. and Ext. Center, Hood River, OR 97031.

Mucor piriformis is an important post-harvest pathogen of pears. The inoculum (sporangiospores) is soil borne and comes into the packing houses with soil on the fruit bins. A survey of soil populations of this fungus indicated that some orchards had high populations while others did not. These populations were monitored monthly for one year showing an increase at fruit harvest time with a subsequent decline in the early Spring. Populations established outside the orchard showed a continuous decline through this same period of time. Sporangiospore distribution in the soil was highest in the top four cms. Work is in progress to determine the causes of these population shifts.

72

THE OCCURRENCE OF BENOMYL RESISTANT ISOLATES OF TRICHODERMA HARZIANUM IN COSTA RICAN SOILS. D. M. Eastburn and E. E. Butler, Department of Plant Pathology, University of California, Davis, CA 95616.

During a screening of Costa Rican banana and pineapple soils for the presence of Geotrichum spp., two isolates of Trichoderma harzianum Rifai were found that were resistant to benomyl at 10 ppm. Characterization of the effect of benomyl on the growth of these isolates revealed that they were resistant to 1024 ppm (the highest concentration tested) on malt agar and in malt broth. The relationship between growth and benomyl concentration was expressed as a bimodal curve. Growth decreased at concentrations from 32 to 128 ppm, increased at 256 and 512 ppm, and declined again at 1024 ppm. Non resistant isolates of T. harzianum were unable to grow at concentrations above 4 ppm. Similar effects on radial growth were seen when carbendazim was used in place of benomyl. Spore germination of the resistant isolates was slightly increased at concentrations of carbendazim above 128 ppm.

73

EFFICACY OF DPX-H6573 AGAINST VENTURIA INAEQUALIS IN GREENHOUSE AND FIELD STUDIES. A.L. O'Leary and A.L. Jones, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, 48824.

In greenhouse studies, apple trees were inoculated with conidia of V. inaequalis and held in a mist chamber for 24 hr. DPX-H6573 at 0.01 g a.i./L was applied at 72 or 120 hr after inoculation. A second application was made on one-half of the trees 7 days after the first application. Only chlorotic lesions developed on sprayed trees, particularly trees sprayed at 120 hr, and trees sprayed twice had fewer lesions. In field tests, DPX-H6573 at 18, 36 and 55 g a.i./ha gave 90.4, 97.4 and 99.4% control of primary scab when applied on a 7-day schedule and 75.1, 93.5 and 95.3% control, respectively, on a 14-day schedule. Only chlorotic lesions developed in the 7-day schedules. Sporulating and more chlorotic lesions developed in the 14-day schedules. DPX-H6573 at 144 g a.i./ha gave 18.3% control on a 21-day schedule, but all lesions were nonsporulating.

74

POTENTIAL OF SYSTEMIC FUNGICIDES FOR THE CONTROL OF PHYTOPHTHORA ROOT ROT AND STEM CANKER OF PEACH. C.H. Graves and R.A. Haygood, Dept. of Plant Pathology and Weed Science and Extension Plant Pathology Dept., Miss. State, MS 39762

A wound-plug technique was utilized to inoculate trunks of one-year-old container-grown peach trees on Lovell rootstock with sterile agar plugs infested with Phytophthora cinnamomi, P. cactorum and P. nicotianae var. parasitica. Metalaxyl was applied as a soil drench 2 and 60 days after inoculation at the rate of 0.71 gm ai/tree. Three foliar applications of fosetyl Al at rates of 9 and 18 gm ai/gal were applied at monthly intervals, beginning two days after inoculation. Lesion lengths were measured two months following the last fungicide applications. Lesion lengths on trees not treated with fungicides ranged from 14 to 35 cm. All of the fungicide treatments significantly reduced lesion development for all isolates with the exception of the low rate of fosetyl Al on one isolate of P. nicotianae var. parasitica. There were no significant differences between the fungicide treatments.

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EFFICACY OF TRUNK APPLICATION OF PHOSPHOROUS ACID AND THREE OTHER FUNGICIDES FOR CONTROL OF PHYTOPHTHORA GUMMOSIS ON CITRUS. M. E. Matheron and J. C. Matejka. Department of Plant Pathology. University of Arizona, Yuma Mesa Agric. Center, Yuma, AZ 85364

Gummosis caused by P. parasitica and P. citrophthora is a serious problem in citrus groves in Arizona. In a 15-year-old Orlando tangelo (C. reticulata x C. paradisi) planting, a 20 cm section of trunk on each tree was painted with phosphorous acid (H_3PO_3), metalaxyl, fosetyl-Al or ethazol. After treatment, pieces of bark were periodically removed from within as well as below the treated area and inoculated with P. parasitica and P. citrophthora. After 165 days, both Phytophthora spp. were effectively inhibited on bark treated with H_3PO_3 or metalaxyl, while fosetyl-Al and ethazol demonstrated no appreciable activity. Canker development was also inhibited on bark tissue 10 cm below the section of trunk treated with H_3PO_3 or metalaxyl. The results suggested that H_3PO_3 applied as a trunk paint could provide effective protection against gummosis on citrus.

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THE REUTER-STOKES APPLE SCAB PREDICTOR PERMITS REDUCED SPRAY APPLICATIONS IN MISSOURI. W.H. Shaffer, Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

The Reuter-Stokes Predictor was evaluated over a 2 year period to determine its usefulness to the Missouri apple grower for reducing fungicide applications. Curative fungicide sprays were applied either 72 or 96 hours after the start of an infection period as determined by the predictor. Disease incidence was compared to a standard protective schedule. The Predictor allowed 2 of 11 sprays to be omitted during 1984 and 3 of 15 in 1985. Curative sprays of Funginex or Topas MZ61 provided significant (P=0.05) control of apple scab when applied as either protective or curative sprays within 72 hours of the start of an infection period. The sterol inhibitors, UBI A-1055 or UBI A-815, gave significant control of primary apple scab even if applied 96 hours after infection. Combination of the Reuter-Stokes Apple Scab Predictor with curative sprays gave an average saving in spray applications of 19% over the 2 year period of the test. The sterol inhibiting fungicides are expensive as compared to fungicides such as Dithane M-45, but use of the Predictor results in savings of spray applications which could make these fungicides cost-effective.

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EFFECT OF STEROL-INHIBITING FUNGICIDES ON FREE FATTY ACID CONTENT IN APPLE LEAVES, Santford V. Overton and Laurence D. Moore, Department of Plant Path., Phys. and Weed Sci., Va. Polytechnic Institute and State Univ., Blacksburg, VA 24061

Sterol-inhibiting fungicides (SIF) were applied to Red Delicious and Jonathan trees at 7 AM or PM. Leaves were collected after 24 and 72 hr and analyzed for individual free fatty acids (FFA). The SIF Vangard and Rubigan® affected the individual FFA concentrations of Red Delicious and Jonathan leaves qualitatively 24 hr after application. After 72 hr, no differences were observed compared to the controls. Changes at 24 hr were recorded as differences in % saturation or in the double bond index (DBI). For both the morning and evening treatments, FFA concentrations in Red Delicious leaves® were affected qualitatively by the SIF Vangard, Rubigan® and Baycor®. Early in the growing season, no effect was observed on the FFA content of Jonathan leaves® treated with the SIF Vangard, Rubigan® and Bayleton® when applied in the morning or evening. However, two applications of these same SIF resulted in significant changes in FFA concentrations of Jonathan leaves.

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STUDIES OF DISEASED ROOT TISSUE USING NUCLEAR MAGNETIC RESONANCE IMAGING. G.A. Johnson, J.E. Bailey, R.I. Bruck, C.A. Matyac, Duke University, Durham, NC and Dept. of Plant Pathology, N.C. State University, Raleigh, NC.
Galled tomato roots, resulting from root knot nematode infection, were visualized through the soil without disturbing the potting medium with use of nuclear magnetic resonance imaging (MRI). Roots from a tomato plant showing root galling after inoculation with Meloidogyne incognita were placed in a plastic tube using 20-30 mesh sand as a potting medium. Once transferred all visualizations of the root tissue were made without disturbing the roots or sand. MRI detects free and bound water protons. Proton relaxation times (T1 and T2) have been correlated by medical researchers with diseased and healthy tissues. Pixel images as small as 100 um were obtained enabling clear definition of some of the root galls. Three dimensional images were produced by taking 16 contiguous 1.2 mm slices. Great potential exists for in situ disease progression studies using this technique.

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A ROLE FOR CA2+ IN THE ELICITATION OF RISHITIN AND LUBIMIN ACCUMULATION IN POTATO TUBER TISSUE BY ARACHIDONIC ACID. M. Zook and J. Kuč, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Ca2+ and Sr2+, but not Mg2+, enhanced rishitin and slightly decreased lubimin accumulation in potato disks treated with arachidonic acid (AA). Disks responded to Ca2+ and Sr2+ up to 24 hr after application of AA. EGTA and La3+ both inhibited AA-elicited rishitin and lubimin accumulation. The addition of Ca2+ overcame the inhibition of AA-elicited sesquiterpernoid accumulation by either EGTA or La3+. Calcium was more effective in overcoming the inhibition by La3+ when applied simultaneously with La3+ than when applied 12 hr after La3+. The data support a role for Ca2+ as a secondary messenger in AA-elicited sesquiterpenoid accumulation in potato.

80

IMMUNIZATION IN CUCUMBER: TRIGGERING THE RAPID LIGNIFICATION RESPONSE. R. A. Dean and J. Kuč, University of Kentucky, Lexington, KY 40546.

Challenge of immunized cucumber leaves with Colletotrichum lagenarium resulted in a more rapid incorporation of ^{14}C t-cinnamic acid and ^{14}C phenylalanine into lignin than in challenged control leaves. Infiltration of ^{14}C into unchallenged immunized leaf discs gave ca 50% greater incorporation than in control discs. Autoradiograms and extraction of the inner and outer regions of the leaf discs revealed that the ^{14}C was concentrated at the wounded edge. Introducing ^{14}C t-cinnamic acid through puncture wounds confirmed ca 50% more rapid incorporation in wounded immunized tissues. These data suggest that immunized leaves have a greater ability to lignify. The rapid lignification response may be triggered by wounding or infection.

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COUPLING EARLY BLIGHT TO A SIMPLE MODEL OF POTATO GROWTH. K.B. Johnson, and P.S. Teng, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

A simple sub-model for early blight (caused by Alternaria solani) was coupled to a potato growth model that partitions dry matter into leaves, stems, roots, and tubers. Increase in early blight severity was modeled with a modified logistic equation that included a term for lesion expansion (dX/dt = Keb * diseased leaf tissue * proportion of green leaf tissue + KIx * lesion expansion function) (Berger & Jones, Phytopathology 75:792). Rate of lesion expansion was determined by a function of whole-plant physiologic-age and the specific physiologic-age of a class of leaf tissue. Disease-induced defoliation occurred when the ratio of infected to total leaf tissue in an age class exceeded 0.35. Values for model parameters were determined from sequentially harvested plant dry matter yields, and disease severity data from diseased and control field plots of Russet Burbank potato. The model (reasonably) explained differences in yield between control and disease treatments.

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COUPLING THE FEEDING EFFECTS OF POTATO LEAFHOPPER TO A MODEL OF POTATO GROWTH. K.B. Johnson*, P.S. Teng*, & E.B. Radcliffe**, Depts. of Plant Path.* & Entomol.**, Univ. of Minn., St. Paul.

Effects of potato leafhopper feeding were coupled to a potato growth model that accumulates and partitions dry matter into leaves, stems, roots, and tubers. Feeding effects on potato were modeled in two ways; as a temporary reduction in the plant's potential net crop growth rate (NCGRT), and as leaf area lost to hopperburn. Daily reductions in potential NCGRT were expressed as an exponential relationship of leafhopper activity, defined as the number of nymphs per 30 mid-plant leaves multiplied by the daily degree day total (% reduction in NCGRT = ((1 - exp-(leafhopper activity * Kpr)) * 100). Rate of hopperburn development was determined from a modified logistic equation (dHB/dt=Khb* % reduction in NCGRT * green leaf dry matter). The model was parameterized with sequentially harvested plant dry matter yields and counts of nymphs from infested and uninfested field plots. Partitioning responses of the modeled crop were similar to the field-grown crop.

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EFFECTS OF TIMING AND LEVEL OF DEFOLIATION ON ONION YIELD. Marvin E. Miller, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596.

Beginning 10 weeks prior to harvest, 0, 25, 50, 75, and 100% of the leaves were removed weekly from different sets of Texas Grano 502 (TG 502) and New Mexico Yellow Grano (NMYG) onion plots to simulate damage caused by Alternaria porri. Yield was not significantly (p = 0.05) reduced by any defoliation level occurring 7-10 weeks prior to harvest. Defoliation of $\leq$ 25% did not significantly reduce the yields of either TG 502 or NMYG. Leaf defoliation levels of $\geq$ 50% on TG 502 and $\geq$ 75% on NMYG occurring 4-6 and 1-6 weeks, respectively, prior to harvest significantly reduced bulb yield. Leaf defoliation levels from 50-100% on TG 502 and 75-100% on NMYG reduced yields 30-53% and 36-64%, respectively, in contrast to the non-defoliated control.

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THE INFLUENCE OF OZONE ON ALTERNARIA LEAF BLIGHT OF MUSKMELON. R.X. Latin, M. Simini, and J.E. Simon. Purdue University, West Lafayette, IN 47907.

Muskmelons in Indiana annually exhibit symptoms of ozone injury and suffer from epidemics of Alternaria leaf blight. This investigation was conducted to determine the effect of ozone on Alternaria leaf blight. Muskmelon seedlings were exposed to three levels of ozone (0.0, 0.1, and 0.2 ppm) in controlled stir tank reactor chambers for 96 hr. Plants were rated visually for severity of ozone injury. After seedlings were removed from the chambers the first true leaf of each plant was inoculated with a conidial suspension of Alternaria cucumerina. Plants were then placed in a moist chamber for 24 hr where leaf wetness was constant and temperature was maintained at 27+/-2C. Lesions were counted 7 days after inoculation. Significant differences in infection occurred among the ozone treatments. Fewer lesions occurred on leaves treated with high (0.2 ppm) rates of ozone. Results concur with other reports of decreased amounts of disease on foliage injured by ozone.

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DEVELOPMENT OF COMMON BLIGHT IN FIELD-GROWN RED KIDNEY BEANS EXPOSED TO HYDROGEN FLUORIDE. K.L. Reynolds and J.A. Laurence. Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.

Field-grown 'California Light Red Kidney' bean plants were spray inoculated with a suspension of rifampin-resistant Xanthomonas campestris pv phaseoli and exposed intermittently to 0, 2, or 4 ugF m^{-3} as HF in open top chambers during the summer of 1984. The experiment was repeated the following year. Plants were exposed for 8 hr, 2 days each week for 9 weeks in 1984 and for 8 hr, 4 days each week for 10 weeks in 1985. Foliar fluoride concentration, disease severity, epiphytic populations of the pathogen and other leaf surface microorganisms were determined weekly. The AUDPC and final disease severity were not affected by exposure to HF, but the apparent infection rate increased with increasing fluoride concentration in air (1985). There was no effect of exposure to HF on epiphytic microbial populations during either year. Yield was not affected by exposure in 1984 but decreased in response to increasing fluoride concentration in foliar tissues in 1985.

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DEVELOPMENT AND RELEASE OF WIS (MDR) 147 BEAN BREEDING LINE. D. J. Hagedorn and R. E. Rand, Department of Plant Pathology, University of Wisconsin, Madison, 53706.

Wis (MDR) 147, a new processing-type bean (Phaseolus

vulgaris) breeding line with multiple disease resistance (MDR) has recently been developed and released. For the first time, resistance to the following diseases has been incorporated into one genotype: 1) Wisconsin's Pythium-Aphanomyces root rot complex, 2) bacterial brown spot (Pseudomonas syringae pv. syringae), 3) halo blight (P. syringae pv. phaseolicola), 4) angular leaf spot (Isariopsis griseola) and 5) soybean cyst nematode (Heterodera glycines). It may be one of the most disease resistant processing-type beans. Wis (MDR) 147 is the result of a 1979 cross between Wis (RRR) 46 and Bush Blue Lake 94. It has normal processing bean maturity, a compact but open bush of medium height with 12 cm pods well off the ground. Pods are dark green in color and slow to develop interlocular cavitation. Yield potential is very good.

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RESISTANCE OF CAPSICUM SP. ACCESSIONS TO VERTICILLIUM DAHLIAE. D. L. Lindsey, Entomology and Plant Pathology Dept. and Jaime Iglesias-Olivas, Horticulture Dept., New Mexico State University, Las Cruces, NM 88003.

Two hundred fifty accessions of five species of chile (Capsicum sp.) were evaluated under greenhouse conditions for resistance to Verticillium wilt. Roots of 3-week-old seedlings were dipped into a conidial suspension (10^6/ml) of a mixture of three isolates of V. dahliae for 1 min., then transplanted into a sand:soil:peat mix. Symptom expression was assessed after 3 weeks. Plants showing no symptoms from the initial inoculation were injected at the cotylendonary node with a conidial suspension (10^6/ml) of V. dahliae. Symptom expression was assessed after 3 weeks. One accession of Capsicum pubescens and 10 accessions of Capsicum annuum were found to be resistant to V. dahliae using this technique.

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QUANTITATIVE DESCRIPTION OF FUSARIUM WILT RESISTANCE IN MUSKMELON. R.X. Latin. Purdue University, West Lafayette, IN 47907.

Inoculum density-disease relationships for eight muskmelon genotypes were determined using the standard root-dip inoculation procedure. Inoculum doses included 10^3, 10^4, 10^5, 10^6, and 10^7 microconidia of Fusarium oxysporum f. sp. melonis race 2 per ml water. Disease incidence (percentage of non-surviving plants) was determined twelve days after inoculation. Probit transformation and regression analysis were used to define resistance in terms of threshold values which were expressed in terms of the inoculum density necessary to kill a certain percentage of plants. The relationship between disease response to inoculum in suspension (standard root-dip procedure) and inoculum in soil was defined to make resistance determinations more meaningful for disease management applications. Ranking genotypes according to wilt resistance on a single quantitative scale eliminates much of the confusion created by assigning genotypes into qualitative categories such as susceptible, resistant, and highly resistant.

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CULTURAL METHODS IN BELL PEPPER: IMPLICATIONS FOR YIELD AND DISEASE CONTROL. V. M. Russo, CALS/AES, University of Guam, Maniglao, Guam 96913.

Bell pepper (Capsicum annuum L. cvs. 'Express Bell' and 'Keystone Giant') were planted in the 1983 and 1984 dry and the 1983 wet seasons on Guam in a north-south and east-west orientation with three in-row spacings, with or without black plastic mulch, and with or without fungicides. Plant mortality was recorded. Total yield, marketable yield, average fruit weight and yield/plant were determined. Seasonal variation indicated large differences in total and marketable yield between the wet and dry seasons and significant differences between dry seasons. Total and marketable yield and the average weekly yield/plant were affected by cultivar, fungicide treatment, spacing and interactions of these. Plant mortality was not consistently affected by treatments. Fungicide treatment affected the average fruit weight of Keystone Giant but not of Express Bell. Yield appeared to be related to precipitation, amount of sunshine and cloudcover.

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THE EFFECT OF PLANTING DATE AND TRIADIMEFON ON POWDERY MILDEW (LEVEILLULA TAURICA) OF TOMATO IN CALIFORNIA. J. C. Correll and V. J. Elliott, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Two fresh market tomato cultivars, 'Royal Flush' and 'Jackpot', were planted at four different times during the 1984 and 1985 growing season in Fresno Co., CA. Each cultivar either received 1 application of triadimefon (0.14 kg ai/ha) 40-50 days after transplanting or was left unsprayed. Treatments were replicated three times and the plot set up in a completely randomized split plot design. Disease incidence and severity was measured on individual leaves and whole plants every 7-14 days. Disease onset occurred at an earlier stage of crop development with each successive planting and apparently was related to available inoculum and not the physiological age of the crop. Disease severity was highest during the warmest part of the season. Powdery mildew caused a significant (75-125%, P=.01) increase in defoliation over triadimefon controls in the late plantings, but did not cause a significant (P=.05) reduction in either fruit number or fresh fruit weight.

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EFFECT OF SOIL SOLARIZATION ON SOILBORNE PATHOGENS OF SWEET POTATO. C. Stevens, A. Y. Tang, V. A. Khan and M. A. Wilson. George Washington Carver Agricultural Experiment Station, Tuskegee University, Tuskegee, Alabama 36088.

Soil solarization of sweet potato (SWP) fields in Tuskegee, Alabama during the summer of 1983 to 1985 was examined for effectiveness in controlling soilborne fungi. Soil solarization increased soil mid-day temperature by 10 to 12 C and up to 57 C in the top 5 cm. Soil samples from solarized (SP) and non-solarized (NSP) plots were collected and assayed for fungi on PDA. Soil solarization for 59 days in 1983, 72 days in 1984 and 98 days in 1985 reduced fungi recoverable on PDA from natural soil by 50, 60, and 99 percent, respectively, at 0-10 cm depth in the soil. Nylon bags containing artificially infested soil with Fusarium solani and F. oxysporum f. batatas were buried in SP and NSP plots. F. solani the causal agent of field and storage rots of SWP and F. oxysporum f. batatas the agent of stem rot were greatly reduced or completely eliminated 0-20 cm in soil solarized for 98 days in 1985.

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THE EFFECT OF VERTICILLIUM WILT ON PAR ABSORPTION AND YIELD LOSS OF POTATOES, S. S. Adams and D. I. Rouse, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Linear relationships between dry matter yields and intercepted radiation are known for healthy potato crops. Our objective was to determine if similar relations hold for wilt-diseased 'Russet Burbank' crops. Replicated field plots were inoculated with different doses of Verticillium dahliae at planting to produce five seasonal epidemic patterns. Sequential leaf area indices (LAI) were determined with a leaf area meter after stratified clip harvests. Flux densities of photosynthetically active radiation (PAR) were measured with a tube quantum sensor. Par absorbed was calculated by subtracting reflected and transmitted PAR from that incident above the crop canopy. Reductions in PAR absorption and canopy LAI were significant during late season and correlated with final yield loss. At the highest inoculum dose, reductions in LAI and absorbed PAR averaged about 40 and 20%, respectively, over the last 60 days of the season and were associated with a 10% yield loss.

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BIOLOGICAL CONTROL OF PSEUDOMONAS AVENAE WITH EPIPHYTIC BACTERIA FROM THE CORN PHYLLOPLANE. C.A. Lopes and R.E. Stall, Plant Pathology Department, University of Florida, Gainesville, FL 32611

Of 179 bacterial cultures isolated from the phylloplane of corn plants, only strains F-11 and F-24 of Pseudomonas fluorescens consistently affected bacterial leaf blight and stalk rot (BLBSR) on sweet corn caused by P. avenae. Disease was reduced by more than 50% when greenhouse-grown plants were treated with 10^8 cfu/ml of either antagonist 12 hours before inoculation with $5x10^6$ cfu/ml of P. avenae. Reduction of BLBSR severity by F-11 at the ED_{50} was equivalent to reducing the inoculum concentration of P. avenae approximately 6000 times, as determined by infectivity titration. In the field, F-11 was able to colonize the whorls of corn plants, the main infection court for P. avenae, and gave better control of BLBSR than 100 ppm streptomycin when both were applied to plants 12 or 24 hours before inoculation with the pathogen.

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EPIPHYTIC FITNESS AND HOST PREFERENCE AMONG ICE NUCLEATION ACTIVE STRAINS OF PSEUDOMONAS SYRINGAE. R. D. O'Brien and S.

E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Eighteen non-pathogenic and pathogenic strains of P. syringae were spray inoculated to 6 plant species in a growth chamber. Bacterial population sizes and ice nucleation frequency (INF) of leaf sonicates and leaf grindings were measured in both wet and dry environments. Significant interactions between environment and both strain and plant species in determining epiphytic population size and INF were observed: this suggests that strains differ quantitatively in ecological adaptability, and that plants differ in relative receptivity to epiphytic bacteria in response to environment. INF measured in vitro is a poor predictor of INF in planta. INF varied with strain and plant. Non-pathogenic strains showed no relative host preference for epiphytic colonization while some pathogenic strains did. Plants supporting largest epiphytic population sizes were not always susceptible hosts.

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COMPETITIVE EXCLUSION OF ICE^+ PSEUDOMONAS SYRINGAE STRAINS ON LEAF SURFACES BY ICE^- DELETION MUTANTS OF P. SYRINGAE CONSTRUCTED IN VITRO. S. E. Lindow and N. J. Panopoulos, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Ice^- deletion mutants of P. syringae constructed in vitro did not differ from their parental strains in growth rate or survival of dessication on plants, in any biochemical reaction tested, freezing survival in vitro, or in soil survival. Bean, potato, tomato, and corn plants having epiphytic populations of Ice^- deletion mutants greater than 10^6 cells/g. fr. wt. only supported populations of homologous or heterologous Ice^+ P. syringae strains (applied as challenge inoculum) 10 to 1000 fold smaller than plants not colonized by Ice^- strains. While Ice^- P. syringae strains differed in their ability to exclude Ice^+ P. syringae strains when applied prior to such strains, no significant interaction between homologous and heterologous genetic backgrounds in reducing the population size of Ice^+ bacteria on leaves was observed.

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THE RELATIONSHIP BETWEEN PATHOGENIC AND NONPATHOGENIC STRAINS OF PSEUDOMONAS SYRINGAE AND BACTERIAL BROWN SPOT DISEASE IN BEANS. R. P. Leite Jr. and D. I. Rouse, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Colonization of the snap bean phylloplane by strains of P. syringae (Ps) nonpathogenic to beans and the effect on populations of naturally occurring Ps pv. syringae were studied under field conditions. The nonpathogenic strains were introduced into the field plots by seed treatment before planting and/or by spray application. Bacterial population sizes were estimated by dilution plating from bulked samples. The nonpathogenic strains of Ps colonized bean leaflets and pods, and reached populations of 10^3 to 10^5 CFU/leaflet or pod. Decreased number of naturally occurring Ps-like bacteria (PSLB) were observed on treatments sprayed with nonpathogenic strains compared with control plots only when nonpathogenic strains were applied prior to establishment of high populations of PSLB. Significant reductions ($p<.05$) in bacterial brown spot severity on leaflets and incidence on pods occurred in these treatments.

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ROLE OF FERRIC ANTAGONIZED INHIBITION (FAI) IN ROOT COLONIZATION AND FUNGAL INHIBITION BY A STRAIN OF PSEUDOMONAS FLUORESCENS. T.V.SUSLOW, D.MATSUBARA, M.DAVIES. Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., Oakland, CA 94608.

Isogenic deficiency mutants, developed by homologous recombination of cloned genes with deletions in regions essential for fluorescent siderophore (Flu) and non-fluorescent ferric antagonized inhibition (Fai) biosynthetic product, have been used to demonstrate the lack of involvement of Flu in the biocontrol of Pythium ultimum and the role of Flu in optimizing rhizosphere colonization of cotton. Genes conferring Flu and Fai were cloned and deficiency mutants developed by Gill and Warren (1986) at AGS. Strains of Pseudomonas fluorescens NZ130R10 (wild type), PRP118 (Flu^+,Fai^-) and PRP68 (Flu^-,Fai^+) were applied to cotton seed cv. Acala SJ2 and planted in fumigated Hesperia Fine Sandy Loam amended with 25 propagules per gram of P.ultimum and maintained at 20°C in a constant temperature tank apparatus. The emergence of cotton seedlings was significantly greater for NZ130 and PRP68 treated seed than for PRP118 or non-treated controls. The colonization of roots by PRP68 was reduced.

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ROLE OF FLUORESCENT SIDEROPHORE PRODUCTION IN BIOCONTROL OF PYTHIUM ULTIMUM BY PSEUDOMONAS FLUORESCENS STRAIN 3551. J. E. LOPER, BIOTECHNOLOGY GROUP, CHEVRON CHEMICAL COMPANY, RICHMOND, CA 94804.

Pseudomonas fluorescens 3551 protected cotton seed from colonization by Pythium ultimum and controlled pre-emergence damping-off. Fourteen non-fluorescent (Flu^-) Tn5 insertion mutants of P. fluorescens 3551 were obtained following matings with E. coli SM10 (pSUP1011). Strain 3551 grew on an iron deficient medium whereas the 14 Flu^- derivatives did not. Southern analysis of EcoR1 digested genomic DNA from these mutants confirmed the presence of single insertions of Tn5 in the chromosomal DNA of each derivative strain which fell into 5 size classes. Cotton seeds treated with strain 3551, or representative Flu^- mutants were evaluated for colonization by P. ultimum 24 hr following planting into infested soil or for their subsequent emergence. While strain 3551 decreased seed colonization and increased emergence to a level statistically equivalent to a standard chemical seed treatment, Flu^- mutants did not.

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EFFECT OF ANTIFUNGAL COMPOUND BIOSYNTHESIS ON COTTON ROOT COLONIZATION AND PYTHIUM SUPPRESSION BY A STRAIN OF PSEUDOMONAS FLUORESCENS AND ITS ANTIFUNGAL MINUS ISOGENIC MUTANT. W. Howie and T. Suslow. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA

A Pseudomonas fluorescens strain (Hv37aR2) and a anti-fungal minus isogenic mutant (WH103) of that strain were compared for seed and root colonization and for in vivo suppression of Pythium ultimum infection of cotton. There were no sigificant differences in colonization between strains. However, seed treatment with Hv37aR2 resulted in a significant increase in emergence and root length and significant decrease in root infection by Pythium as compared to WH103 and an untreated control. Seed treatment with WH103 resulted in an increase in emergence and slight decrease in root infection as compared to the untreated control. These results indicate that two components are involved in disease suppression, primarily antibiotic production and secondarily seed and root colonization.

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BACTERIAL Tn5 MUTANTS AFFECTED IN ANTIBIOSIS TO GAEUMANNOMYCES GRAMINIS VAR. TRITICI. A. R. Poplawsky, Y. F. Peng, and A. H. Ellingboe. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Tn5 mutagenesis was performed on three bacterial strains (Pseudomonas fluorescens NRRL B-15135, NRRL B-15133 and gram-negative strain I11) effective in controlling take-all disease of wheat (causal agent Gaeumannomyces graminis var. tritici, Ggt), and capable of inhibiting the growth of Ggt in vitro (antibiosis). Mutations were observed at the following frequencies: auxotrophic, 0.72%; increased antibiosis, 0.83%; loss of antibiosis, 0.38%; loss of fluorescence on King's medium B (NRRL B-15135 only), 0.50%. Southern hybridizations showed that 47 of 51 mutants affected in antibiosis had insertions in a single Eco R1 fragment. Of thirteen I11 antibiosis mutants, insertions were in at least 7 different Eco R1 fragments. Likewise, of 14 NRRL B-15135 and 20 NRRL B-15133 antibiosis mutants, insertions were in at least 7 and 16 different Eco R1 fragments, respectively.

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EFFECT OF TWO ORGANIC CARRIERS ON VIABILITY AND GROWTH IN VITRO OF SEVERAL SOIL MICROORGANISMS. M. M. Gates and W. H. Wills, Dept. of Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

The viability of several soil microorganisms antagonistic to Phytophthora parasitica in vitro was determined in two carrier materials, carboxymethyl cellulose (CMC) and methylcellulose (MC) at 4, 8 or 15 C for 8 to 17 wk. In general, populations of several fungi (Aspergillus sp., Penicillium sp., and two non-sporulating isolates) and three Actinomycetes showed no decrease in viability. Furthermore, cfu of Trichoderma sp. increased significantly ($P=0.05$) in CMC at 4 C. Two bacterial isolates also showed no loss of viability after 10 wk. In a carbon substrate-utilization study at 25 C, sporulating fungal isolates increased in biomass in both MC and CMC after 2 wk. Two of the bacterial isolates increased approximately 1.5-fold in cell

number in both C sources, whereas another bacterium decreased 5-fold in MC. Most of the microorganisms tested were capable of utilizing CMC or MC as a carbon source.

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PSEUDOMONAS PUTIDA ANTAGONISTIC TO SOFT ROT BACTERIA AND OTHER PHYTOPATHOGENS. C. H. Liao, USDA/ARS, Rutgers University, New Brunswick, NJ 08903

Twenty-three strains of bacteria antagonistic to Erwinia carotovora were isolated from plants, and identified as P. putida, P. fluorescens, and Flavobacterium sp.. One strain (P. putida PP22) produces an antibiotic against soft-rotting erwinias, pseudomonads and xanthomonads, and pathovars of X. campestris and P. syringae . The antibiotic is non-volatile, heat-stable, protease-inactive, and non-siderophore-related. The molecule of the antibiotic is small, as judged by dialysis properties, and has been detected in broth and agar media and in plant tissues inoculated with PP22. A mutant of PP22 resistant to rifampicin and streptomycin is able to colonize potato tubers either on surface or in rhizosphere. This antibiotic-resistant mutant suppresses the growth of 3 genera of phytopathogenic bacteria artificially inoculated onto potato slices. These results suggest a potential use of PP22 as a biocontrol agent for phytopathogens.

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ISOLATION AND CHARACTERIZATION OF DIVERSE GROUPS OF SOIL BACTERIA AS WHEAT ROOT COLONIZERS. M. Elliott Juhnke, D.E. Mathre and D.C. Sands. Montana State University, Bozeman, MT 59717

Bacteria characterized as efficient wheat root colonizers have been isolated. They include not only fluorescent pseudomonads but also non-fluorescent pseudomonads, Bacillus spp., Streptomyces spp. and an unidentified aerobic, gram-positive species. In 1985 sixty antibiotic resistant strains of these bacteria were tested as seed treatments under field conditions. Twenty were shown to persist on wheat roots and were recovered at appreciable numbers through harvest. At six weeks the average CFU per mg root was 580. A subsequent growth chamber test with these isolates confirmed the field data with an average bacterial population of 3200 CFU per mg root at four weeks. Combinations of pseudomonads, bacilli, streptomycetes and the unidentified species were found to be compatible on seeds and roots in growth chamber studies. By isolating and characterizing efficient wheat root colonizers, these organisms will be useful for developing strains with various biological control properties.

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POPULATION DYNAMICS OF AGROBACTERIUM TUMEFACIENS BIOVAR 3 IN SOIL ASSOCIATED WITH HOST AND NON-HOST PLANTS. A. L. Bishop, and T. J. Burr, Dept. of Plant Pathology, Cornell University, N. Y. S. Agric. Expt. Station, Geneva, NY 14456.

Soil infested with grape crown gall tissue, or a suspension of Agrobacterium tumefaciens biovar 3 (At3, strain CG48), was planted with grapes (host, cv. Chardonnay), oats (non-host, cv. Astro), or left fallow. Populations of virulent At3/g soil were estimated by plating soil dilutions on selective media and testing virulence of strains recovered. No At3 was detected in uninfested control soil. Populations (log cfu/g) in soil infested with gall declined from 5.0 at 3 days to 4.6, 3.7, and 3.4, respectively, in grape, oat, and fallow soils at 70 days. Populations in soil infested with CG48 were undetectable at 14 days. 70 days after infestation with CG48, populations (log cfu/g) had increased to 3.5 and 2.1, respectively, in grape and oat soils, but remained undetectable in fallow soil. Greater At3 populations were associated with host and non-host plants than with fallow soil, regardless of infestation technique.

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RAPID INDEXING OF GRAPE CUTTINGS FOR FREEDOM FROM CROWN GALL WITH AN INEXPENSIVE WATER DISPLACEMENT PRESSURE CHAMBER. R.N. Goodman, F.A. Tarbah, and D. Butrov. University of Missouri, Columbia, MO 65211.

Forcing sterile water through hard wood and green wood stem cuttings permitted detection of comparatively few, randomly distributed Agrobacterium tumefaciens cells in xylem vessels. Displaced xylem visual fluid (0.1 ml) is plated on Roy-Sasser selective medium (Phytopathology 73:810). Stem pieces, 2-10 mm diam., can be rapidly (25-30/h) processed in the chamber. A. tumefaciens has been detected in Vitis labrusca, V. vinifera and hybrid species commonly used in wine production and as rootstocks obtained from California, Missouri and Virginia. Our earlier study suggested terminal segments of lengthy canes harbored fewer and often no A. tumefaciens cells. These more recent data suggest in addition, that some cultivars may be uniformly laden with the pathogen. We have proposed a system that indexes successively the newest growth of a rooted cutting three times to produce an A. tumefaciens-free grape "mother plant".

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EPIPHYTIC BEHAVIOR OF XANTHOMONAS CAMPESTRIS pv. PRUNI ON PEACH AND PLUM. D. Petra Shepard and E. I. Zehr, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, SC 29631.

Epiphytic populations of Xanthomonas campestris pv. pruni (Xcp) were monitored on twigs, leaves, buds, flowers and fruit of 16-yr-old Blake peach and Methley plum trees. Xcp was epiphytic on twigs of peach and plum on all sampling dates throughout the year and on leaves during the growing season. Populations of Xcp on leaves increased rapidly only after periods of rainfall and decreased during extended hot, dry periods. Xcp was epiphytic on buds, flower and fruits in populations ranging from 10^1 to 10^5 colony-forming units (cfu) per gram fresh weight. Large epiphytic populations (10^3 to 10^5 cfu) accompanied symptom development, but symptoms did not always follow large numbers of Xcp on plant surfaces.

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THE EFFECT OF CULTURAL PRACTICES ON THE GENERATION OF BACTERIAL AEROSOLS IN TOMATO TRANSPLANTS. T. B. McInnes, R. D. Gitaitis, and S. M. McCarter, Dept. of Plant Pathology, Coastal Plain Experiment Station, Tifton, GA 31793 and Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

Bacteria suspended in aerosols were monitored using an Andersen air sampler by sampling air above tomato transplant fields at morning, noon, and afternoon periods throughout the 1984 season. Clipping, irrigation, harvesting, and a cupric hydroxide-mancozeb chemical treatment were evaluated for their effects on generation of bacterial aerosols in a tomato transplant field inoculated with Xanthomonas campestris pv. vesicatoria (XCV) in 1985. Numbers of total bacteria in aerosols were greatest in the afternoon. Rainfall and irrigation reduced, but clipping and harvesting increased the number of bacterial aerosols. Cupric hyroxide-mancozeb had no apparent effect on the generation of XCV aerosols.

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A BACTERIAL LEAF SPOT OF GYPSOPHILA PANICULATA (BABY'S BREATH) INCITED BY PSEUDOMONAS ANDROPAGONIS. J. B. Jones and A. W. Engelhard, GCREC, 5007-60th St. E., Bradenton, FL 34203.

A leaf spot was observed in the summer of 1985 on field-planted baby's breath, Gypsophila paniculata. The spots were round, up to 1mm in diameter and tan to dark brown in color. Some had a narrow, dark margin and/or a halo surrounding the individual spots. When the spots were numerous on a leaf (1-2 mm apart), general chlorosis followed by necrosis of the tissue occurred. Tissue isolations onto medium B of King et al. yielded a white, non-fluorescent Gram negative bacterium that was negative for oxidase and arginine dihydrolase. The bacterium is a strict aerobe and has one polar flagellum. It produced a hypersensitive reaction in tomato leaves. Upon reinoculation by misting a bacterial suspension over G. paniculata plants, the symptoms observed in the field were reproduced. The leaf spots which were predominantly associated with the younger foliage, began developing on both leaf surfaces 7-10 days after inoculation when plants were incubated in high humidity at 28 C.

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A BLIGHT OF WILD AND CULTIVATED EUPHORBIA SPECIES CAUSED BY AMPHOBOTRYS RICINI. G. E. Holcomb and D. W. Wells, Dept. of Plant Pathology & Crop Physiology and Hammond Research Station, La. Agric. Exp. Stn., L.S.U. Agric. Center, Baton Rouge 70803.

A blight of prostrate spurge and cultivated poinsettia was observed in the fall of 1984 and 1985. Prostrate spurge plants, growing in bermuda turfgrass plots and containers of ornamental plants, were blighted and killed. Shoots and leaves of poinsettia were killed, but entire plants were not killed. Fungal mycelia and conidia were produced on blighted plants. Pure cultures of the fungus were obtained, and pathogenicity tests were positive on Euphorbia supina, E. heterophylla, E. hirta and detached leaves of E. pulcherrima. The pathogen was identified as Amphobotrys ricini (teleomorph Botryotinia ricini), a pathogen of castor bean.

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EFFECT OF HOST NUTRITION ON ALTERNARIA LEAF SPOT OF THREE FOLIAGE PLANTS. A. R. Chase and R. T. Poole, University of Florida, Agricultural Research and Education Center, Apopka, FL 32703.

The effect of host nutrition on severity of Alternaria leaf spot of Brassaia actinophylla, Dizygotheca elegantissima, and Schefflera arboricola was tested using an isolate of Alternaria panax Whetzel originally obtained from B. actinophylla. Plants were grown with various rates of Osmocote 19:6:12 for 2 mo prior to inoculation: 5, 10, 15, 20, 25, or 30 g/15-cm pot (6 g/pot is recommended rate). Plants were misted (5 sec/30 min, 12 hr/d) starting 24 hr prior to inoculation and until test completion. Plants were sprayed with a conidial suspension ($1x10^4$/ml) and placed in plastic bags for 3 d. Height, fresh weight of tops, quality, and number of leaves for B. actinophylla and S. arboricola were unaffected by fertilizer rate. Ratings for these parameters for D. elegantissima were inversely related to fertilizer level. Severity of Alternaria leaf spot was inversely related to fertilizer rate for B. actinophylla and S. arboricola but was unaffected for D. elegantissima.

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LEAF SPOT AND BLIGHT OF CISSUS RHOMBOFOLIA CAUSED BY CYLINDROCLADIUM SP. C. R. Semer IV, B. C. Raju, J. C. Trolinger and P. Randhawa. Technical Business Group, Yoder Brothers, Inc. Alva, Florida 33920.

Leaf and stem blight symptoms were observed in cutting propagation, 12 week-old plants and hanging baskets of Cissus rhombofolia growing in a greenhouse. Symptoms of affected cuttings and plants were crown rot at the soil line extending into stems, petioles, leaf bases and leaf spots. Severly diseased plants showed wilting and died in 2-3 weeks. Whitish mycelium with abundant conidia were present on the stem, petiole and leaf surfaces. Red-orange perithecia of Cylindrocladium sp. were present at and just below the soil line and the affected roots exhibited reddish lesions. In vitro isolations yielded Cylindrocladium sp. Pathogenicity studies revealed the isolate recovered was pathogenic on cissus, hibiscus and azalea but not on spathiphyllum. The isolate of Cylindrocladium was examined and found to have taxonomic characteristics in common with Calonectria illicicola and C. crotalariae.

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Spatial distribution of dogwood canker in Tennessee nurseries. M. Windham and M. Montgomery. Dept. of Ent. & Pl. Path., Univ. of Tennessee, Knoxville, TN 37901-1071.

Dogwood canker is the most important disease in dogwood production in Tennessee nurseries but the etiology of this disease is unknown. Sixteen blocks of dogwoods ranging from 850 to 4000 trees each were surveyed for canker in 1985 and 1986. Disease incidence ranged from less than 1 to 86% and correlated with tree age (R=0.83). The spatial distribution of diseased trees was clumped in seven of nine fields of 3 year or older trees, according to ordinary runs analysis. In fields of younger trees, the spatial distribution of diseased trees was random. The clumped distribution of this disease in blocks of older trees supports the hypothesis that the cause of dogwood canker is a biotic agent.

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A histological study of dogwood canker in the flowering dogwood, Cornus florida. M. Windham, E. Graham, and M. Montgomery. Dept. of Ent. & Pl. Path., Univ. of Tennessee, Knoxville, TN 37901-1071.

Forty 3-year-old dogwood stems that exhibited symptoms of dogwood canker and thirty nonsymptomatic stems were examined histologically. In trees with very small cankers, symptoms were limited to the stem's periderm. In more advanced cankers, vascular rays on the side of canker were much darker stained, but still nucleated. In the most advanced cankers, abnormal xylem-like cells were observed in addition to the other symptoms. Signs of various potential pathogens of this disease were noted, but no one sign was observed in all cankered stems.

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BIOLOGICAL CONTROL OF BOTRYTIS CINEREA ON GREENHOUSE ROSES. J. C. Redmond, J. J. Marois, J. D. MacDonald, Department of Plant Pathology, University of California, Davis, CA 95616.

Botrytis cinerea is a serious fall and winter disease problem of greenhouse roses. Its management is complicated by buildup of resistance to several classes of fungicide. To determine the potential for biological control, numerous bacteria, yeasts and filamentous fungi were isolated from petals of field and greenhouse grown roses. The most common bacterial and yeast isolates then were screened for their activity against Botrytis using an in vivo system which involved spraying the isolates on detached rose petals and then following with an inoculation of the pathogen. Two bacteria and two yeasts which markedly reduced infection were further evaluated by spraying on whole cut roses kept at 25 C and 95% RH. One day later the roses were sprayed with a conidial suspension of 10^3 spores/ml of B. cinerea. The yeast Exophiala jeanselmei reduced disease lesions by an average of 63%, compared to the fungicide iprodione which reduced disease by an average of 74%.

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Phytophthora Leaf and Stem Blight of Periwinkle in Florida. T. S. Schubert, R. M. Leahy, E. Chichester, Div. of Pl. Ind., Fl. Dept. of Ag. and Cons. Serv., P. O. Box 1269, Gainesville, FL 32602.

Phytophthora parasitica Dast. has been diagnosed as the cause of a recently severe leaf and stem blight of periwinkle (Catharanthus roseus (L) G. Don) in Florida. The disease has been very infrequently reported elsewhere, most recently in California in 1977. (PDR 61:182-4, 560-1). Initial symptoms are dark water-soaked blotches on leaves and young stems, followed by rapidly progressing cankers and dieback on larger stems. Under warm, humid conditions, sporangia can appear as a white bloom on recently infected tissues. Lesions are quickly colonized by Colletotrichum which may confound diagnosis. Isolation of the pathogen on a medium selective for Oomycetes is considered essential for accurate diagnosis. In pathogenicity tests, both zoospores and mycelial fragments have proven to be satisfactory sources of inoculum. Fungicide trials are underway.

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FUNGICIDES FOR THE CONTROL OF ROSE POWDERY MILDEW (SPHAEROTHECA PANNOSA VAR. ROSAE) AND RUST (PHRAGMIDIUM MUCRONATUM). A. O. Paulus, J. Nelson and S. Besemer, Plant Pathology Department, University of California, Riverside, CA 92521.

Fungicides were evaluated from 1983-1985 to determine effective materials for the control of powdery mildew and rust of rose. Chemical sprays were applied to runoff with a 7.5 l CO_2 Hudson sprayer at 207 kPa. One hundred eighteen ml of Rohm and Haas Triton B-1956 spreader-sticker per 378 l of water were added to all fungicide suspensions. In 1983 excellent control of powdery mildew was obtained with diniconazole-80%, and rust was controlled with diniconazole and triforine-95%. Rust was not prevalent in the 1984 trial but DuPont 6573 gave 93% powdery mildew control, penconazole-93%, triforine-90% and no treatment -45%. Powdery mildew was controlled in the 1985 trial by myclobutanil-95%, triadimenol-93%, diniconazole and Maag 15-1297-88%. Effective control of rust was obtained with diniconazole 88%, myclobutanil-86%, Mobay 1608-85% and no treatment-15%. Severe rust developed in the Maag 15-1297 plots and was not significantly different from no treatment.

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BLIGHT OF POTHOS CAUSE BY THREE FUNGI. J. C. Trolinger, C. R. Semer IV, and B. C. Raju. Technical Business Group, Yoder Brothers, Inc., Alva, FL 33920.

Leaf and stem blight symptoms were observed on mature 36-inch totem pole plants of pothos (Epipremnum aureum (Lind & Andre) Bunt.) growing in a greenhouse. Three types of symptoms were observed. Brown dry lesions on white variegated portions of lamina consistently yielded Colletotrichum gloeosporioides, whereas gray-brown dry blotches extending from leaf margins into centers, sometimes showing concentric rings, yielded Rhizoctonia solani. The most frequently observed symptom was black brown wet blotches on leaves and stems which yeilded Phytophthora parasitica and R. solani (about 50% of isolations). Pathogenicity tests were conducted with each organism separately on healthy tissue and the described symptoms were reproduced in three to five days. Combination sprays of benomyl (1.2 g/L) and metalaxyl (0.2 ml/L) alternated with mancozeb (2.4 g/L) at weekly intervals provided control.

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BOTRYTIS CINEREA SPORE CONCENTRATION AMONG GERANIUM STOCK PLANTS IN THE GREENHOUSE. M. K. Hausbeck, S. P. Pennypacker, and R. E.

Stevenson. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Botrytis cinerea spore concentration was monitored in a commercial greenhouse using a Burkard recording spore trap within a geranium (Pelargonium x hortorum) stock system planted in July, 1984. Maximum average hourly spore concentrations occurred most frequently between 2200 and 1000 hours, during the 17 July to 8 August, 1985, sampling period. Diurnal spore concentration differed from previous reports and may, in part, be explained by the use of a computer-controlled dehumidification fan system during late evening and early morning hours. Air temperature above and below the dense, closed canopy was recorded simultaneously at two locations in the greenhouse. Temperatures differed between the two locations and the temperature below the canopy was lower than the temperature above the canopy (α=0.01). Daily fluctuations were similar regardless of measurement site.

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TEMPERATURE AND MOISTURE EFFECTS ON LEAF SPOT OF PHOTINIA x. FRASERI CAUSED BY ENTOMOSPORIUM MESPILLI. L. G. Brown and L. W. Baxter, Jr., Dept. of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Photinia x. fraseri in nurseries under overhead irrigation are highly susceptible to leaf spot caused by Entomosporium mespilli. Rate of symptom development was examined over a temperature range of 7-35 C. Plants were inoculated ($1x10^4$ conidia/ml) and incubated in darkness during wetting periods followed by a 12 hr light/dark regime. Infection at 7 C occurred between 12 and 24 hr after inoculation and symptoms were visible after 14 days. At 14, 21, or 28 C a 9-hr wetting period was required for infection and symptoms appeared 5-7 days later. After wetting for 48 hr at 21 C, symptoms failed to appear on plants exposed to 35 C for 72 hr. Under continuous leaf wetness at 35 C conidia were viable for 12 but not 24 hr. These temperature and moisture relationships may have application in fungicide management of photinia leaf spot.

120

MATRIC POTENTIAL RELATIONS IN CONTAINER MIXES OF PINE BARK AND SUPPRESSION OF PHYTOPHTHORA ROOT ROT OF RHODODENDRON SPP. B. OWNLEY GINTIS and D. M. BENSON. Department of Plant Pathology, NCSU, Raleigh, NC 27695-7616.

The disease suppressive nature of container mixes of pine bark has been attributed to physical properties of the media which affect water retention characteristics. Patterns of change in water content and matric potential were determined for irrigated pine bark mixes that differed in composition and particle size distribution. At intermediate and low inoculum densities, significant reduction in the incidence and/or severity of Phytophthora root rot of Rhododendron spp. was observed for combinations of media and irrigation in which matric potential was not maintained in the -20 to -50 mb range. Two patterns of matric potential relations were associated with suppression of Phytophthora root rot. Mixes with matric potential characteristics that did not decrease below -20 mb, and mixes that decreased well below -50 mb were suppressive. The disease suppressive effect was overcome with high inoculum densities.

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PRODUCTION AND SPATIAL ARRANGEMENT OF SCLEROTIA OF SCLEROTIUM ROLFSII ON APPLE ROOTSTOCK. S. F. Tomasino and K. E. Conway, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Sclerotial production by Sclerotium rolfsii (Sr) was enumerated in a 5 cm zone of soil around the bases of 10 recently killed apple rootstocks maintained in microplots. Soil samples were taken at 1 cm increments to a depth of 4 cm. One hundred-fifty sclerotia were recovered from the entire zone with 72% confined in the 0-1 cm zone. Sclerotial densities (SD) declined as distance (D) from the tree increased. A polynomial regression equation, Y=17.2-10.4(X1)+1.5(X2), was obtained where Y=SD and X=D. In containers, 22 trees were inoculated with 1 to 4 sclerotia of Sr within a 3 cm distance from the base of the trees. Relationship between SD and time elapsed between death and sampling was analyzed. No correlation between initial inoculum number and final SD was evident. A linear regression equation, Y=-.52(X)+65.7, was obtained where Y=SD and X=elapsed time. Thus, as time increased between the sampling date and tree death, the SD decreased.

122

INTERACTION BETWEEN THE SATELLITE VIRUS OF TOBACCO MOSAIC VIRUS AND ITS HELPERS IN DIFFERENT HOSTS. Rodrigo A. Valverde and J. A. Dodds, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

A satellite virus of tobacco mosaic virus (STMV) is found naturally in California in association with TMV-U5 infecting Nicotiana glauca. Other tobamoviruses able to support STMV replication are TMV-U1, and green tomato atypical mosaic virus. The host range of STMV could be extended by changing the helper. Presence of the satellite did not alter symptoms induced by the helpers, except for those in N. rustica which reacted with lethal systemic necrosis when infected with TMV-U1 and STMV. Single infections with TMV-U1 induced only necrotic local lesions. Yields of STMV virions were not significantly affected by the host or helper. Highest yields of STMV dsRNA were obtained when STMV was associated with TMV-U5. After passage of TMV-U5 through N. tabacum or N. glauca STMV reappeared in some single local lesions subcultures which were originally freed of detectable satellite.

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VARIABILITY IN COWPEA CHLOROTIC MOTTLE VIRUS. O. R. PAGUIO, C. W. Kuhn, and H. R. Boerma, Departments of Plant Pathology and Agronomy, Univ. of Georgia, Athens, GA 30602.

The primary objective of this study was to determine which RNAs of cowpea chlorotic mottle virus (CCMV) control virus replication, virus movement, and disease reactions in resistant soybean genotypes. Reactions caused by seven strains of CCMV were relatively similar in individual soybean genotypes. However, strain BYS was the most distinctive strain, and two new isolates of CCMV (N and D) were derived from it. Isolate N caused no necrosis on hypersensitive soybean cultivars, and virus concentration in uninoculated leaves was similar to susceptible soybean genotypes. Furthermore, isolate N caused reactions unique to CCMV in susceptible cowpea cultivars: systemic necrosis and inhibition of replication at 30 C. Isolate D caused severe systemic reactions in hypersensitive soybeans, even though relatively little virus was produced in the leaves: vein necrosis, leaf malformation, and stunt. These new isolates should be valuable in future studies with pseudorecombinants.

124

TWO COMPONENTS ASSOCIATED WITH CITRUS RINGSPOT VIRUS. K.S. Derrick, Dept. of Plant Pathology and Crop Physiology, La. Agric. Exp. Sta., La. State Univer. Agr. Ctr., Baton Rouge, LA 70803 and R. H. Brlansky, R. F. Lee, L. W. Timmer, and T. K. Nguyen, Univ. of Fla., Citrus Res. and Ed. Ctr., Lake Alfred, FL 33850.

Citrus ringspot virus (CRSV) is readily transmitted to many herbaceous plants by sap inoculation and produces local lesions on Chenopodium quinoa, but the infectivity in extracts is unstable, and the presumed virus has not been characterized. Infectious preparations of CRSV were prepared by differential centrifugation, but individual fractions following sucrose density gradient centrifugation (10-40%, 2.5 h at 38 K rpm, SW 41 rotor) were not infectious when assayed on C. quinoa. Mixtures of fractions approximately 3.5 cm deep (top component) and 5.5 cm deep (bottom component) in the gradients were highly infectious. The two components appeared to depend on each other for infectivity. Efforts to infect citrus plants with single components were not successful.

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USE OF VIRUS CONCENTRATION, SYMPTOMATOLOGY, AND YIELD AS A MEASURE OF "RESISTANCE" TO MDMV-A IN DIFFERENT SORGHUM CULTIVARS. L. M. Giorda and R. W. Toler, Dept. of Plant Path. & Microbiology, Tex. Agr. Exp. Sta., College Station, TX 77843.

Maize dwarf mosaic virus titer in extracts from 12 field-grown sorghum cultivars was measured by enzyme-linked immunosorbent assay (ELISA) at preboot stage. Virus concentration, visual assessment of symptoms, yield, and number of seeds/panicle were compared. Significant differences were obtained within and among cultivars with mosaic or red leaf symptoms. Clear relationships among high virus content, symptom severity, or yield decrease were less obvious in such genotypes as RTx430 and BTx378. The association between "resistance" to MDMV-A, as previously determined, with reduced virus concentration in infected plants was cultivar specific. Correlation between virus concentration and visual assessment was low (R^2=0.399);

however, a trend was observed when cultivars were grouped according to mosaic or red leaf. Yield reduction of 11-79% was positively correlated with severe red leaf and lower virus titer.

126

SUSCEPTIBILITY AND RESISTANCE OF SOYBEANS TO PEANUT STRIPE VIRUS. Dulce Warwick, and J. W. Demski. Dept. of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

Seed of 158 soybean (Glycine max [L.] Merr.) lines were obtained from the INTSOY collection. Fifteen seedlings from each soybean line were inoculated with each of three isolates of peanut stripe virus (PStV). Three weeks after inoculation the lines were rated susceptible or resistant based on visual observation, tests using ELISA, and/or recovery of virus by mechanical transmission. Fifty eight percent of the lines were resistant; systemic symptoms were not present nor could virus be detected in expanding leaves. Susceptible lines were grown to maturity and seeds collected. Positive ELISA values were obtained from green immature seed. After seeds matured (dried), positive ELISA values were obtained from the seed coat but not from the cotyledon or embryo tissue. Over 15,000 seed from infected parents were planted and the seedlings tested for PStV. The virus was not detected in any of the seedlings.

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NURSERY MANAGEMENT TO SELECT BEANS FOR RESISTANCE TO BEAN GOLDEN MOSAIC. Josias C. de Faria and Maria José de O. Zimmermann, CNPAF, EMBRAPA, Caixa Postal 179, 74000 Goiânia, Goiás, Brasil.

Bean Golden mosaic virus is a whitefly transmitted disease. In Brazil, most of the BGMV nurseries show symptoms of leafhopper and other insects injuries. Six cultivars with four different inseticide treatments were studied in a completely randomized block in split plot design with four replications. Disease incidence and severity were assessed at every 10 days beginning 20-days after sowing. Yield was also obtained. Approximately 95% of the plants exhibited BGMV symptoms at 45 DAP. All inseticide treatments increased yields of the six test cultivars. Significant differences among the cultivars was obtained with carbofuran soil treatment plus three applications of Monocrotophos at 15 days interval. Insect damage was reduced in all insecticide treatments. Application of carbofuran in the soil induced delayed appearance of disease symptoms and increased yield. A tolerant Brazilian line 10 988 MDS 76, was superior in yield among the six cultivars when untreated, while Porrillo Sintetico was superior overall.

128

CUCUMBER, RIBGRASS AND TURNIP MOSAIC VIRUSES IN HESPESIS MATRONALIS. **R. E. Ford, R. I. Hamilton and L. Beczner. Dept of Plant Pathology, University of Illinois, Urbana, IL 61801, Agriculture Canada Research Station, Vancouver, B.C. and Plant Protection Institute, Budapest, Hungary.**

Cucumber - To strain (CMV), ribgrass (RMV) and turnip (TuMV) mosaic viruses were isolated from Hesperis matronalis in British Columbia. All three viruses infected H. matronalis seedlings following mechanical inoculation. TuMV caused mosaic but CMV or RMV were symptomless. Various combinations of TuMV in mixed infection with CMV or RMV caused more severe mosaic, necrosis, and leaf distortion. In seed transmission studies, CMV but not TuMV was isolated from immature ovules excised from naturally infected H. matronalis seedlings. Seedlings grown in the greenhouse from mature seed were symptomless and CMV, RMV and TuMV were not detected by infectivity assay or ELISA. This is the first report of CMV or RMV in H. matronalis in British Columbia.

130 Withdrawn

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DOUBLE INFECTION OF Clitoria ternatea WITH CUCUMBER MOSAIC VIRUS AND A POTYVIRUS IN THE STATE OF CEARÁ, BRAZIL. J. A. A. Lima, D. E. Purcifull, and M. J. B. Cavalcante. Universidade Federal do Ceara, C. P. 3038, Fortaleza-CE, 6000-Brazil and Dept. Plant Pathology, Univ. Florida, Gainesville,FL.

A severe mosaic was observed in plants of Clitoria ternatea growing as a forage crop in Ceara, Brazil. Extracts from affected plants reacted with antisera to cucumber mosaic virus (CMV) and to a potyvirus, bean common mosaic virus. Serological analysis of mechanically inoculated plants revealed that Cassia occidentalis was infected only by the potyvirus and Macroptilium lathyroides only by CMV. Vigna unguiculata 'Macaibo' was infected by both viruses. Light microscopy revealed the presence of cytoplasmic inclusions typical of potyviruses in infected tissues of C. ternatea and Cassia occidentalis. The potyvirus was seed-transmitted in C. ternatea. Among 33 species inoculated in the greenhouse, 13 were infected by the potyvirus, including 10 varieties of V. unguiculata and 4 of Phaseolus vulgaris.

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SEED TRANSMISSION OF ZUCCHINI YELLOW MOSAIC VIRUS IN SQUASH. R. F. Davis and M. K. Mizuki, Dept. of Plant Pathology, Cook College, N.J. Agric. Exp. Sta., Rutgers Univ., New Brunswick, N.J. 08903, and Dept. Pl. Virol., Inst. Biologico-S. Paulo, Brazil, C.P. 7119, respectively.

Transmission of zucchini yellow mosaic virus (ZYMV) was detected by ELISA in a total of 246 of 1299 (18.0%) Cucurbita pepo 'Black Beauty' seedlings derived from 15 naturally infected fruits. Transmission through seeds of individual fruits ranged from 0-81.1%, with higher rates occurring in seeds of reduced size and malformed shape. In 17 of these seedlings, detection of ZYMV decreased from the cotyledonary leaves to the fourth true leaf and was not detected in the upper leaves or in the flowers. Symptoms in plants infected by seed transmission were very mild and dilution end point by ELISA or bioassay was much lower than in plants experimentally inoculated with a known isolate of ZYMV. Virus was efficiently transmitted by aphids from two of these seedlings to healthy squash plants.

133

ASSOCIATION OF VIRUS PARTICLES WITH MEALYBUG-WILT OF PINEAPPLE. **U.B. Gunasinghe and T. L. German, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822**

Mealybug-wilt of pineapple is a widely distributed and severely damaging disease that was described over thirty years ago (Carter, W., Phytopath:23:207-242, 1933). The name of the disease is derived from its association with mealybugs, but the causal agent has not been described. We have shown that double-stranded RNA is present in infected plant tissues, but absent in healthy tissue. Attempts to demonstrate the presence of virus by solvent clarification or PEG precipitation were unsuccessful. After ammonium sulfate fractionation, long flexuous rod-shaped virus particles were recovered from infected tissues and observed in the electron microscope. These particles were not seen in identically treated healthy material. Characterization of the nucleic acid, protein coat, and immunologic properties of this virus will be described.

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MECHANICAL TRANSMISSION OF RHYNCHOSIA MOSAIC VIRUS. Joseph M. Jilka and Joseph G. Utermohlen. Department of Plant Pathology. University of Illinois, Urbana, Illinois 61801.

Rhynchosia mosaic virus, a white-fly transmitted geminivirus, was mechanically transmitted for the first time to Glycine max

cv. 'Williams'. The source of the inoculum was frozen leaf tissue of Phaseolus vulgaris cv. 'Topcrop' infected with inoculum collected in Puerto Rico. Isolated viral DNA from Topcrop was partially characterized by endonuclease restriction mapping. The infected tissue was triturated in 0.05 M phosphate buffer (pH 7.5) and 0.1% 2-mercaptoethanol. Several different ages of soybean seedlings, ranging from 7-10 days, were dusted with 600 mesh carborundum and inoculated mechanically. A total of 40 plants were inoculated. Systemic infections developed in two of the 9 and 10-day old inoculated plants about four weeks following inoculation and symptoms were identical to those resulting from white-fly transmissions of the virus to soybeans. The symptoms included stunting and a yellow-green leaf mosaic.

135

ALTERATION OF UNIFOLIOLATE LEAF ANGLES AND CIRCADIAN LEAF MOVEMENTS IN SEEDLING SOYBEANS INFECTED BY TOBACCO RINGSPOT VIRUS. B. W. Kennedy. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Mechanical (sap) inoculated first leaves of Glycine max L. cv Bansei grown in controlled environments at 25 C and 12 hr light followed by 12 hr dark resulted in downward curvature of inoculated leaves and a lateral twisting of emerging trifoliolate leaves within 3-4 days. Angles of affected unifoliolate leaves, as measured by horizontal position of the midvein, approached the vertical and remained during both light and dark periods; circadian rhythmic movements stopped. Healthy control plants moved over a mean range of 35-75 degrees during light/dark cycles. Both healthy and diseased leaves showed ultradian rhythmic movements with a phase between 25 and 120 min; amplitude varied from 1-5 degrees during light to a maximum of 15 degrees during dark periods. This is the first known report of a plant virus altering circadian leaf movement rhythm in its host.

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MOLECULAR CLONING AND ANALYSIS OF STRAWBERRY VEIN BANDING VIRUS DNA. D. C. Stenger, T. J. Morris, and R. H. Mullin, Department of Plant Pathology, University of California, Berkeley, CA 94720.

DNA with Caulimovirus properties was isolated from virion enriched preparations of strawberry vein banding virus (SVBV). Native viral DNA was double stranded and consisted of both circular and linear forms 7.8 kilobase pairs (kbp) in size. Each DNA strand contained one discontinuity positioned at 0.0 or 0.5 map units on the circular molecule. EcoRl digested SVBV DNA was cloned into E. coli using the vector pUC8. A recombinant plasmid (pSVBV-E3) contained a 7.8 kbp EcoRl insert which hybridized to SVBV DNA but not to cauliflower mosaic virus DNA, and had a restriction map identical to SVBV DNA. EcoRl digested pSVBV-E3 was not infectious when mechanically inoculated to Fragaria vesca. pSVBV-E3 was used as a probe to detect SVBV infection. Dot hybridization tests indicated SVBV DNA titre varied greatly between leaflets sampled from the same plant.

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CORRELATIONS AMONG GREENHOUSE TESTS AND BETWEEN FIELD AND GREENHOUSE EVALUATIONS FOR BEET NECROTIC YELLOW VEIN VIRUS (BNYVV) RESISTANCE IN BETA MARITIMA. E. D. Whitney, ARS, USDA, 1636 E. Alisal St., Salinas, CA 93905.

Rhizomania, a fungus, Polymyxa betae, transmitted virus, BNYVV, disease complex identified in California in 1983 is a serious disease of sugar beet in Europe, Japan, and USA. Non adapted tolerant cultivars from Europe have not performed well in limited testing in rhizomania infested areas of the USA. New sources of resistance and a procedure for the identification of BNYVV resistance by ELISA are reported in this paper. Correlations were 0.69, 0.65, and 0.70 (P = 0.001) for resistance among three greenhouse tests of 61 B. maritima accessions grown in infested soil and 0.77 (P = 0.001) between the mean greenhouse test scores and a field test of 15 resistant types. Resistance in some accessions appears to be simply inherited.

138

RAPID BIOLOGICAL EVALUATION OF THE DECLINE INDUCING POTENTIAL OF CITRUS TRISTEZA VIRUS (CTV) ISOLATES. **S. M. Garnsey and R. K. Yokomi, U. S. Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803, and R. H. Brlansky, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850.**

Rapid expression of the budunion disease syndrome of CTV occurred when small sour orange seedlings (Citrus aurantium) (.5-.7 cm diam) were simultaneously budded with healthy Hamlin orange (C. sinensis) buds and graft-inoculated with CTV. Plants were topped 2 wks post inoculation (PI) to force uniform scion bud growth. Stunting of the Hamlin scions and/or leaf chlorosis was observed 8-10 wks PI in plants inoculated with known, severe CTV isolates. Mild isolates did not induce visible symptoms 25 wks PI. CTV infection was confirmed in symptomless inoculated plants by ELISA. Cytological examination of budunions of plants infected with severe isolates confirmed association of visual symptoms with phloem necrosis at the budunion.

139

PRELIMINARY STUDIES ON LOCATION OF BLUEBERRY SHOESTRING VIRUS (BBSSV) IN HIGHBUSH BLUEBERRY CV. 'JERSEY' USING INDIRECT FLUORESCENT ANTIBODY AND DOT-BLOT IMMUNOASSAY TECHNIQUES. L.A. Urban, K. Klomparens, and D.C. Ramsdell. Dept. of Bot. and Plant Path., Michigan State University, E. Lansing, MI 48824.

Known BBSSV diseased blueberry leaves, stems, and roots were embedded in Tissue-tek and sectioned using a cryostat. Sections were stained with anti-BBSSV IgG, anti-tobacco ringspot virus IgG, or normal serum. Sections were then stained with sheep anti-rabbit fluorescein isothiocyanate(FITC) and observed with an epifluorescent microscope(450-490nm excitation,520nm barrier filter). Fluorescence was observed in both epidermal layers, palisade and mesophyll cells, and vascular tissue of the leaf. Xylem elements fluoresced in both stems and roots. Autofluorescence was common in most tissues, but was not the same color as FITC. Blossoms, roots, berries, seeds, xylem, and phloem tested positive for the virus using dot-blot immunoassay.

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MOVEMENT OF BLUEBERRY SHOESTRING VIRUS IN APHID-INOCULATED HIGHBUSH BLUEBERRY LEAVES. L.A. Urban, D.C. Ramsdell, and K. Klomparens, Michigan State University, E. Lansing, MI 48824

Blueberry leaves were aphid-inoculated with blueberry shoestring virus (BBSSV). Blueberry aphids, Illinoia pepperi (MacGil.), were placed on symptomatic diseased bushes for a 48-hour acquisition access period. Seven to ten of these aphids were then placed in a 2.5 cm dia. cage on each of ten leaves on a healthy plant (8 plants total) for an inoculation access feeding period of 48 hours. Leaves were tested by indirect fluorescent antibody staining and dot-blot immunoassay or ELISA at intervals of 1,3,7,14,21,35, and 63 days post-inoculation. In 1984, BBSSV was detected in epidermal and mesophyll cells by epifluorescence microscopy beginning on day 7. By day 14 fluorescence was found in palisade and vascular tissue and was found throughout the leaf by day 35. ELISA tests of leaf samples were negative. In a repeat experiment in 1985, fluorescence was found throughout the leaf by day 7. Virus was detected in leaves on day 63 by dot-blot immunoassay.

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GRAFTING AND GIRDLING TECHNIQUES USED TO DETERMINE MOVEMENT OF BLUEBERRY SHOESTRING VIRUS IN HIGHBUSH BLUEBERRY CV. 'JERSEY'. L.A. Urban, D.C. Ramsdell, and K. Klomparens, Dept. of Bot. and Plant Path., Michigan State University, E. Lansing, MI 48824

Blueberry shoestring virus (BBSSV) is transmitted by the blueberry aphid Illinoia pepperi (MacGil). Being aphid vectored, BBSSV is hypothesized to be phloem translocated. This study was undertaken to determine if the virus moves in phloem, xylem, or both. Three or four diseased buds from field-grown plants were grafted onto healthy, potted, 3-year old test plants. The plants were then girdled above the grafts, girdled below the grafts, or not girdled. Leaf samples taken monthly from grafted stems tested virus positive above (up to 40%) and below (up to 80%) the girdle on plants girdled above the graft. Up to 90% of the non-girdled, grafted plants tested virus positive. Dot-blot immunoassay was used to test leaves on non-grafted stems of grafted plants; all samples tested virus negative. This study suggests the virus is moving in both xylem and phloem.

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GRAFT TRANSMISSION OF DEAD SPUR. C.L. Parish and M.W. Williams. Tree Fruit Research Laboratory, 1104 N. Western Avenue, Wenatchee, WA 98801.

Dead spur disease of apple appears to be caused by a graft-transmissible agent. In controlled tests the apple cultivars 'Earlistripe Delicious', 'Royal Red Delicious;, 'Tydeman' and 'Ottawa 292' all induced symptoms of dead spur, when they were used as inocula and grafted on 9 cultivars of 'Red Delicious', both spur and non-spur types. The long, leggy or willow-like growth exhibited by some cultivars ('Tydeman', 'Lodi', 'Ottawa', etc.) appears to be related to the dead spur disorder. When cultivars with this type of growth are top-worked or used as inocula, symptoms of dead spur are observed in the new scion cultivar or the receptor host. Dead spur symptoms being eliminated in new trees propagated from heat-treated dead spur-affected scions, further suggests a graft-transmissible pathogen is the cause of the disorder.

143

DETECTION AND QUANTIFICATION OF SOYBEAN DWARF VIRUS BY ELISA. A. D. Hewings and V. D. Damsteegt, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701

Double-antibody sandwich and indirect ELISA were used to study a dwarfing (SDV-D) and a yellowing (SDV-Y) strain of soybean dwarf virus from Japan. Each strain was transmitted to 7-day old Wayne soybean seedlings with Aulacorthum solani. Eleven, 19, 26, 33, 40, and 54 days after inoculation, tissue from various levels of the canopy was harvested and analyzed for presence of virus. In all tissues, virus concentration was highest at 19 days and decreased steadily thereafter. At all dates virus concentration was highest in unifoliolates and in the youngest trifoliolates. In a second series of experiments, 7-, 14-, 21-, and 28-day-old Wayne soybeans were inoculated by caging 10 viruliferous aphids on one unifoliolate for 48 hr. The youngest trifoliolates were harvested and analyzed for infection 16 days after inoculation. The data suggest that susceptibility to virus infection decreases with plant age.

144 Withdrawn

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PROPERTIES AND CYTOPATHOLOGY OF A VIRUS CAUSING MOTTLING SYMPTOMS ON EUONYMOUS EUROPAEUS. R. C. Larsen, R. C. Gergerich and K. S. Kim, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

A virus causing leaf mottling on Euonymous europaeus (L.) has been recently discovered in Arkansas. The virus, Euonymous mottle virus (EuMV), was transmitted mechanically to species of Chenopodium, Vigna, Cucumis and Nicotiana. The virus was also seed transmitted. The virus has a multipartite genome with isometric particles ca. 28 nm in diameter. Preliminary virion molecular analysis revealed a single protein with M_r of 53,000 and two ssRNAs with M_r of 2.4×10^6 and 2.0×10^6 determined under non-denaturing conditions. The virus is serologically unrelated to tomato ringspot virus and tobacco ringspot virus. Ultrastructural studies revealed the presence of viruslike particles within membranous tubules in the cytoplasm as well as in the plasmodesmata. Tubules with the particles were often embedded in abnormal cell wall protrusions. Based on symptomatology, genomic properties and cytopathic effects, EuMV may be a member of the nepovirus group.

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EVALUATION AND GENETIC ANALYSIS OF WSMV RESISTANCE IN WHEAT GERMPLASM BY ELISA AND SLOT-BLOT HYBRIDIZATION. S. L. Stoddard, B. S. Gill, and S. A. Lommel. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

ELISA, slot-blot hybridization, and symptomatology assays were used to evaluate a large number of wild wheat species for resistance to wheat streak mosaic virus (WSMV). No resistance was found among Triticum or Aegilops species. Nine WSMV resistant registered germplasms were evaluated; 8 were immune. Five wheat X Agropyron amphiploids were resistant to WSMV. The genetic expression of WSMV resistance was studied in a wheat X A. intermedium and wheat X A. elongatum amphiploid and their derived disomic addition lines. The amphiploids were immune to WSMV whereas a single addition line from each amphiploid was resistant but the resistance was heat sensitive. Chromosome dosage effects appeared to be involved in WSMV resistance, and it was concluded that there was one major and at least one minor genetic factor involved in the WSMV resistance observed in the two Agropyron species.

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PROPERTIES OF FOUR PREVIOUSLY UNCHARACTERIZED CASSAVA VIRUSES. B.D. Harrison, A.M. Lennon and M.M. Aiton, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

Four mechanically transmissible viruses were obtained from cassava. Cassava virus X is a potexvirus found in plants from Colombia with frogskin disease. It has a narrow host range that includes Nicotiana benthamiana, possesses particles 480 nm long and is distantly serologically related to potato virus X but not to cassava common mosaic virus. Cassava green mottle virus occurs in the Solomon Islands, has a very wide host range, is seed transmissible, produces 26 nm diameter isometric particles which sediment as three components, and has a bipartite RNA genome. Despite its resemblance to them, no serological relationship to definitive nepoviruses was detected. Cassava plants from Kenya with brown streak disease contained two viruses that are transmissible to the same range of solanaceous species and have filamentous particles. One is serologically related to cowpea mild mottle virus and the other is a potyvirus related to wild potato mosaic virus.

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EFFECT OF FREEZING ON INFECTIVITY OF WHEAT STREAK MOSAIC VIRUS FROM WHOLE PLANT AND EXTRACTS OF WHEAT INFECTED FOR DIFFERENT TIMES. D. A. Karr and D. L. Seifers. Fort Hays Branch Agricultural Experiment Station, Hays, Kansas 67601-9228.

Under short-term freezing, wheat streak mosaic virus (WSMV) was most stable in whole tissue rather than extracts. This stability was greatest in whole tissue 7 days post-inoculation (DPI) and to a lesser extent in 14 DPI whole tissue where infectivity comparable to fresh plant extracts could be maintained for 2 and 1 wk, respectively. WSMV in 21 DPI whole tissue and extracts from 7, 14, and 21 DPI infected plants, with exception of 7 DPI extracts after 1 wk freezing, were least stable as demonstrated by infection percentages significantly lower than those of fresh tissue after only 1 wk of freezing.

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EFFECTIVENESS OF ROOT INOCULUM FROM MAIZE WHITE LINE INFECTED PLANTS. Raymond Louie, USDA-ARS, Dept. of Plant Pathology, OSU-OARDC, Wooster, OH. 44691

Roots from 3-7 field-grown corn plants (Rupp 1624, planted 8 May) with symptoms of maize white line mosaic virus (MWLMV) were collected at intervals of 1-5 wk (12 collections, 25 Jun-13 Dec). Washed roots (1-2 mm, 0.75 gm/sample) were placed at 6, 12, and 18 cm from the bottom of, or mixed throughout, a 6 x 25 cm cylinder filled with vermiculite. Various root regions of corn test plants (Seneca Chief) grown in the root-infested vermiculite were tested for MWLMV by enzyme-linked immunosorbent assays at 2-3, 5-6, and 7-8 wk after planting. Of 2477 assays that were analyzed with respect to physiological age of inoculum, its placement in a growing medium, or length of inoculation period, MWLMV was most often detected in roots of test plants inoculated with root inoculum from infected plants collected after flowering, when the root inoculum used was mixed throughout the growing medium, and when the test plants were assayed at 5-6 wk after planting.

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INFECTIVITY NEUTRALIZATION OF RICE TUNGRO-ASSOCIATED VIRUSES ACQUIRED BY VECTOR LEAFHOPPERS. H. Hibino and P.Q. Cabauatan. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Rice tungro bacilliform virus (RTBV) depends on rice tungro spherical virus (RTSV) for its transmission by the vector leafhopper *Nephotettix virescens*. The leafhopper transmits RTBV and RTSV in a semipersistent manner. Leafhoppers carrying both RTBV and RTSV fed for 16 hr through membranes on anti-RTBV or anti-RTSV IgG diluted 25 times. Leafhoppers that fed on anti-RTBV IgG lost RTBV and transmitted only RTSV. Leafhoppers that fed on anti-RTSV IgG lost most RTSV and transmitted mostly RTBV. When leafhoppers carrying RTSV alone fed on anti-RTSV IgG, they lost most RTSV but retained the ability to acquire and transmit RTBV. When the leafhoppers carrying RTBV and RTSV were forced to feed on a mixture of the IgGs and then transferred to plants infected with both viruses, they reacquired both viruses. These results indicate that RTSV itself may not be the bearer of the helper function for RTBV transmission by the leafhopper.

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INCREASE LEVELS OF MAIZE DWARF MOSAIC IN INSECTICIDE TREATED SORGHUM (Sorghum bicolor (L.) Moench). D. L. Seifers and T. L. Harvey. Fort Hays Branch, Kansas Agricultural Experiment Station, Hays, Kansas 67601.

Significant ($P<0.05$) increases in levels of natural infection by maize dwarf mosaic virus (MDMV) occurred in insecticide (carbofuran) treated compared to untreated sorghum in 1985. Data were collected at two sites, each having a different hybrid, planting date, and four treated and untreated replications. Site 1 (Dekalb DK18, carbofuran 1.12 kg/ha) and site 2 (DK28, carbofuran 2.24 kg/ha) had average infection percentages of 10 and 4; and 23 and 10 percent for treated and untreated plots, respectively. The virus was identified as MDMV-strain A by its reaction to antisera of MDMV-A&B in the enzyme-linked immunosorbent assay and on the basis of host range. This is the first report of increased infection of sorghum hybrids by MDMV associated with systemic insecticide treatment.

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Tn3-HoHo1 mutagenesis of a gene from *Xanthomonas campestris* pv. *malvacearum* that confers avirulence against a cotton line possessing bacterial blight resistance gene B_5. K. McNally[1], M. Essenberg[1], and D. Gabriel[2]. Dept. of Biochem., Oklahoma State University, Okla. Agricultural Expt. Station, Stillwater, OK 74078[1] and Dept. of Plant Pathol., University of Florida, Gainesville, FL 32611[2].

Xanthomonas campestris pv. *malvacearum* (Xcm) strain H (widely avirulent) was used as the source of DNA for the construction of a clone bank by D. Gabriel. Screening of individual clones by triparental matings from *E. coli* HB101 to Xcm strain N (widely virulent) revealed that cosmid pUFA809 carries an avirulence gene against cotton line AcB_5. This cosmid was chosen to be analyzed by transposon mutagenesis using the Tn3-HoHo1 system (S. Stachel *et al.*, 1985, EMBO J 4:891-898). Screening Xcm strain H pUFA809::Tn3-HoHo1 transconjugants in AcB_5 revealed that about 1 in 40 transconjugants were virulent whereas spontaneous mutants virulent against AcB_5 were about 1 in 10^4 of the Xcm strain H population tested. Analysis of a number of these transconjugants is underway.

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DIFFERENTIATING PATHOVARS OF XANTHOMONAS CAMPESTRIS WITHOUT PATHOGENICITY TESTS. G. R. Lazo, D. W. Gabriel, and R. Roffey. Plant Pathol. Dept., Univ. of Florida, Gainesville, FL 32611.

No method, other than direct plant inoculations, has proved reliable for distinguishing the over 125 pathovars of Xanthomonas campestris (Xc). Different laboratory tests were evaluated for the ability to differentiate Xc pathovars. All biochemical tests used were unsatisfactory. Total protein profiles on SDS-PAGE gels and total DNA digests on agarose gels revealed variations between strains, but pathovars were not clearly resolved. Profiles of plasmid DNA digests were surprisingly useful, but of 26 pathovars tested, only 13 contained plasmids. When randomly selected cosmid clones of Xc were used as probes against Southern transfers of Xc genomic digests, restriction fragment length polymorphisms (RFLPs) were characteristic for all strains of a given pathovar. These genetic markers allowed presumptive diagnosis in the absence of plant inoculations for 40 strains tested, comprising 7 pathovars. RFLPs may allow taxonomic clarification of Xc pathovars named for the "host from which first isolated".

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XANTHOMONAS CAMPESTRIS PVS. CITRI, ALFALFAE AND PHASEOLI ARE GENETICALLY AND PATHOLOGICALLY RELATED. D. W. Gabriel, A. R. Burges, G. R. Lazo and R. Roffey. Plant Pathology Department, University of Florida, Gainesville, FL 32611.

Over 125 pathovars of X. campestris are recognized on the basis of the hosts from which they were first isolated. The recent Florida citrus canker outbreak was caused by at least nine pv. citri strains of unknown origin. Two cloned chromosomal fragments of pv. citri were used as probes against genomic digests of DNAs extracted from over 40 strains of nine pathovars. The probes revealed restriction fragment length polymorphisms that were pathovar-specific genetic markers for six of the pathovars. A seventh genetically distinct group included pvs. citri (Florida and A strains), alfalfae and phaseoli. When pv. citri strains from Florida were spray or syringe inoculated into bean leaves, growth kinetics and disease symptoms were indistinguishable from those caused by pvs. phaseoli and alfalfae. These results suggest that beans, alfalfa and citrus may be common hosts for the same pathovar.

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POSITIVE REGULATION OF CAPSULAR POLYSACCHARIDE SYNTHESIS IN ERWINIA STEWARTII. D. L. Coplin, R. D. Frederick, D. R. Majerczak, and P. J. Dolph, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Genes for capsular polysaccharide biosynthesis (cps), from Erwinia stewartii SS104 have been cloned in plasmid pES2144. Transcriptional and translational cps::lacZ gene fusions were constructed by Tn3HoHoI mutagenesis. The three cps gene clusters on pES2144 were expressed in wild-type strains but not in Cps^- avirulent mutant MU14110. In contrast, the galE gene on pES2144 was constitutive and expressed in MU14110. A 1 kb region of recombinant plasmid pRF121 complemented both MU14110 and an rcsA mutant of E. coli (rcsA is a strong positive regulator of capsule synthesis in E. coli). Conversely, a clone of the E. coli rcsA gene, pATC352, restored both Cps^+ and virulence to MU14110. Homology between pRF121 and pATC352 was not detected in Southern blots and pRF121 did not hybridize to E. coli genomic blots. Production of the E. stewartii rcsA product was autoregulated in a pRF121 rcsA::lacZ/rcsA E. coli strain and was not affected by rcsB or rcsC.

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INDUCIBILITY OF COPPER RESISTANCE IN PSEUDOMONAS SYRINGAE PV. TOMATO. D. A. Cooksey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

We have perviously isolated copper-resistant strains of Pseudomonas syringae pv. tomato that will grow in defined media containing 1.2-1.6 mM copper sulfate. Plasmids were identified in one strain (PT23) that conferred similar levels of resistance to copper when transferred to copper-sensitive P. syringae pv. syringae strains (J. Bacteriol. 165: 534-541). When copper sulfate (1.2 mM) was added to log-phase cultures of PT23 or the copper-resistant P. syringae pv. syringae transconjugants, the cultures entered an extended lag phase before the exponential growth rate was resumed. However, if cultures were previously grown in sub-inhibitory concentrations of copper sulfate (0.05-0.1 mM), the lag phase was reduced or eliminated. These results suggest that plasmid-determined copper resistance in PT23 is inducible by copper.

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THE BASIS OF THE COMPETITIVENESS EXPRESSED BY RHIZOBIUM LEGUMINOSARUM BV. TRIFOLII STRAIN T24. T. M. Barta, C. L. Jenkins, and E. W. Triplett, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Rhizobium leguminosarum bv. trifolii strain T24 induces nodules that are incapable of symbiotic nitrogen fixation, is very competitive for nodulation of clover roots, and constitutively produces a bacteriostatic compound that inhibits other Rhizobium strains in vitro (Arch. Mikrobiol. 64: 130-145). Transposon mutants of strain T24 lacking antibiotic production are less competitive than the wild-type strain. However, non-nodulating mutants of strain T24 which still produce the antibiotic do not inhibit nodulation by other strains in mixed inoculum. An E. coli cosmid clone bank of the strain T24 genome is being screened for the genes encoding antibiotic production and immunity. Although strain T24 carries three large indigenous plasmids, there is no evidence that the antibiotic genes are plasmid-borne. Isolation and characterization of the antibiotic is in progress. The antibiotic produced by strain T24 has been concentrated and partially purified by reverse phase chromatography.

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EFFECTIVENESS OF VARIOUS DISINFESTANTS IN ELIMINATING XANTHOMONAS CAMPESTRIS PV. CITRI FROM CITRUS BUDWOOD. G. C. Wisler, M. E. Meadows, and L. L. Breman. Division of Plant Industry, Gainesville, FL 32602.

As part of the effort to eradicate citrus canker in Florida, several budwood disinfestation treatments were evaluated. Budwood was cut from potted 'Swingle' citrumelo (Poncirus trifoliata X Citrus x paradisi) plants. Budsticks were placed for 30 minutes in a suspension of X. campestris p.v. citri (XCC) containing 10^6 colony forming units (cfu)/ml, blotted dry, and placed in disinfestant baths. After treatments, buds were cut from the sticks and all buds from each treatment were placed in 1.0 ml of tap water in a sterile centrifuge tube. Tubes were placed on a shaker for 30 min. All the liquid in each tube was plated on nutrient agar. Citric acid (0.1M, pH4.0 at 55C) for 10 min was the only treatment which resulted in 100% kill of XCC. Clorox (10.0% for 30 min) and Gallex 900 (0.2% for 10 min) were the next best treatments. The currently used treatment (0.02% Clorox for 10 min) was ineffective using an inoculum load of 10^6 cfu/ml.

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THE INFLUENCE OF OSMO-SENSITIVITY ON SEED AND ROOT COLONIZATION OF COTTON BY FLUORESCENT PSEUDOMONADS. W. Howie, T. Suslow, and N. Gutterson. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA.

Pseudomonas putida strain MK280 was treated with nitroso-guanidine to obtain mutants which were sensitive to an osmotic potential of - 10 bars (obtained by amending a minimal medium with NaCl, Na_2SO_4, KCl, or sorbitol). There were no significant differences between the seed populations of MK280 and its osmo-sensitive mutant (NP179) after drying bacterial suspensions onto cotton seeds in a laminar flow hood. Likewise, osmo-sensitivity did not correlate with colonization since populations of strain NP179 were not significantly different from strain MK280. P. fluorescens strain B10, which naturally is osmo-sentive, colonized seeds and roots as well as strain MK280 when cotton was grown in soil with low microbial activity, yet when cotton was grown in soil with high microbial activity its population was significantly lower than MK280.

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COMPUTER AIDED SPECTROPHOTOMETRIC MEASUREMENTS OF COLOR AND COLOR INFRARED TRANSPARENCIES OF TOMATO BACTERIAL LEAF SPOT. C. H. Blazquez, L. E. Hedley, A. J. Benary, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

Color and color infrared photographs of tomato leaves infected with Xanthomonas vesicatoria were scanned with a spectrophotometer at 10 nm intervals from 400-700 nm. A computer program was written in BASIC for an Apple II+ to input light as percent intensity and compute the spectral curves in either one or two intensity peaks. A sub-program tabulated intensity values and expressed results as integrals. Color transparencies of X. vesicatoria produced one spectral curve, while color infrared transparencies gave two peaks which were displayed as the ratio of the first to the second. Spectrophotometric measurements of disease stages were more accurate than visual interpretations of the transparencies.

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EFFECT OF CUT SURFACE AREA AND PUNCTURES ON THE SEVERITY OF BACTERIAL SOFT ROT OF POTATO SEEDPIECES. D. A. Fontem and J. A. Bartz, Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Freshly cut potato seedpieces with a varying number of cut surfaces or manually-induced punctures were evaluated for soft rot following 1-min immersion in suspensions of different concentrations of Erwinia carotovora pv. carotovora and 48 h mist-chamber incubation. The severity of soft rot, measured as percentage weight loss, was greatest in seedpieces with the most number of cut surfaces or punctures. No significant differences in disease severity were observed between punctures made through the cut surfaces and those made through the intact periderm. Storage of the seedpieces for 18, 24, or 48 h at 7 or 24 C prior to inoculation significantly reduced soft rotting as compared to immediate inoculation. The correlation between percentage weight loss and inoculum concentration was better fit to a power function than to a linear equation, indicating no minimum threshold level of inoculum for infection of freshly cut or wounded tubers.

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ANALYSIS OF RESISTANCE IN SUGAR BEET CULTIVAR C13 TO ERWINIA CAROTOVORA BETAVASCULORUM. E. D. Whitney and B. E. Mackey, ARS, USDA, 1636 E. Alisal St., Salinas, CA 93905 and 800 Buchanan St., Berkeley, CA 94710.

Two systems of resistance to Erwinia carotovora betavasculorum have been reported in sugar beet. A single dominat gene confers high resistance. Quantitatively inherited resistance has been reported but not analyzed. Twelve week old plants of C13 were cut in half. One half of each pair was tested for reaction to the pathogen and scored on a scale of 0 to 4. The corresponding halves were photo-thermally induced and pair crossed to produce full-sib seed. Forty plants within 33 full-sib families were injured-inoculated and scored for reaction of roots and foliage to E. carotovora betavasculorum on a scale of 0 to 4 and by days of survival to 3 months. Significant differences in disease reaction occurred among these populations. Analysis suggested at least three additive factors for resistance. Greater discrimination between families occurred for visual (0 to 4) ratings than for days of survival. A single dominant gene also was confirmed in this analysis.

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DEVELOPMENT OF STRAIN SPECIFIC ANTISERA FOR AGROBACTERIUM. Hacène Bouzar and Larry W. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Antisera (AS) were developed in New Zealand White rabbits against crude ribosomes of Agrobacterium strains K84, U11, B6, C58 and A323. These AS were tested by immunodiffusion against phenol extracts (PE) of 30 Agrobacterium strains, 4 Rhizobium strains, and 8 unrelated species. Each of these AS was strain specific, producing a single precipitin band with only the PE from the homologous strain. The presence or absence of plasmids in the homologous strain did not affect the reaction. Bare-rooted plants were inoculated with the biocontrol agent K84 and planted at two nursery sites. Antiserum to K84 was used to confirm the identity of K84 strains recovered after one growing season from roots and tumors of these plants. Because these strain specific AS can be used to rapidly and accurately identify agrobacteria introduced into the environment, they offer another alternative to antibiotic resistant strains which may be genetically impaired.

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APPLICATION OF MONOCLONAL ANTIBODIES IN THE IDENTIFICATION AND DETECTION OF *ERWINIA AMYLOVORA*. C. P. Lin, T. A. Chen, J. M. Wells, Plant Pathology Department and USDA, ARS, Rutgers University, NJAES, New Brunswick, NJ 08903 and T. van der Zwet, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

Monoclonal antibodies (MA) specific to *Erwinia amylovora* were used to identify and detect *E. amylovora* in bacterial cultures and infected apple fruits by indirect immunofluorescent staining. Pure cultures of bacterial isolates were air-dried and heat-fixed on slides or collected on polycarbonate membranes before application of MA and fluorescein isothiocyanate (FITC) conjugated anti-mouse immunoglobulin. Nine of the ten species specific antibodies reacted with the external antigens of *E. amylovora* to yield strong positive fluorescence and were further used in the *in situ* detection. Diseased apple fruit tissues were first freehand sectioned and then fixed with acetone before staining with MA. Positive fluorescent staining was easily observed under epifluorescent microscope in infected cells but not in healthy controls.

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DIFFERENTIATION OF SOFT ROT BACTERIA USING MONOCLONAL ANTIBODIES. M. J. Klopmeyer, R. S. Livingston and A. Kelman, Department of Plant Pathology, University of Wisconsin-Madison, 53706.

Monoclonal antibodies (MAbs) against the major extracellular endopectate lyases (PL) of Erwinia carotovora subsp. carotovora (Ecc) were used to differentiate other Erwinia spp., several pectolytic pseudomonads and a pectolytic fungus. Using a dot blot immunoassay, selected MAbs detected pectic enzyme preparations from Ecc, E. carotovora subsp. atroseptica (Eca) and some strains of E. chrysanthemi (Echr). However, no positive reactions were obtained with pectic enzyme preparations from a corn strain of Echr, several pectolytic pseudomonads and commercially available pectin lyase and polygalacturonase from Aspergillus japonicus. Polyclonal rabbit antiserum against purified Ecc

PL's reacted the same as the MAbs except that all soft-rotting Erwinia spp. that were tested gave a positive reaction.

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UNIQUE STRAINS OF XANTHOMONAS CAMPESTRIS PV. CITRI ASSOCIATED WITH MEXICAN CITRUS BACTERIOSIS. A.M. Alvarez, A.A. Benedict, and E.L. Civerolo, University of Hawaii, Honolulu, HI 96822 and USDA Fruit Laboratory, Beltsville, MD 20705

Since the initial outbreak of bacteriosis on Mexican lime in 1981, remarkably few cultures of the pathogen have been isolated. High populations of oxidative, yellow-pigmented bacteria prevented recovery of more fastidious xanthomonads associated with Mexican lime disease. An inhibition assay was developed using monoclonal antibodies (MCA) to identify Xanthomonas strains in infected leaf tissue, and additional strains were isolated on selective media. The recently recovered Xanthomonas strains showed MCA reactivity patterns identical to the serologically unique Mexican T-strains (T20-T24) recovered from earlier outbreaks. They were serologically unrelated to a Mexican strain, M3, or X. campestris pv. citri strains from other geographical origins.

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MONOCLONAL ANTIBODY ANALYSIS FOR MONITORING DISSEMINATION OF BLACK ROT OF CABBAGE. G. Y. Yuen, A. M. Alvarez, and A. A. Benedict. University of Hawaii, Honolulu, HI 96822.

The simultaneous spread of different strains of Xanthomonas campestris pv. campestris (Xcc) was monitored in cabbage fields using four monoclonal antibodies (MCA). Four strains of Xcc, which differed in their MCA reactivity patterns, were introduced into replicate field trials through infected transplants. The dissemination of each strain from primary inoculum sources was followed independently throughout the cabbage cropping period. To identify the pathogens, extracts of black rot lesions were spotted onto a semi-selective medium. Without further purification, resulting cultures of Xanthomonas were tested with MCA using indirect ELISA. Rates of spread and spatial patterns were found to differ between strains. In addition, the experimentally introduced strains were detected in lesions not recognized as black rot. Pathogenic and nonpathogenic xanthomonads having MCA patterns unlike those of the introduced strains also were identified.

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SELECTION OF PSEUDOMONAS SPP. INHIBITORY TO POTATO SEED TUBER DECAY. D.J. Rhodes, C. Logan* and D.C. Gross, Dept. of Plant Pathology, Wash. St. Univ., Pullman WA 99164-6430 and Dept. of Plant Pathology, Queen's Univ. of Belfast, N. Ireland BT9 5PX*.

A method was developed to select Pseudomonas strains which inhibited potato tuber rots caused by Erwinia carotovora subsp. atroseptica (Eca) and Fusarium sambucinum. Wells cut in tuber slices were inoculated with 10 µl of Pseudomonas (10^8 cfu/ml) combined with 10 µl of Eca (10^7 cfu/ml), Fusarium (1.5×10^6 conidia/ml), or a 1:1 (v/v) mixture. Rot was measured after 3 days of incubation at 20 C. To test the effectiveness of the assay in selecting Eca-inhibitory strains, pseudomonads in powder formulation were dusted onto seed pieces previously dipped in 10^8 cfu/ml Eca and planted in steamed soil. Rot was measured after 7 days at 15 C. A significant correlation (r= 0.64) was observed between the degree of suppression of Eca in tuber slice assays and on seed pieces in soil. Two strains selected by this assay reduced blackleg-affected stems by 44% in the field and gave better control of tuber decay, non-emergence and yield reduction than previously tested strains.

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RESISTANCE OF *XANTHOMONAS CAMPETRIS* PV. *MALVACEARUM* (XCM) TO THE COTTON PHYTOALEXIN 2,7-DIHYDROXYCADALENE (DHC) : POSSIBLE INVOLVEMENT OF REACTIVE OXYGEN SPECIES. Tzeli J. Sun and M. Essenberg, Dept. of Biochemistry, Oklahoma State University, Oklahoma Agricultural Experiment Station, Stillwater, OK 74078.

The phytoalexin DHC, produced when cotton is inoculated with incompatible races of Xcm, inhibits the growth of bacteria. Ten strains of Xcm, representing 5 races were tested for resistance to DHC. Race 1, Race 2, strain N, Race 4 and strain H were most resistant. Bacterial growth of more resistant strains exhibited a 1-2 hr lag before the onset of logarithmic growth. Race 3 is more sensitive to DHC than Races 1,2,4 and strians H, N. Two race 3 rif^R mutants, RS4, RS8 were very sensitive to DHC, both showing a 2-3 log killing during the first 4 hr of the bioassay. DHC is photoactivated to nick DNA, and oxygen is also involved in this activation. Known photodynamic reagents eosin(E) and methylene blue(MB) were used to select E^R, MB^R or EMB^R Xcm from RS4 and RS8, and a 280 to 2800-fold increase in the percentage of the resistant population toward DHC resulted. These compounds are known for producing singlet oxygen, superoxide and H_2O_2. When RS4, RS8 were raised in a Mn(II)-enriched nutrient broth and then bioassayed in the same medium a similar increase in resistance to DHC was observed. The results suggest the involvement of detoxification of active oxygen species in the bacterial resistance to DHC.

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SURVIVAL OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI IN BEAN STUBBLE AND DEBRIS WITH THREE TILLAGE SYSTEMS. R. L. Gilbertson, E. Carlson, R. E. Rand, D. J. Hagedorn and D. P. Maxwell, Department of Plant Pathology, University of Wisconsin, Madison, 53706.

Bean plants colonized by Xanthomonas campestris pv. phaseoli (XCP) in summer were either left no till, or disked or deep plowed. Bean stubble from each tillage treatment was collected in late October and monthly thereafter. Leaves, stems and pods were assayed for XCP on MXP medium, a semi-selective medium for XCP. XCP was recovered throughout the winter; population densities (PDs) declined as winter progressed. Leaves yielded more XCP than stems or pods, and PDs were greater from no-till than disked or plowed areas. Representative XCP colonies were tested for pathogenicity after each sampling date. Pathogenic cfus gradually declined until very low %s were found in March 1986.

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HYPERSENSITIVITY OF PEPPER TO XANTHOMONAS CAMPESTRIS PV. VESICATORIA ASSOCIATED WITH PARASITISM. R. E. Stall and G. V. Minsavage. Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611

Mutants that did not cause disease in pepper were selected from nitrosoguanidine-treated cells of race 2 of the pepper group of Xanthomonas campestris pv. vesicatoria. Even though the mutants were prototrophic they did not increase in number in pepper leaves and were labelled as nonparasitic. The mutants also did not cause a hypersensitive reaction in pepper plants with the Bs1 gene for resistance, which is not typical for cells of race 2. However, transconjugants of the nonparasitic mutants mated with a wild-type strain that was virulent in Bs1 plants were both parasitic in susceptible plants and avirulent in Bs1 plants. Thus, the avirulence gene was not altered in the mutants, but its phenotypic expression was affected by mutations that prevented population increases in pepper leaves.

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EFFICACY OF FIVE METHODS OF INOCULATING POTATO PLANTS WITH PSEUDOMONAS SOLANACEARUM. E.R. French, International Potato Center, Apartado 5969, Lima 100 - Peru.

Stem puncture (inserting a dissecting needle through a drop of inoculum into the axil of a leaf), soil infestation (irrigating 10 cm pots with 40 ml of inoculum), cutting a terminal leaflet with dissecting scissors dipped in inoculum, infiltrating a terminal leaflet with a needleless 5 ml disposable syringe with a perforated plunger from another syringe on its tip, and using carborundum (600 mesh) were compared utilizing 10^8 cfu of P. solanacearum/ml of water and ten plants per treatment. The experiment was done twice with temperatures averaging 25.3 and 27.6°C. After 12 days stem puncture and scissor-cut inoculated plants were all severely wilted, carborundum and infiltration were inconsistent, and soil infestation resulted in slower symptom development with only initial wilting in nine out of ten plants. The scissor-cut inoculation method was equally consistent to the stem puncture method, but it has the advantage of being a faster method to inoculate.

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CHARACTERIZATION OF STREPTOMYCETE-LIKE ISOLATES FROM POTATO TUBERS WITH SYMPTOMS OF COMMON SCAB. R. Loria, B. A. Kempter, and A. A. Jamieson, Dept. of Plant Pathology, Cornell Univ., Long Island Horticultural Research Laboratory, Riverhead NY 11901

Tubers with symptoms of common scab were collected from potato production areas in the Northeastern US and Canada. Streptomycete-like isolates were recovered from lesions, tested for pathogenicity on potato, and the physiological and morphological

characteristics of isolates were evaluated. Most of the pathogenic isolates had characteristics typical of Streptomyces scabies: spiral spore chains, gray aerial mass color, and the ability to produce melanoid pigments and utilize D-glucose, L-arabinose, D-fructose, D-mannitol, raffinose, rhamnose, sucrose and xylose. The remaining pathogenic isolates, which appear to be a different Streptomyces spp., had flexuous spore chains, pale orange-gray aerial mass color, and did not produce melanin or utilize raffinose. All nonpathogenic isolates could be distinguished from S. scabies based on spore chain type, aerial mass color, ability to produce melanoid pigments, or utilization of certain carbon sources.

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A PROCEDURE OF PEA BIOASSAY FOR VIRULENCE OF RHODOCOCCUS RUBROPERTINCTUS. S. H. Kim, PA Dept of Agr, Harrisburg, 17110.

Virulence of Rhodococcus rubropertinctus has commonly been determined by inoculating pea (Pisum sativum). Inconsistent results led to evaluation of several methods using isolates from geranium, hebe, impatiens, shasta daisy, and ATCC 12794 & 12795 representing virulent and avirulent R. rubropertinctus. The following procedure consistently induced a multishoot at the cotylendonal node by the virulent isolates. 'Alaska' peas were surface sterilized with 70% ethanol for 1 min, then 10% Clorox for 10 min, and rinsed 3X with sterile distilled water (SDW). The treated seeds were then soaked in SDW for 3 hr. The turgid seeds were germinated in 1.5% water-agar plates for 3 days, 26C. The seedlings were soaked for 2 hr in a 3 day-old inoculum grown in an agitated liquid medium composed of 7g K_2HPO_4, 3g KH_2PO_4, 0.1g $MgSO_4 \cdot 7H_2O$, 10g sucrose, 2g L-asparagine and 0.01g thiamine HCl per liter. The inoculated seedlings were transferred to individual test tubes containing Hoagland's solution with 0.5% agar, and incubated with a 12 hr light/dark cycle at 20C for 7 days to induce macroscopic multishoot.

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THE USE OF DRY INOCULUM OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI FOR TESTING FOR COMMON BLIGHT RESISTANCE AND XANTHOMONAS COLONIZATION OF BEAN CULTIVARS.
R. L. Gilbertson, E. Carlson, R. E. Rand, D. J. Hagedorn and D. P. Maxwell, Department of Plant Pathology, University of Wisconsin, Madison, 53706.

Dry inoculum of Xanthomonas campestris pv. phaseoli (XCP) was prepared from infected bean leaves from field or greenhouse, dried, ground into a powder and stored at 4 C. Inoculum concentration was 109-1010 cfu/gram. Beans in field plots were inoculated at planting with a liquid slurry prepared from dry inoculum, or foliage was dusted with inoculum after wounding with carborundum. Common blight developed with all inoculum treatments and disease severities were similar to inoculation with liquid XCP suspensions. XCP colonized primary trifoliolate leaves and pods; controls were not colonized. Colonization of susceptible 'Topcrop' was greater than for tolerant 'Harris'. Use of dry inoculum was an efficient technique for evaluation for common blight resistance.

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MICROCENTRIFUGE SAP EXTRACTION FOR RATOON STUNTING DISEASE ASSAYS.
S.J. Kostka[1], P.W. Reeser[1], and A.G. Gillaspie, Jr.[2], [1]Crop Genetics International, Hanover, MD 21076 and [2]ARS, U.S. Department of Agriculture, Beltsville, MD 20705.

A microcentrifuge-based method was developed for large scale seedcane indexing for Clavibacter xyli subsp. xyli (Cxx), the causal agent of ratoon stunting disease. Cores (5 x 20 mm) were removed parallel to the stalk axis from the basal internode, placed in an extraction apparatus (a 0.5 ml microfuge tube with cap and base removed inserted in a 1.5 ml microfuge tube), then centrifuged for 1 min at 10,000 x g. Sap yields were approximately 100µl per core. High speed microcentrifugation was compared to low speed centrifugation (1000 x g; 50 ml conical tubes; 15 x 100 mm samples) for cell recovery from Cxx-infected, greenhouse grown CP44-101, CP53-1 and CP65-357. Cxx concencentrations, determined using a Petroff-Hauser counting chamber and phase-contrast microscopy, were 6-fold higher from low speed-extracted sections than from high speed-extracted cores. Reduced cell recovery is offset by improved handling efficiency and increased sample capacity.

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DISTRIBUTION OF RATOON STUNTING DISEASE IN THE LOUISIANA SUGARCANE RELEASE PROGRAM.
K. E. Damann, Jr., Dept. of Plant Path. and Crop Physiol., La. Agri. Expt. Sta., La. State Univ., Agric. Ctr., Baton Rouge, LA 70803.

Plantings of sugarcane cultivar CP 76-331, for release to Louisiana growers, were assayed for ratoon stunting disease (RSD) by alkaline-induced metaxylem autofluorescence. Plots in the eastern and western areas were the culmination of 14 years of selection and increase from a common source. The frequency of diseased stalks assayed in plant cane (1984) and ratoon cane (1985) was 0% and 3% in the eastern area and 15% and 33% in the western area, respectively. The RSD increase from plant cane to ratoon crops was probably due to mechanical spread since RSD has no known vector. The higher frequency of RSD in the western area was probably the result of infections from a contaminated harvester in the seed increase process. Since much less RSD was found in the eastern area, the initial infections probably occurred at a later time or with less frequency than in the western area and resulted in the asymmetric distribution of RSD between the areas.

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EFFECT OF TEMPERATURE ON SYSTEMIC SPREAD OF TAN SPOT OF SOYBEAN FROM SEED TO UNIFOLIATE LEAVES.
J. M. Dunleavy, U.S. Dept. Agriculture, ARS, 417 Bessey Hall, Iowa State University, Ames, IA 50011.

Curtobacterium flaccumfaciens pv. flaccumfaciens the cause of bacterial tan spot of soybean, is seed transmitted, but has not been observed to move systemically in plants. One hundred seeds, that had been exposed to natural infection, of each of four resistant and four susceptible soybean cultivars were surface-sterilized, sown in sterile soil, and placed in growth chambers at 10, 15, 20, 25, 30, and 35 C. Bacterial tan spot lesions occurred on unifoliate leaves of some plants of all susceptible cultivars at 25 C, and all but one, at 30 C. They also formed on leaves of some plants of all but one of the resistant cultivars at both 25 and 30 C. Lesions did not form on leaves at lower and higher temperatures. Mean percentages of susceptible plants with lesions were 13.5% at 25 C and 7.0% at 30 C, contrasted to 5.5% at 25 C and 2.5% at 30 C for resistant plants.

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EFFECT OF DISEASE ONSET AND SEVERITY OF STEWART'S AND GOSS' WILT ON SWEET CORN.
Suparyono and J. K. Pataky. Department of Plant Pathology, Univeristy of Illinois, Urbana, IL 61801.

Plant height and number of marketable ears of sweet corn were affected severely by Stewart's wilt (Erwinia stewartii [Es]) and Goss' wilt (Corynebacterium michiganense pv. nebraskense [Cmn]). When plants were inoculated with Es at the 3-5 leaf stage, disease severity was 72, 31 and 25%; plant height was reduced 19, 7 and 1%; and, marketable ears were reduced 34, 4 and 4% for Jubilee (susceptible), Gold Cup (moderate) and Miracle (resistant), respectively. When plants were inoculated at the 3-5 leaf stage with Cmn, disease severity was 63, 46 and 23%, plant height was reduced 17, 8 and 5%; and marketable ears were reduced 25, 8 and 0% for Jubilee, Gold Cup and Miracle, respectively. Disease severity, plant height and marketable ears were affected slightly for Gold Cup and Miracle when plants were inoculated at 5-7 and 8-10 leaf stages. Marketable ears from Jubilee were decreased by 16 and 14% when inoculated with Es and by 5 and 0% when inoculated with Cmn at 5-7 and 8-10 leaf stages, respectively.

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SPLASH DISPERSAL OF PHYTOPHTHORA CACTORUM WITHIN A STRAWBERRY CANOPY BY SIMULATED RAIN. K. M. Reynolds, M. A. Bulger, L. V. Madden, and M. A. Ellis, Department of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Splash dispersal of propagules of Phytophthora cactorum was investigated using simulated rain. Drop volume distributions produced by low, medium, and high volume spray nozzles were very similar to drop volume distributions observed for natural rainfall intensities of 15, 30, and 60 mm/hr, respectively. Fruit infection within two concentric circles of strawberry plants was evaluated to determine effects of rainfall intensity, plant distance from a point source of inoculum, presence of plant barriers or straw mulch, and fruit elevation on successful dispersal of inoculum. The effect of rainfall intensity on dispersal was strongly influenced by the use of straw mulch (even in the absence of soil-borne inoculum), and whether or not fruit were touching the ground. Canopy foliage acted as an effective barrier to dispersal of inoculum-bearing splash droplets.

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RELATIONSHIP OF WOUND-INDUCED PEROXIDASE ACTIVITY TO EPICARP LESION DEVELOPMENT IN PISTACHIO (PISTACIA VERA L.) FRUIT. R. M. Bostock, C. S. Thomas, J. M. Ogawa, R. E. Rice, and J. K. Uyemoto. Departments of Plant Pathology and Entomology, University of California, Davis, CA 95616.

Needle-puncture through the pericarp of maturing pistachio fruit induced symptoms similar to those observed in fruit with epicarp lesion (EL) caused by insect feeding. Mechanical or insect feeding wounds through the pericarp markedly stimulated peroxidase activity (PO) in tissues of 'Kerman' and 'Trabonella' fruit. The induced PO was histochemically detected within 24 hr of wounding, prior to symptom development and at a distance several mm from the wound site. Penetration of the inner surface of the endocarp was essential for stimulation of PO and lesion development. Both events were inducible during April and May but not after the onset of endocarp lignification. Gallotannins, the principal pericarp phenols and components readily oxidized by peroxidases, declined early in the season. Wounding induced at least three cathodic isoperoxidases not present in unwounded fruit.

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EVALUATION OF SACCHARUM GENOTYPES FOR RESISTANCE TO USTILAGO SCITAMINEA. Benigno Villalon, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596

Thirty-four sugarcane breeding lines were evaluated for resistance to smut over a 3-year period by inoculating seed pieces. Percent smut ranged from 3 to 63 in the 3rd-year crop. Thirty-three of 34 lines exhibited smut whips. One line, Mex-3, remained symptomless and was categorized as resistant, 2 as tolerant, 7 as intermediate and 25 as susceptible. Significant differences occurred between infected and healthy stools in stalk weight, diameter and number of stalks per stools. Overall yield of all lines varied from 40.9-84.8 kg through the 3-year test. The highest yielding NCo 310 exhibited 37% infected stools was rated smut susceptible. Five lines receiving a rating of tolerant or intermediate were below average yielders. The top seven yielding lines were rated susceptible with high percent infected stools. Line TCP 81-3066 yielded well despite having the highest percent smut infected stools (83%).

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PATHOGENICITY OF A FUSARIUM SP. ISOLATED FROM SOYBEAN PLANTS WITH SUDDEN DEATH SYNDROME. J. C. Rupe and G. J. Weidemann, University of Arkansas, Dept. of Plant Pathology, Fayetteville, AR 72701.

In 1985, extensive isolations of fungi were made from roots of soybean plants with sudden death syndrome (SDS). A slow growing Fusarium sp. was the most frequently isolated fungus from SDS affected plants collected at two fields. It was also isolated from plants grown in the greenhouse in naturally infested soil from a third field. This Fusarium sp. produces macroconidia which range from 30-65 X 6-8 um and have 3-5 septa. No microconidia are produced in culture and chlamydospores form slowly in sterile water. On potato dextrose agar, fungal colonies are appressed, cream colored later turning blue and stain the agar reddish-brown to brown. The latter color varies in intensity. Pathogenicity was tested with cultivars McCall and Lee 74 at 24¢ with 12 hr photoperiod. Foliar symptoms developed within one m month and were identical to those of adjacent plants growing in naturally infested field soil. The root systems were limited and discolored with dark reddish-brown lesions forming at and below the soil line.

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BIOLOGICAL SPECIES OF ARMILLARIA MELLEA ISOLATED FROM SOUR CHERRY IN MICHIGAN. Tyre J. Proffer, A.L. Jones, and G.R. Ehret. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, 48824-1312.

A root rot caused by Armillaria mellea is responsible for the death of Montmorency sour cherries growing on sandy soils in western Michigan. In the autumn of 1985, 112 basidiocarps of A. mellea were collected from 65 sour cherry trees in 20 orchards. Single basidiospore cultures were established. A total of 930 isolates were obtained. An additional 133 isolates were collected from sweet cherry, peach, apple, and oak. Based on isolate pairings, three biological species of A. mellea were recovered from sour cherry. These biological species correspond to North American Biological Species I, III, and VI. Field observations indicated that biological species VI produced basidiocarps some weeks before the other biological species and was also found on oak. Biological species I was recovered most frequently and was also isolated from sweet cherry and peach.

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THE BIOLOGICAL SPECIES OF PHYTOPHTHORA MEGASPERMA FOUND IN ALFALFA FIELDS. E.M. Hansen and P.B. Hamm, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331

Three distinct forms of P. megasperma have been recovered from alfalfa fields in North America and Japan. They differ in protein electrophoretic profiles, pathogenicity, karyotype, and morphology; they apparently represent reproductively isolated populations. One form, ALF, (=P. megasperma f.sp. medicaginis) is strongly pathogenic only on alfalfa, has n=12-15 chromosomes, and small oospores. The second form, DF, causes only slight damage to alfalfa upon inoculation, but it is strongly pathogenic to Douglas-fir and to soybean in stem inoculations. DF isolates have 17-24 chromosomes and small oospores. The last form, BHR, are moderately pathogenic to alfalfa and Douglas-fir. They have 26-34 chromosomes and large oospores. The common association of two and occasionally three of these forms in alfalfa fields raises important questions about using forma specialis designations, the role of agricultural commerce in pathogen distribution, and the process of speciation in Phytophthora.

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ANALYSIS OF RACE-SPECIFIC VARIATION IN FUSARIUM OXYSPORUM F. SP. PISI. J. B. Mullen, D. J. Hagedorn and S. A. Leong. Department of Plant Pathology, USDA-ARS, University of Wisconsin-Madison.

Fusarium oxysporum f. sp. pisi causes wilt of pea (Pisum sativum). Four pathogenic races (1,2,5, and 6) are identified by inoculation of host cultivar differentials. We are examining restriction fragment length polymorphisms (RFLPs) in the mitochondrial and nuclear DNA within and between these races. RFLPs were identified by comparing mitochondrial DNA restriction endonuclease digestions and by probing nuclear DNA restriction endonuclease digestions with cloned nuclear DNA fragments from a race 2 isolate. Data will be presented on the variability found.

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Cell wall regeneration does not affect the sensitivity of Neurospora protoplasts to cercosporin. K. D. Gwinn and M. E. Daub. North Carolina State University. Raleigh NC. 27695

Cercospora species produce cercosporin, a toxin which does not affect their growth, but does inhibit growth of Neurospora crassa. Protoplasts from C. nicotianae and N. crassa were equally sensitive to the the toxin. Cell wall glucan production as evidenced by the binding of Tinopal 5BM was identical in both fungi. In C. nicotianae, production of

glucans was correlated with increased resistance to cercosporin, but this was not true for N. crassa. Patterns of binding of Con A suggest that mannose production is concurrent in these fungi. Regenerating C. nicotianae protoplasts bound both wheat germ and Bandeiraea simplicifolia agglutinins after shorter incubation periods than did N. crassa protoplasts, indicating differences in the timing of chitin synthesis in the two fungi.

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THE RELATIONSHIP BETWEEN LATE LEAFSPOT SEVERITY AND DEFOLIATION IN THREE PEANUT CULTIGENS. G. R. Watson, T. A. Kucharek, F. M. Shokes, and D. W. Gorbet, Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL, NFREC, Quincy, FL, and Dept. of Agronomy, AREC, Marianna, FL.

Two cultigens, Southern Runner (SR) and UF 81206 (206), that express resistance to late peanut leafspot, caused by Cercosporidium personatum, were compared to the cultigen Florunner (FLO) in the field for an association between leafspot severity and defoliation. Significant positive correlations existed between late leafspot severity and defoliation within the top canopy for the last 3 assessment dates for all 3 cultigens. Within the lowest canopy region such a correlation occurred for 4 of 6 assessment dates on FLO and only 1 of 6 assessment dates on SR and did not occur on 206. Statistical comparisons of regression coefficents showed more defoliation occurred at lower late leafspot severities with SR and 206 when compared to FLO. Defoliation in lower canopy regions of SR and 206 may be due to natural leaf abscision caused by higher amounts of leaf area in the top canopy region and thus not be related to leafspot resistance.

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FACTORS AFFECTING DEVELOPMENT OF BLUEBERRY STEM BLIGHT CAUSED BY BOTRYOSPHAERIA DOTHIDEA. T.C. Creswell and R.D. Milholland. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Effects of host tissue maturity, inoculum density and isolate virulence on development of blueberry stem blight was determined. Lesion extension was measured weekly after inoculation with five isolates of Botryosphaeria dothidea on one rabbiteye and three highbush blueberry cultivars. Differences were detected among cultivars in degree of susceptibility to the pathogen and among isolates in virulence on individual cultivars. Length and rate of development of lesions was greater on succulent than woody stems. Wounding stems beneath a droplet of conidial suspension allowed inoculum uptake by the plant's vascular system and proved to be an effective inoculation method. Inoculations using 1,000 conidia per stem resulted in fewer and shorter lesions than inoculations using 5,000 or 10,000 conidia per stem. Lesion length and number were similar for inoculations using 5,000 or 10,000 conidia per stem.

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PRODUCTION OF HYDROXAMATE SIDEROPHORES BY VERTICILLIUM DAHLIAE AND STEMPHYLIUM BOTRYOSUM. Shulamit Manulis[1], Rachel Zion[1], Yoel Kashman[2] and Isaach Barash[1], Departments of Botany[1] and Chemistry[2], Tel Aviv University, Tel Aviv, Israel.

Various isolates of V. dahliae and S. botryosum f. sp. lycopersici have been found to secrete at least 3 hydroxamate siderophores. Two of the major siderophores produced by each of these pathogens were identified as coprogen B and dimerum acid by means of comparative spectroscopy (H-NMR and IR) and chromatography with authentic compounds. The secretion of siderophores in both fungi was completely repressed in the presence of 1.8 µM iron. In V. dahliae a direct correlation was established between increase in pH from 4.5 to 7.5 and the rate of siderophores secretion. However, the amount of coprogen B was inversely related to elevation of pH as opposed to increase in dimerum acid. The siderophore-mediated iron transport in S. botryosum has been characterized and will be described. It was found that the coprogen B-iron complex is transported intact into the cell and accumulated presumably for storage purposes.

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ANALYSIS OF ALCOHOL DEHYDROGENASE LEVELS DURING RHIZOMORPH DIFFERENTIATION OF ARMILLARIA MELLEA. S. D. Cohen and J. J. Motta, Department of Botany, University of Maryland, College Park, Maryland 20742.

An external chemical signal, ethanol, stimulates a programmed sequence of events culminating in rhizomorph differentiation of Armillaria mellea. Since ethanol induces this morphological change, it is suggested that alcohol dehydrogenase (ADH) is involved in differentiation. Enzyme activity remained low in mycelia (4 µmol/min/mg d.w.) and the first two stages of rhizomorph development (3 µmol/min/mg d.w.) after alcohol induction. During rhizomorph initiation and elongation stages, enzyme activity was significantly higher (331 µmol/min/mg d.w.) than in mycelia (97 µmol/min/mg d.w.). An inhibitor of the enzyme (ADH), pyrazole (170 mM), effectively blocked rhizomorph development but not mycelial growth. Several ADH isozymes were detected by gel electrophoresis in the rhizomorph elongation stage. These data suggest that ethanol metabolism may be important in rhizomorph differentiation.

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PATHOGENICITY AND IDENTIFICATION OF SOME ALASKAN ISOLATES OF ARMILLARIA. C. G. Shaw III and E. M. Loopstra. USDA Forest Service, Forestry Sciences Laboratory, Pacific Northwest Forest and Range Experiment Station, P.O. Box 909, Juneau, AK 99801.

Nine isolates of Armillaria spp. from somatic mushroom tissues, spores, or decaying wood were inoculated onto Sitka spruce seedlings (10 per isolate), which were inspected after 2 and 3 years for Armillaria infections. Isolates were also tested in vitro for compatibility with monosporous (haploid) isolates of known North American Biological Species (NABS) of Armillaria. One set of two single-spore isolates and their parent mushroom belonged to NABS V; another such set belonged to NABS IX. Surprisingly, single-spore isolates of both NABS V & IX infected substantially more seedlings (x = 65% and 40%, respectively) than did the parent mushroom isolates (10% and 0%, respectively). The three isolates from wood infected $\geq$ 50% of the inoculated seedlings. Compatibility of these isolates with known NABS could not be verified, possibly due to difficulties with assessing compatibility reactions between diploid and haploid isolates or, perhaps, because these isolates are new NABS.

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SEROLOGICAL DETECTION OF PHOMOPSIS LONGICOLLA IN SOYBEAN SEEDS. M. L. Gleason, S. A. Ghabrial and R. S. Ferriss. Plant Path., Seed & Weed Sciences, Iowa State University, Ames, IA 50011 and Dept. of Plant Path., University of Kentucky, Lexington, KY 40546.

Antisera to mycelium of Phomopsis longicolla were used in indirect ELISA and a modified immunoblot assay for detecting seedborne infections. A seed immunoblot assay (SIBA) was used to overcome nonspecific interference from seed extracts in indirect ELISA. In SIBA, mycelium of P. longicolla growing onto nitrocellulose paper from infected soybean seeds produced a colored blotch after the paper was assayed. Results of SIBA for incidence of P. longicolla and Diaporthe phaseolorum v. sojae in halved seeds from ten seed lots correlated significantly ($P<0.001$) with agar plate bioassay of corresponding seed halves, but did not correlate with incidence of symptomatic seeds. Indirect ELISA absorbance values for bulked samples of seed coat halves from the same ten seed lots correlated weakly ($0.10>P>0.05$) with agar plate bioassay, but correlated strongly ($P<0.01$) with incidence of symptomatic seeds. Because the two serological assays apparently measure different aspects of the disease, both may be useful in evaluating soybean seed lot quality.

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PATHOTYPES OF PSEUDOPERONOSPORA CUBENSIS. C. E. Thomas, T. Inaba, and Y. Cohen. USDA, ARS, U. S. Vegetable Laboratory, Charleston, SC 29407; National Institute of Agro-Environmental Sciences, Tsukuba, Japan; and Dept. of Life Sciences, Bar-Ilan Univ., Ramat-Gan, Israel.

Host specificities of local isolates of the cucurbit downy mildew pathogen, Pseudoperonospora cubensis, were determined through controlled inoculations of 26 cultivars representing 13 species within seven genera of the family Cucurbitaceae in Israel, Japan, and the USA. Five pathotypes could be distinguished based on high compatibility with specific hosts. All were highly pathogenic to Cucumis sativus and C. melo var. reticulatus and pathotype 1 was limited to them. Pathotype 2 was limited to these hosts and C. melo var. conomon. Pathotype 3 was limited to these three species and subspecies plus C. melo var. acidulus. Pathotype 4 was pathogenic to all of the aforementioned hosts plus Citrullus lanatus. Pathotype 5 was pathogenic to all hosts mentioned above plus Cucurbita spp. Research supported by BARD.

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EFFECT OF TEMPERATURE ON GROWTH OF DIAPORTHE/PHOMOPSIS ISOLATES FROM SUNFLOWER. S. M. Yang, USDA-ARS, P.O. Drawer 10, Bushland, TX 79012, L. L. Black and G. E. Holcomb, Dept. Plant Pathology, Louisiana State University, Baton Rouge, LA 70803

Temperature-growth studies were conducted on 19 isolates of the sunflower stem pathogen Diaporthe/Phomopsis. These isolates were obtained from Minnesota (2 from J. Baumer), North Dakota (3 from T. Gulya), Ohio (2 from P. Lipps), Texas (3 from S. Yang), and Yugoslavia (4 from M. Acemovic, 2 from A. Maric, and 3 isolated by S. Yang from Yugoslavia-grown sunflower). Growth of the 19 fungal isolates was determined over a temperature range of 5-35C at five degree intervals. All isolates grew at 5C and 35C except one from Ohio which did not grow at 35C. Best mycelial growth occurred between 20-25C for 12 isolates and between 20-30C for 8 isolates. Mycelium color at 25C was white for 6 isolates, creamy yellow-white for 4 isolates, gray-white for 4 isolates, fluffy gray-white for 4 isolates, and brown for 1 isolate. This variation among the 19 fungal isolates suggests that they may belong to more than one biotype or species.

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BOTRYODIPLODIA STEM LESIONS IN TEXAS CITRUS. R. M. Davis. Texas A&I Univ. Citrus Center, P.O. Box 1150, Weslaco, TX 78596.

Two of 12 isolates of Botryodiplodia theobromae cultured from trunk lesions on young citrus trees were pathogenic on grapefruit and sour orange seedlings. Troyer citrange seedlings were resistant to infection while symptom expression was intermediate in sweet orange seedlings. In inoculated field trees lesions advanced downward in grapefruit scions but stopped at the bud union with the sour orange rootstock. Lesion development was arrested in trees treated with benomyl. To determine the relative ability of the 12 isolates to decay citrus wood, sterilized blocks were inoculated, incubated for 16 weeks, and weighed. One of the two pathogenic isolates reduced the weights of the blocks by 12.5%; all other isolates reduced the weights of the blocks by no more than 4%. Some characteristics of cultures of the two pathogenic isolates were distinguishable from those of the other isolates.

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LIFE CYCLE OF A SPIREA LEAF SPOT PATHOGEN Margaret A. Williamson, Institute of Ecology, University of Georgia, Athens, GA 30602, Ernest C. Bernard, and Charles H. Hadden, Dept. of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37901

Cylindrosporium filipendulae was isolated from diseased Spiraea x vanhouttei for the first time in Tennessee and proven to be the cause of the leaf spot disease. Numerous apothecia observed on overwintered S. x vanhouttei leaves were determined to be the perfect state of C. filipendulae by cultural methods. Conidia produced in culture by ascospore isolates infected spirea leaves and induced symptoms identical to those caused by C. filipendulae. The newly discovered teleomorph could provide primary inoculum.

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SPHAEROTHECA FULIGINEA SEXUAL REPRODUCTION ON CUCURBITS IN ISRAEL. Yigal Cohen and Helena Eyal, Life Sciences Department, Bar-Ilan University, Ramat-Gan 52100, Israel

Cleistothecia of S. fuliginea (Schlecht. ex Fr.) Poll., a causal agent of powdery mildew in cucurbits, found on mildewed muskmelon (Cucumis melo L. cv. Ananas-Yokneam) plants in the greenhouse in January 1983 were identified to belong to race 1 of the fungus (Phytoparasitica 11:216,1983). Spontaneous reoccurrence of cleistothecia on muskmelons of race 2 of the fungus was observed in January 1986 in growth chambers. At 14-17°C, cleistothecial production was abundant on muskmelon cotyledons (81%) and moderate on cucumber cotyledons (31%), whereas at 22-25°C, no cleistothecia were produced on either host. Cleistothecia were brown, round (about 100 um), bore simple melanized appendages and contained a single ascus with 8 ascospores. Ascospores inoculation tests are currently being conducted.
Research supported by BARD Project US 752-84C.

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PATHOGENICITY OF GLOEOCERCOSPORA SORGHI TO SORGHUM ROOT TISSUE. J. C. Borbón Reyes and L. E. Trevathan, Dept. of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS.

The frequency of Gloeocercospora sorghi in root tissue of sorghum seedlings, and the response of sorghum growing in soil infested with sclerotia under shadehouse conditions, were determined in a two-year study. Soil infestations were made with 1, 10, 25, 50, and 100 sclerotia/g of soil. Plant height was measured weekly for 5 wks. Top and root fresh and dry weights were made on plants harvested at the end of the growing period. G. sorghi was isolated on potato dextrose agar from surface sterilized symptomatic root tissue and crown cross sections. The fungus was isolated from an average of 70% of the plants growing in infested soil. Sorghum growth was reduced when as few as 10 sclerotia/g were incorporated into the soil. Growth reduction occurred for at least 5 wks during culture under shadehouse conditions. G. sorghi also caused pre- and post-emergence damping-off of sorghum seedlings.

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Deposition efficiency of Venturia inaequalis ascospores. Jonathan D. Kaplan and William E. MacHardy, Dept. of Botany and Plant Pathology, University of New Hampshire, Durham, NH 03824.

The deposition efficiency of airborne ascospores of Venturia inaequalis was examined in a glasshouse experiment under light wind conditions produced by a portable fan. Two volumetric spore traps were placed at 0.9, 1.8, and 2.7 m from an inoculum source of approximately 200 heavily scabbed leaves. A McIntosh seedling was placed just below the intake of each spore trap, and one 22 x 22 mm glass coverslip was affixed to the upper surface of each of two leaves. Area dose (spores cm^{-2}) was calculated for each spore trap location. The deposition efficiency was calculated by dividing the number of spores deposited cm^{-2} by the area dose. Deposition efficiency was low, with a mean of 3.8, 2.5, and 0.8% at wind speeds of 0.23, 0.45, and 0.89 m s^{-1}, respectively. The small proportion of airborne ascospores deposited may explain the low incidence of primary scab lesions recorded on unprotected tissue in an orchard with a large source of inoculum.

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INFLUENCE OF TEMPERATURE, INOCULUM DENSITY, AND CULTIVAR SUSCEPTIBILITY ON THE INFECTION OF SOYBEAN SEEDLINGS BY SOUTHERN Diaporthe phaseolorum. R.C. Ploetz, and F.M. Shokes. Route 3, Box 4370, Quincy, FL 32351.

The influences of temperature, inoculum density, and cultivar susceptibility on the infection of soybean seedlings by ascospores and α-conidia of the fungus causing soybean stem canker in the southeastern United States (southern Diaporthe phaseolorum) were studied under high moisture conditions (free water maintained on plant surfaces for 48 hr). Maximum levels of infection occurred at 28 and 34 C; lower levels of infection occurred at 10, 16, and 22 C, and infection did not occur at 40 C. Frequency of infection and $\log_{10}$ inoculum density were positively correlated (r^2=0.97) within a range of 1 X 10^3 to 1 X 10^6 spores per mL. No relationship was found between the field susceptibility or resistance of a given cultivar to stem canker and the frequency with which a cultivar was infected. Events responsible for resistance in soybean to stem canker apparently occur after infection has taken place.

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ESTIMATING INFECTION EFFICIENCY OF PLASMOPARA VITICOLA ON GRAPE. **N. Lalancette, M. A. Ellis and L. V. Madden, Plant Pathology Dept., The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691**

Infection efficiency, the ratio between the number of lesions produced and the number of spores applied, provides an overall measure of the germination, penetration, and colonization subphases of the infection cycle. To estimate the number of sporangia of P. viticola applied to a given grape leaf, a relationship was developed between the leaf area and the volume of inoculum that it can retain without runoff. Using linear regression, leaf area described 93% of the variation in the volume of liquid retained by the leaf. Three groups of five plants each were inoculated with suspensions at 10, 100, and 1000 sporangia/ml, one suspension per group. Given the inoculum concentration, the volume of inoculum applied, a count of the number of lesions per leaf, and an estimate of the number of zoospores produced per sporangium, the infection efficiencies were 0.025, 0.061, and 0.055 for each of the three plant groups, respectively.

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THE EFFECTS OF PRUNING ON INFECTION OF APPLE FRUIT BY ZYGOPHIALA JAMAICENSIS AND GLOEODES POMIGENA. C. M. Ocamb-Basu, T. B. Sutton, and L. A. Nelson, Departments of Plant Pathology and Statistics, North Carolina State University, Raleigh, NC 27695-7616.

The influence of tree pruning on disease development of Zygophiala jamaicensis (causal agent of flyspeck) and Gloeodes pomigena (causal agent of sooty blotch) on apple fruit was investigated in unsprayed blocks within commercial orchards and in abandoned orchards from 1983-1985. The severity of sooty blotch and flyspeck was less in pruned trees than in unpruned trees within commercial orchards. Sooty blotch also was less severe in pruned trees in abandoned orchards but there was either no difference in flyspeck severity or flyspeck was more severe in pruned trees in abandoned orchards. Pruning effects were greatest during the wet growing seasons of 1984 and 1985. Fruit position and height within the canopy had a significant effect on sooty blotch and flyspeck.

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EFFECT OF CULTIVAR, PLANT AGE AND LEAF POSITION ON THE EXPANSION RATE OF EARLY BLIGHT LESIONS ON POTATO. J.R. Pelletier and W.E. Fry. Department of Plant Pathology, Cornell University. Ithaca, NY. 14853.

Plants in field plots of Kennebec, Norchip and Rosa potatoes were inoculated 35, 47, and 62 days after planting with isolate 4R of Alternaria solani. Leaves were marked and photographed at 2- to 7-day intervals for up to 33 days after inoculation. Lesion areas on the photographs were determined by computer image analysis. Lesions expanded most rapidly on the lower leaves in all three cultivars and inoculation dates. Expansion rates for the first inoculation on Rosa were markedly slower than those on Kennebec or Norchip. Expansion rates were not significantly different between cultivars for the second and third inoculations.

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THE SPATIAL PATTERN OF PHYTOPHTHORA ROOT ROT IN A GINSENG CROP IN WISCONSIN. M. K. Rahimian and S. S. Adams, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The spatial pattern of ginseng root rot, caused by Phytophthora cactorum was assessed in a 3-year-old garden. The garden was divided into 6400 of 1.5 m^2 cells (alleys not included). Each cell contained about 200 plants. The disease incidence in each cell was recorded five times during the growing season. The contiguous cells were grouped into quadrats of 37.5 m2 to 150 m2 sizes. Early in the season, Morisita's index of dispersion was 4.81 and 2.30 for 37.5 m2 quadrat sizes, respectively. Late in the season, this index was 3.16 and 1.90 for 37.5 m^2 and 150 m^2 quadrat sizes, respectively. The F statistic of the index was significant at $P < 0.005$ for all quadrat sizes and all five ratings during the growing season indicating that the disease in the garden had an aggregated dispersion pattern.

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FACTORS AFFECTING EPIDEMICS OF SOUTHERN BLIGHT OF PROCESSING CARROT IN NORTH CAROLINA. V. L. Smith, S. F. Jenkins, and D. M. Benson, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695-7616

The effect of air temperature (AT), soil temperature, relative humidity (RH) and moisture on epidemics of southern blight of processing carrot, caused by Sclerotium rolfsii, was determined. Two to 10 disease foci were established within rows, using colonized oat grains, such that maximum interfocus distance was achieved. Soil temperature 2 cm below the soil surface was monitored with thermistors, and RH and AT near the soil surface was determined with aspirated wet bulb-dry bulb thermistors. Disease severity was evaluated twice weekly, by measuring the length of diseased area along the rows. Incremental increase in disease severity was influenced by fluctuating RH > 95 % in the interval 3-4 days prior to a disease rating. Disease severity, measured by area under disease progress curves, was significantly affected by the initial number of foci and plant density. These factors contribute to extensive crop destruction caused by S. rolfsii.

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DISEASE GRADIENTS OF CERCOSPORA ARACHIDICOLA ON PEANUT. C. A. Matyac, J. E. Bailey and S. C. Alderman, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616

Disease gradients were examined on a susceptible variety and a genotype with a high level of rate limiting resistance. Two plants with sporulating lesions were placed within a row at four sites per genotype, 30 m apart. Lesion numbers were counted on plants in 4 directions from source plants at 21, 39, and 63 days after planting source plants in the field. Gradients were calculated using linear regression using the models of Gregory and Kiyosawa. The average gradients at the three dates of observation were 5.79, 1.70 and 1.26, respectively. Early season gradients had no significant diferences due to genotype, location or direction. A downwind gradient of 1.29 at midseason was significantly lower than upwind or across row gradients of 1.75, 1.87 and 1.88, respectively. End of season gradients varied significantly with location, direction and genotype. Differences between genotypes were much less than those between locations and the direction of prevailing winds.

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AN EXPERT SYSTEM FOR PREDICTING CROP DISEASE EPIDEMICS. D.C. Sands, E.L. Sharp, A.L. Scharen, and L.S. Slater, Dept. of Plant Pathology, Montana State University, Bozeman, Montana, 59717, and J. Caristi, Dept. of Mathematics and Computer Science Valparaiso University, Valparaiso, Indiana 46383.

An expert system has been developed having knowledge bases for two wheat diseases caused by Puccinia striiformis and Septoria nodorum. The program is rule based and written for IBM compatible personal computers. The inference engine can work with weather data input on a daily basis, or in a batch mode. Each cycle of infection ending in spore production is clocked, and as the season progresses, the number of disease cycles and subsequent yield reduction is predicted. A comparison of stripe rust infections at Pullman, WA. for four years gave average differences of only 3% between computed and observed values in disease coefficients. An additional feature of the system is transparency, enabling adaptive changes in the programming.

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XYLEMELLA FASTIDIOSUM, GENUS NOVUM, SPECIES NOVUM, OF THE FAMILY PSEUDOMONADACEAE: GRAM-NEGATIVE, XYLEM-LIMITED, FASTIDIOUS BACTERIA FROM PLANTS. J. M. Wells, USDA/ARS, Rutgers U., New Brunswick, NJ 08903; B. C. Raju, Weyerhaeuser Tissue Culture Center, Apopka, FL 32703; and W. G. Weisburg, Dept. Genetics and Development, U. of Illinois, Urbana 61801.

Twenty-four strains of Gram-negative, xylem-limited fastidious bacteria from plants, isolated from different disease sources including Pierce's disease of grapevine (PCE), plum leaf scorch (PLM) and periwinkle wilt (PWT) were positive for 6 physiological tests, negative for 18 and variable for 1 (β-lactamase). Strains reacted serologically to ascites fluid from mouse immunized against the PCE bacterium, and to monoclonal antibodies prepared from spleenic cells of mouse immunized with the PLM bacterium. DNA homology among PCE, PLM and PWT strains was above 90% with no relatedness to members of the Enterobacteriacea or Vibrionaceae. Comparisons of ribosomal RNA sequences relate the fastidious bacteria most closely to the genus Xanthomonas in the Pseudomonadaceae.

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IMMUNOFLUORSCENCE MICROSCOPY USING MONOCLONAL ANTIBODIES TO STUDY SPIROPLASMA WITH PRESERVED HELICAL CELL SHAPE. M. Konai, R.E. Davis, and R. Jordan, USDA, Agricultural Research Service, Microbiology and Plant Pathology Laboratory and Florist and Nursery Crops Laboratory, Beltsville, MD 20705

A technique was developed for immunofluorescence of in vitro cultured spiroplasma cells fixed on slides while retaining helical cell shape. An aliquot of culture was placed on a poly L-lysine coated slide and the cells were killed and fixed with an equal volume of 1% glutaraldehyde in PBSS (0.02M phosphate, 0.85% NaCl, 7% sorbitol, pH 7.4). Nonspecific antibody binding was blocked by 2% bovine serum albumin in PBSS. Monoclonal antibodies (McAbs) (ascities fluid) against S. citri and corn stunt spiroplasma (CSS) were added to slides followed by fluorescein isothiocyanate conjugated rabbit anti-mouse antibodies. Slides were observed for fluorescence using a Zeiss epifluorescence microscope. Of the six McAbs investigated, all reacted with S. citri and/or CSS of Spiroplasma Group I, but not with spiroplasmas of 7 other Groups.

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PURIFICATION OF MYCOPLASMA-LIKE ORGANISMS FROM LETTUCE AFFECTED WITH ASTER YELLOWS AGENT. Y. P. Jiang and T. A. Chen, Plant Pathology Department, NJAES, Rutgers University, New Brunswick, NJ 08903

A technique was developed to purify the mycoplasma-like organisms (MLOs) associated with the aster yellows disease (AY). Symptomatic diseased lettuce leaf veins were isolated and homogenized in an isolation medium, and followed by a low and high speed centrifugation. The pellet was resuspended in a suspension medium and layered on discontinuous percoll density gradients. Electron microscopy of AY-MLO fraction collected after centrifugation showed high density of MLOs with well preserved cellular organelles identical to those observed in the phloem of diseased plants. Electrophoresis of the purified AY-MLO showed several protein bands corresponding to those from infected plant preparation. The purified AY-MLO had been used to develop monoclonal antibodies which, when used as a probe, not only reacted against AY-MLO but also monospecifically to an electrophoretic band.

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NATURAL OCCURRENCE OF ASTER YELLOWS IN VEGETABLE CROPS OF OKLAHOMA. D. Errampalli[1], J. Fletcher[1], and C. Eastman[2]. Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285[1], and Section of Economic Entomology, Illinois Natural History Survey, Champaign, IL 61820[2].

Aster yellows (AY), first reported in Oklahoma in daisy fleabane and lettuce in 1945, has occurred frequently in vegetable plantings in the state. Carrot, lettuce, and intercropped carrot and barley were planted in two locations in Oklahoma in fall 1984, spring 1985 and fall 1985. Incidence of AY, diagnosed by symptomatology and positive Dienes' staining, was higher at Bixby (up to 88% in one lettuce plot) than at Stillwater, and varied from year to year. Two AY isolates from carrot in 1984 and one each from carrot, lettuce and daisy fleabane from 1985 were transmitted to asters, periwinkle, and celery via the aster leafhopper Macrosteles fascifrons. All five isolates infected celery, with symptoms appearing in less than 45 days, but at least four symptom types are distinguishable based on the reaction of aster.

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INITIAL STAGES OF X-DISEASE IN PEACH: WITHIN-TREE SPREAD AND EFFECTS ON GROWTH. S. M. Douglas, Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven, CT 06504.

X-disease, associated with a mycoplasmalike organism, causes chronic decline and eventual death of stone fruit trees. Rates and patterns of within-tree spread and the effects of initial stages of disease development on vigor and production of potential fruiting wood were studied in a 5 yr old commercial peach orchard in Connecticut. Naturally infected trees first exhibited X-disease symptoms in 1983, 1984, or 1985. Initial disease severities of individual trees ranged from 0.9 to 64% and increased an average of 24%/yr. Terminal growth decreased linearly (r^2=.63) with increasing disease severity. Scaffolds and major branches from symptomatic trees were smaller and produced significantly less fruiting wood than those from apparently healthy trees. Nonsymptomatic portions of diseased trees also had significantly less terminal growth than their counterparts of apparently healthy trees.

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Effect of substrate and simulated acidic rain on early seedling development of red spruce. G. R. Stanosz and R. I. Bruck. Dept. of Plant Path., NC State Univ., Raleigh, NC 27695-7616.

Litter (L), fermented material (F), and mineral soil (M) were collected from a high elevation (1950 m) conifer stand and a lower elevation (1580 m) conifer-hardwood stand in the Pisgah National Forest, NC. Red spruce (Picea rubens) seeds of a commercial, highly germinable lot were placed on the surface of each substrate in pots and were exposed twice weekly to simulated rain (SR) adjusted with H_2SO_4 and HNO_3 to pH 5.5, 4.0, or 2.5. Regardless of the elevation of substrate collection, after 4 weeks exposure to SR of pH 5.5 or 4.0 approx. twice as many viable seedlings were present in F and M than L. Exposure to SR of pH 2.5 however, resulted in fewer viable seedlings in F and M than L. Numbers of viable seedlings in F and M receiving SR of pH 2.5 were also much lower than in F and M receiving less acidic SR. The most acidic treatment caused discoloration, distortion, and necrosis of radicles. Injury was associated with accumulation of SR on the surface of M during rains, and the longer period of time surfaces of F and M remained moist.

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OCCURRENCE OF AIR POLLUTION SYMPTOMS ON EASTERN WHITE PINE IN THE SOUTHERN APPALACHIAN MOUNTAINS. R. L. Anderson[1]; B. I. Chevone[2], A. H. Chappelka[2] and H. D. Brown[1]; USDA Forest Service, Region 8, Atlanta, GA[1]; Dept. of Plant Path., Physiol. and Weed Sci., VPI & SU, Blacksburg, VA[2].

Fifty eastern white pines each on 201 uniformly spaced plots in Georgia, Kentucky, North Carolina, South Carolina, Tennessee and Virginia were surveyed in the fall of 1985 for visible symptoms of air pollution (primarily O_3). Incidence was highest in Kentucky and Tennessee with 77 and 33% of the stands exhibiting needle tipburn symptoms. Lowest incidence occurred in Georgia (10%). Twenty-three percent of all stands exhibited symptoms. Plantations had a higher percentage of affected trees than natural stands and symptomatic trees were most common on sandy soils on southwest slopes. Symptom incidence was not

correlated with elevation. Forty-eight paired trees of the same age, one symptomatic and the other symptomless, were examined for growth differences. Symptomatic trees were 0.7 m shorter, 2 cm smaller in diameter and had 49% less volume than non-symptomatic trees.

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BIOINDICATOR SURVEY FOR OZONE INJURY IN GEORGIA, NORTH CAROLINA AND SOUTH CAROLINA. [1]A. H. Chappelka, [1]B. I. Chevone, [2]H. D. Brown and [2]R. L. Anderson, [1]Dept. of Plant Path., Physiol. and Weed Sci., VPI & SU, Blacksburg, VA and [2]USDA, Forest Service, Region 8, Atlanta, GA.

During September 1985, 60 plots, located in 47 counties in the Piedmont regions of Georgia, North Carolina and South Carolina were surveyed for visible symptoms of ozone injury. The bioindicator species observed were ash (white and green), black cherry, poison ivy, wild grape and yellow-poplar. Black cherry and yellow-poplar were the most widely distributed of the species observed. Species sensitivity, from most sensitive to tolerant was yellow-poplar > poison ivy > black cherry > ash > wild grape. There was considerable geographic variation in plant response, with the most severely damaged plots occurring adjacent (30-50 km) to large metropolitan areas (> 100,000 people). All bioindicator species were most severely affected in Georgia and least affected in North Carolina. The survey indicated that sensitive plants, in their native habitat, are affected by ambient atmospheric pollutant concentrations.

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REACTION OF RESISTANT SLASH PINES TO CRONARTIUM QUERCUUM F. SP. FUSIFORME FROM PROGENY OF SUSCEPTIBLE AND RESISTANT PINES. C. H. Walkinshaw, Southern Forest Experiment Station, P. O. Box 2008 GMF, Gulfport, MS 39505.

Resistant slash pines were inoculated with 12 fungus isolates from Jackson County, Florida. These isolates were randomly selected from those collected from stem galls on 43 open-pollinated slash pine families in the planting. At the time of spore collection (6 years) 31% of the living trees in this planting had stem galls and the rust mortality was 23%. In greenhouse tests, incidence of galls on inoculated seedlings of four resistant pine families was not significantly different among the 12 single gall inocula. Rust isolates from susceptible families had a larger variance in pathogenicity than those from resistant trees in the planting. The most infectious isolates in our studies since 1975 have been from trees of susceptible pine families. Results support application of resistant slash pines to control fusiform rust on high hazard sites.

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IN VITRO RESISTANCE SCREENING OF PINUS TAEDA SEEDLINGS INOCULATED WITH CRONARTIUM QUERCUUM F. SP. FUSIFORME AS EVALUATED BY ELISA AND LIGHT MICROSCOPY. P. C. Spaine and H. V. Amerson, Depts. of Botany and Forestry, respectively. North Carolina State University, Raleigh, NC 27695.

Control pollinated Pinus taeda seedlings with high, intermediate and low rust resistance field ratings, inoculated in vitro with basidiospores of Cronartium quercuum f. sp. fusiforme (Cqf) were compared for incidence and severity of infection by both ELISA and histological methods. ELISA and histological assessment gave comparable rankings for the families for both incidence and severity. The resistant family had a 20% infection rate as evaluated by both histological and ELISA procedures. Intermediate and susceptible families had a 50% infection rate as assessed by histological methods and a 66% infection rate detected by ELISA. In ELISA assessment the resistant family was statistically separable from the intermediate and susceptible families which were not statistically separable from each other.

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THE DURATION OF EFFICACY OF THIABENDAZOLE IN CONTROLLING PITCH CANKER ON LOBLOLLY PINE SEEDLINGS. G.B. Runion and R.I. Bruck, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Six-month-old loblolly pine seedlings were placed within a five-year-old plantation of loblolly pines infected with the pitch canker fungus, Fusarium subglutinans. Seedlings of 10 half-sib families were treated with 0, 0.134, 0.201, or 0.268 kg(ai)/ha thiabendazole immediately following placement in the plantation. Seedlings were exposed to natural inocula for 3, 6, 9, or 12 wk. Terminals of surviving seedlings from each exposure period were assayed for infection by isolation on Nash and Snyder's Fusarium selective medium. The percentage of infected seedlings increased significantly from the 3 (6.2%) to the 6 wk (14.4%) exposure period but did not increase with additional exposure. The percentage of infected seedlings decreased significantly with an increase in the amount of thiabendazole applied up to the 0.201 kg/ha rate (0 kg/ha=19.5%, 0.134 kg/ha=13.7%, 0.201 kg/ha=8.8%, 0.268 kg/ha=11.4%). No significant differences were observed among half-sib families.

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EFFECT OF SHORT-TERM STORAGE OF TRIADIMEFON-TREATED SEED ON INCIDENCE OF FUSIFORM RUST ON LOBLOLLY PINE SEEDLINGS. W. D. Kelley, Dept. of Botany, Plant Pathology, and Microbiology, Ala. Agric. Exp. Stn., Auburn University, AL 36849.

Loblolly pine seed usually are sown within 48 hr after being treated with triadimefon; however, protection from fusiform rust on emerging seedlings was not diminished by storing the treated seed for short periods before sowing. Stratified seed were dressed with triadimefon (2.5 g Bayleton 50-WP/kg seed), briefly air-dried, and stored for 0, 3, 10, 17, and 24 days at 5 C. Young seedlings were inoculated with basidiospores of Cronartium quercuum f. sp. fusiforme 31 days after seed were sown, and seedlings were examined for rust galls seven months later. No galls were found on seedlings from triadimefon-treated seed, regardless of length of storage; however, 63% of seedlings from non-treated seed were galled. Results of this study demonstrate the stability of triadimefon on pine seed, thus permitting a less rigid time schedule for forest tree nursery personnel during the spring sowing season.

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EFFECT OF TEMPERATURE AND AMMONIUM NITRATE ON CYLINDROCLADIUM ROOT ROT OF PINUS STROBUS. C. Stevens, Tuskegee University, M. A. Palmer, North Central Forest Experiment Station, St. Paul MN 55108, and A. Y. Tang, Tuskegee University, AL 36088.

Seedlings of Pinus strobus were transplanted into soil inoculated with Cylindrocladium scoparium and unidentified Cylindrocladium sp. and incubated in separate growth chambers at 15, 24, 27, and 33 C for two months. The optimum temperature for disease development and production of microsclerotia (MS) was 27 C. In a separate experiment, inoculated seedlings were fertilized with four levels of ammonium nitrate (N) (0, 58, 116, and 231 µgN/g soil), and incubated at 27 C for two months in the growth chamber. Higher rates of N on roots of seedlings reduced the disease severity. The number of MS/cm root, and the percent roots containing MS were negatively correlated with increasing levels of N. This information can be used to plan for integrated management strategies for controlling root rot in forest tree nurseries.

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ANTAGONISTIC INTERACTION OF RACES OF MELAMPSORA MEDUSAE ON CULTIVARS OF POPULUS. C.S. Prakash, Department of Forestry, University of Kentucky, Lexington, KY 40546-0073.

Two races of M. medusae were mixed in equal proportions and inoculated on a compatible cultivar of poplar. The aggressiveness (various traits) of the racemix was significantly less than that of either component race, suggesting antagonism between these races when mixed. Similarly, an avirulent race inoculated 4 days prior to a virulent race on a resistant cultivar resulted in incompatibility of this cultivar to the virulent race, suggesting a host mediated interaction of races leading to induced resistance. Antagonism in the pathogen population may be an important factor in the stability of natural pathosystems because of the observed high pathogen polymorphism for virulence.

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RELATIVE SUSCEPTIBILITY OF TWENTY PINUS SPECIES TO FUSIFORM RUST. F.H. Tainter and R.L. Anderson, Department of Forestry, Clemson University, Clemson, SC 29634-1003; and Resistance Screening Center, USDA Forest Service, Asheville, NC 28804.

Twenty species of Pinus and two standards were inoculated with basidiospores of Cronartium quercuum (Berk.) Miy. ex Shirai f. sp. fusiforme at the Resistance Screening Center with the standard operational procedures for slash pine. Fourteen species: Pinus attenuata (96%), P. cubensis (94%), P. canariensis (89%), P. caribaea hondurensis (88%), P. contorta murrayana (88%), P. contorta latifolia (86%), P. eldarica (84%), P. longifolia (77%), GA SL [susceptible Georgia slash] (75%), P. glabra (73%), P. engelmannii (73%), P. brutia (67%), P. halepensis (65%), and P. greggii (58%) had symptoms of rust infection greater than that of the rust resistant slash pine standard [FA-2] of (53%). P. aristata (the only Haploxylon species tested) and P. montana had symptom incidences of 23% and 25%, respectively. P. banksiana, P. densiflora, P. montezumae, P. hartwegii, and P. mesogeensis had symptom incidences of from 4 to 8%.

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USE OF A REMOTE PILOTED AIRCARFT SPORE SAMPLER (REPASS) OVER A COTTONWOOD PLANTATION. F. I. McCracken, U.S. Forest Service P. O. Box 227, Stoneville, MS 38776

Design of REPASS included a forward air intake to avoid turbulent air, an aspirated trap system, stable flight, and simple construction. Specifications were: length 150 cm, wingspan 183 cm, weight of 4.5 kg, power 10 cc engine, air speed 25-88 kph. A vacuum motor pulled air over 1-12 plastic rods at 0.106 m^3/min. Single rods were exposed 1 min at 5 altitudes to 60 m over 97 ha of 1-yr-old cottonwood near Fitler, MS seasonally. Fungus spore quantity and genera varied with altitude and collection date. In summer, spores of the genera Cercospora, Cladosporium, Epicoccum, Helminthosporium, Melampsora, Monilochaetes, Phialophora, Trichothecium plus 12 other identified genera were trapped. Total spores decreased as altitude increased. Spore genera decreased with onset of winter. A few Bipolaris spores were collected in January. Urediospores of M. medusae were not trapped over 50 m, after leaf fall, or in spring. A REPASS can meter spore density over crop trees within visual range of the operator.

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ELISA AND ITS APPLICATION TO SCREENING OF MICROBES FOR PRODUCTION OF CERATO-ULMIN, A TOXIN OF *CERATOCYSTIS ULMI*. Nordin, J.H.[1], Mason, T.L.[1], Smith, L.L.[1], Willman, P.A.[1], Richards, W.C.[2] and Takai, S.[2]. [1], Department of Biochemistry, University of Massachusetts, Amherst, MA 01003. [2] Great Lakes Forestry Centre, Canadian Forestry Service, Sault Ste. Marie, ON P6A 5M7 Canada

Polyclonal antibodies from hyperimmune ascitic fluid of mice immunized with cerato-ulmin (CU), a protein toxin of *C. ulmi*, have been utilized to develop a sensitive and specific assay to detect CU. As little as 20 ng (1.5 picomoles) of CU per assay well can be detected by this method. Employing extracts of plate cultures of a number of microbial species including many isolated from elm tissue, as well as several other *Ceratocystis* species, it was noted that (a) only extracts of *C. ulmi* contain antibody-reactive material and (b) that among 86 *C. ulmi* isolates screened for CU production, 56 were positive numbers. Using both extracts of plate cultures and cell-free culture filtrates of 10 *C. ulmi* isolates, the ELISA was compared with the turbidimetric method (Takai and Richards 1978) for detecting their CU production. Isolates classified either as producers or nonproducers of CU by the ELISA using extracts of plate cultures were found to group in the same categories when their culture filtrates were assayed either by the ELISA or turbidimetry.

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NEW SYSTEMS FOR DELIVERY OF BIOCONTROL AGENTS TO SOIL. J. B. Bahme, S. D. Van Gundy, and M. N. Schroth. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Soil incorporation of bacterial granules and soil injection of microorganisms by a drip irrigation system were evaluated on field-grown potatoes as methods to improve delivery of biocontrol agents. Average colonization of roots and daughter tubers by Pseudomonas fluorescens strain A1 increased 60-fold with the granular application versus the standard seedpiece treatment. Periodic introduction of strain A1 and conidia of Trichoderma viride isolate T-1-R9 in drip irrigation water maintained high populations of the antagonists in the top 30 cm of beds, with means of 5×10^5 and 1×10^4 cfu g^{-1} soil for the bacterium and fungus, respectively. Average populations of T-1-R9 recovered from surfaces of roots and tubers were 25 cfu cm^{-1} and 5.5×10^4 cfu g^{-1} (dry wt.), respectively. Mean populations of drip-applied A1 on these plant parts were 300 times greater than with seedpiece inoculation. Interactions of drip-introduced P. fluorescens and Erwinia carotovora will be discussed.

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CHARACTERIZATION OF ANTIBIOTIC MINUS MUTANTS OF ERWINIA CARTOVORA SUBSP. BETAVASCULORUM. Paige E. Axelrood, Manuela Rella and M. N. Schroth, U. of California, Berkeley, CA 94720

Antibiotic producing (Ant^+) strains of Erwinia cartovora subsp. betavasculorum (ECB) inhibit the growth of sensitive strains of Erwinia carotovora subsp. carotovora (ECC) when co-inoculated into potato tubers. Transposon mutagenesis of ECB Ant^+ was done using the vector pME9 containing Tn5-751. Eleven antibiotic minus mutants (Ant^-) were identified. Preliminary genetic characterization shows that at least six loci are involved in antibiotic production. The mutants were characterized for their ability to prevent the growth of ECC in mixed ECB Ant^-/ECC potato wound co-inoculations. The growth of ECC was not inhibited in nine of eleven ECB Ant^-/ECC treatments. The remaining two mutants inhibited the growth of ECC. However, they showed an Ant^-, rot minus phenotype in single inoculations and preliminary evidence indicates that these could be regulatory mutants. These results suggest the antibiotic produced by ECB plays an important role in preventing the growth of ECC.

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SURVIVAL OF A PSEUDOMONAS SYRINGAE PV. LACHRYMANS INA ISOLATE IN BUDS OF CUCUMBER SEEDLINGS. Curt Leben, Department of Plant Pathology, The Ohio State University/OARDC, Wooster, OH 44691.

Cells of the pathogen (Psl), which have ice nucleation activity (INA), were placed on terminal buds (TB) of seedlings with 1-2 leaves (L1-L2). After 24 hr in a dew chamber, pots were put in a greenhouse (low RH=30-50%) or in a chamber in the greenhouse (high RH=80->95%). Tests were made in the winter with supplemental light (day/night=27/21 C), and only soil was watered. After 21-24 days, there were 9-11 more leaves. Lesions usually were on L3 and L4, and occasionally on higher leaves at high RH. The TB, axillary buds (AB), and underside of leaves were assayed with selective agar. At high RH, Psl was in the TB and on the two youngest leaves of all plants, on most other leaves, and in most AB (three tests, 18 plants total). At low RH, Psl was absent in TB and AB, and from lesion-less leaves (20 plants in three tests). These and other data from moderate RH tests indicate that Psl was carried in the TB, distributed on unfurling parts, and died when exposed to dry air.

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TREATMENT OF TOMATO FRUIT WITH BIOCIDES PRIOR TO HYDRO-HANDLING REDUCES POSTHARVEST DECAY. J. A. Bartz and G. A. Simone, Plant Pathology Dept., University of Florida, Gainesville, 32611.

Fresh market tomato fruits hydro-handled at packinghouses (flumed/dumped in water) may become infiltrated with water and subsequently decay. Bacterial soft rot in fruits artificially infested with Erwinia carotovora pv. carotovora (Ecc) was reduced 65 to 81% if fruit were immersed in an aqueous solution of 500 ppm chlorine or 2.5 g/l a.i. Kocide plus 0.3 g/l a.i. mancozeb within 24 h before infiltration with sterile tap water. Reducing the period between chlorine treatment and infiltration from 24 to 4 h improved decay control in fruits naturally infested with Ecc, Geotrichum candidum and Rhizopus stolonifer. The ratio of primary decay lesions caused by Ecc versus the fungi was not altered by chlorine treatment. Pre-packinghouse treatment of tomato fruits with broad spectrum biocides will reduce postharvest decay during commercial handling.

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FIRST REPORT OF SPINACH LATENT VIRUS IN NORTH AMERICA. Hsing-Yeh Liu and James E. Duffus. USDA-ARS, Salinas, CA 93905.

During studies on the purification of a beet virus in spinach (Spinacia oleracea) a distinctive UV absorption peak containing virus-like particles was found. Subsequent studies have shown the origin of these particles to be a latent virus seedborne in spinach. Electron microscopy showed the virus particles to be irregularly spherical, c. 28 ~ 32 nm in diameter, and very similar in appearance to ilarviruses. The virus was shown to be serologically related and apparently very similar to the spinach latent virus (S_pLV) reported from Yugoslavia and the Netherlands. The S_pLV is thus present in some commercial spinach seed lots in USA and has rates of seed transmission of up to 50%. The virus is seedborne and pollen borne in several Chenopodiaceae species including wild beet, Beta macrocarpa. The economic significance of the S_pLV in commercial spinach production has not yet been determined.

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SELECTIVE ISOLATION OF PSEUDOMONAS CICHORII FROM SOIL AND LEAF TISSUE WITH TWO SELECTIVE MEDIA. J.B. Jones, GCREC, Bradenton, FL 34203, P.S. Randhawa, Yoder Bros., Inc., Alva, FL 33920, and M. Sasser, Univ. Delaware, Newark, DE 19711.

Two selective media were developed for isolation of P. cichorii from contaminated soil or leaf tissue. Medium 1 (PMCL) consists of: K_2HPO_4 0.8 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KH_2PO_4 0.8 g, L(+)-tartrate 2.0 g, SDS 0.1 g, $(NH_4)_2SO_4$, boric acid 0.25 g, cycloheximide 100 mg, cephalexin 20 mg, novobiocin 4 mg, malachite green 4 mg, dodecyltrimethylammonium bromide 30 mg, ampicillin 5 mg, potassium tellurite 0.2 mg in one liter of H_2O. Medium 2 consists of Medium B of King et al. (KMB) minus glycerol, plus malachite green oxalate 10 mg, tergitol P-28 1.5 ml, L(+)-tartrate 2.0 g, vancomycin 400 mg, cephalexin 100 mg, novobiocin 25 mg, and bacitracin 400 mg in one liter of H_2O. The media were tested for recovery of P. cichorii using inoculum prepared from laboratory inoculum. Of 12 cultures tested with PCM1 and rison to recovery on KMB. The two media reduced foliar and soil contaminants 87.2-100% and 50-100%, respectively.

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FUSARIUM ROOT ROT OF PEAS EFFECTED BY INOCULUM DENSITY AND PLACEMENT. Charles M. Rush and John M. Kraft, USDA-Agricultural Research Service, IAREC, P.O. Box 30, Prosser, WA 99350

A greenhouse study was conducted to evaluate the effects of inoculum density and placement on Fusarium root rot of peas caused by Fusarium solani f. sp. pisi. When Fusarium inoculum was placed in the lower 10 cm of 30 cm containers, pea roots became infected, but no disease symptoms appeared. Even the high inoculum density of 5000 cfu/g soil, when placed in the lower 10 cm, failed to cause any measurable plant stress. When inoculum was placed in the upper 10 cm or mixed throughout the containers, dry top and root weight, plant height, and leaf area were all significantly lower than check plants growing in noninfested soil. Plants growing in the high inoculum density soil exhibited severe root rot symptoms earlier than plants growing in soil infested with 150 cfu/g soil. There was no significant difference between plant stress measurements whether inoculum was mixed throughout the container or placed in the upper 10 cm.

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COMPARISON OF PERCENT LEAF AREA WITH WHITE RUST LESIONS AND TWO OTHER METHODS FOR EVALUATING PARTIAL RESISTANCE TO ALBUGO OCCIDENTALIS IN SPINACH. M. C. Black and F. J. Dainello, Texas A&M University Agricultural Research and Extension Center, Uvalde, TX 78802-1849.

White rust of spinach, caused by Albugo occidentalis, was evaluated three ways. A visual plot rating (0-5) detected large differences in resistance but can have inteference from freeze and aphid damage. Destructive sampling of whole plants and sorting leaves into two classes (with and without lesions) was time consuming and did not directly reflect small lesion size and number of lesions per leaf under moderate and severe disease conditions. Sampling individual leaves of similar maturity and sorting into classes (0,1,5,10,20 and $\geq$ 50 percent leaf area with lesions) was superior. More initial training was needed but small differences in partial resistance were detected. Green Valley 11, XPH 1452, 4CX-119 and Coho had high levels of resistance. Melody, Crystal Savoy, 4CX-87 and 4CX-88 were highly susceptible. Leaf area with lesions was also useful in evaluating fungicide tests.

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FIELD AND GREENHOUSE REACTION OF BEAN GERMPLASM TO Rhizoctonia solani. M.A. Pastor-Corrales and G.S. Abawi, CIAT, Cali, Colombia and Cornell University, Geneva, NY, 14456.

Two evaluations were conducted in an experimental field with a history of severe Rhizoctonia root rot near Popayán, Colombia, S.A. A total of 136 accessions were included in the first nursery with 3 replications. Each replicate consisted of two 1-m rows with 15 seeds/row. Seeds in one row were covered with 1 liter of Rhizoctonia-infested soil (2%, V:V; potato-soil inoculum : field soil) prior to closing the row. Emergence and disease severity rating were recorded at 2 and 5 weeks after planting. Stand counts and seed yield were recorded at harvest time. A total of 65 lines were re-evaluated in the same field. Greenhouse evaluation included 78 lines and was by planting seeds in 2% Rhizoctonia-infested soil. Emergence and disease severity ratings were recorded at 10 and 21 days after planting. A 300, BAT 1753, RIZ 21, RIZ 30, and EMP 81 were among the accessions considered resistant to R. solani in all evaluations.

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EVALUATION OF SELECTED BEAN ACCESSIONS FOR RESISTANCE TO Macrophomina phaseolina. M.A.Pastor-Corrales, and G.S. Abawi, CIAT, Cali, Colombia and Cornell University, Geneva, NY 14456.

The reaction of 60 bean accessions to Macrophomina phaseolina (Mp) was evaluated in a field near Quilichao and a greenhouse near Palmira, Colombia, S.A. Field evaluations consisted of non-inoculated and Mp-inoculated treatments. Inoculations were made by placing 4 g or whole rice grains colonized with Mp (2 wk)/2 m rows of beans (approx. 2-3 colinized rice grains/bean seed). Two nurseries were conducted, each with 3 replications. In greenhouse evaluations, bean seeds were covered with soil infested with dried sclerotia of Mp (2 g/kg soil). Based on emergence, Mp-incidence and severity ratings; 15 accessions were classified as highly resistant; 15 were considered intermediate and the rest as highly susceptible. A 300, BAT 332, BAT 447,BAT 1385, EMP 86, G 5059 and San Cristobal were among the highly resistant. In one test, San Cristobal 83 and G 5059 had average disease severities of 1.0, whereas A 294 (susceptible) had a score of 7.7 on a scale of 1-9 (1=no disease symptoms and 9=dead plant).

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CYCLOHEXIMIDE AND INDUCED RESISTANCE IN CUCUMBER. S.M. Rutter and R. Hammerschmidt, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Systemic induced resistance of cucumber has been demonstrated to be associated with enhanced levels of a group of acid, cell wall associated peroxidase isozymes (PO). The same PO are also enhanced in etiolated cucumber seedlings that have had resistance induced by a prior heat shock. Treatment of etiolated seedlings with cycloheximide (CH) resulted in increased activity of the same PO induced by heatshock. Increasing the concentration of the metabolic inhibitor from 1 to 100 uM resulted in increasing activity of the PO. Injection of a 10 uM solution of CH into the first true leaf of green cucumber plants resulted in a systemic increase in the activity of the PO that was similar to the systemic increase in activity observed when the first true leaf was inoculated with Colletotrichum lagenarium. Plants treated with CH, however, did not exhibit induced resistance against a challenge by C. lagenarium. These results suggest that increased activity of the PO are not causally related to induced resistance in cucumber.

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INDUCED RESISTANCE IN THE CLADOSPORIUM FULVUM - TOMATO INTERACTION. M.Arlt and V.J.Higgins, Department of Botany, University of Toronto, Toronto, Ontario, Canada, M5S 1A1.

Inoculation of tomato plants with an avirulent race of Cladosporium fulvum, the causal agent of leaf mould of tomato, significantly reduced sporulation of a virulent race inoculated from 1 to 7 days later at the same site. Benomyl application 2 days after inoculation with a benomyl-sensitive avirulent race inhibited the induction of resistance to a benomyl-tolerant virulent race. When the avirulent race was killed by exposure to u.v. light prior to inoculation, its ability to reduce sporulation of the virulent race was inhibited. DeWit and Spikman (Physiol.Pl.Path.21:1-11) found that race and cultivar specific elicitors of chlorosis and necrosis are present in the intercellular fluids of compatible race-cultivar interactions of C. fulvum and tomato. Injection of intercellular fluids from an avirulent race failed to induce resistance to a virulent race. The data suggests that a continuous elicitation of the resistance responses by a viable avirulent race is necessary for the induced resistance phenomenon.

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INDUCING PLANT DISEASE EPIDEMICS: THEORY AND PRACTICE. F. W. Nutter, Jr., Department of Plant Pathology, University of Georgia, Athens, GA 30602

Multiple treatment levels are needed to provide a wide range of stimulus-response levels from which it is intended to develop reliable empirical or mechanistic yield loss models. Disease development over time for pathogens with a logistic growth pattern is described by $\frac{dy}{dt} = ry\ (1-y)$ where 'r' is the apparent infection rate, 'y' is the present level of disease severity and '1-y' is the amount of healthy tissue. A knowledge of this relationship can be used to optimize the range of disease severity levels. For example, a pathosystem with a high 'r' is manipulated best by methods that differentially affect 'r', whereas pathosystems with low 'r' values may respond more to methods that result in different levels of inoculum. In some pathosystems, the time period the host and pathogen are associated may be manipulated to affect disease severity. Examples will be provided.

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DEVELOPMENT OF A COMPREHENSIVE IPM PROGRAM FOR THE DAIRY FARM. J. Keith Waldron. Integrated Pest Management Support Staff, 5130 Comstock Hall, Cornell University, Ithaca, NY 14853.

A New York dairy/field crops integrated pest management (IPM) program was initiated in 1982. The program is unique among existing IPM programs, in that it is aimed at enhanced management of overall dairy production units rather than simply at improved management of dairy cattle or individual crops. Benefits include both increased and timely information on all major components of the dairy farm. Pilot IPM programs have been managed locally by cooperating county extension personnel; over 100 farms participated in 1985. During the summer, corn and alfalfa fields are intensively monitored for insects, diseases and weeds on a regular schedule. Dairy cattle are also closely monitored for insect pests in barns and pastures. Winter dairy cattle monitoring for insects and mites was initiated in 1986. Optional services include soil and forage sampling and analysis, and comprehensive crop records keeping. Future inclusion of IPM applications to limited acreage crops and stored products is planned. Two Crop Management Associations were formed in 1986, as outgrowths of county projects.

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STATISTICAL MODELS FOR THE PREDICTION OF MICROSCLEROTIA OF MACROPHOMINA PHASEOLINA IN MISSOURI SOILS. S.M. Rosenbrock and T.D. Wyllie. Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Effects of soil texture, soil fertility, weather, and field location on microsclerotia of Macrophomina phaseolina were investigated in commercial fields in Missouri. Statistical models were developed to predict numbers of microsclerotia as a function of data collected in the spring and fall 1984 and evaluated with data collected in the spring and fall 1985. Most of the observed variability ($R^2 = 0.60$, $P = 0.0001$) appears to be related to field location, the level of phosphorus and potassium, and pH of the soil. When levels of microsclerotia for a field are known and incorporated into the model, as much as 80% of the observed variability ($P = 0.0001$) can be explained. This information is important for commercial growers to do management planning. Evaluations of risk levels from calculated population estimates combined with rotation practices can be effective in reducing populations or maintaining them at tolerable levels.

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INFLUENCES OF LOCATION AND ENVIRONMENT AS THEY EFFECT DISTRIBUTION OF MICROSCLEROTIA OF MACROPHOMINA PHASEOLINA IN MISSOURI SOILS. S.M. Rosenbrock and T.D. Wyllie. Department of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

Six areas, each with 14-23 fields, were sampled twice in 1984 and again in 1985 for number of microsclerotia of Macrophomina phaseolina/gram of soil. Temperatures in four categories (expressed as degree days >27, >21, <0, & <-18 C) and precipitation were collected from October 1983 to October 1985 for each area. Correlation and regression analyses indicated that populations are significantly lower in northern areas of the state which experienced fewer accumulated degree day temperatures (ADD) at both >27 and >21 C and greater ADD at both <0 and <-18 C. Adequate summer rainfall stabilized populations at their previous levels even if degree day temperatures over 21 C were high. The largest increases in numbers of microsclerotia occurred in areas having the greatest amounts of ADD >21 C and less than normal amounts of precipitation. Populations appear to be stable in years of adequate precipitation and hot weather but increase in years with combined hot weather and drought.

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SPATIAL AND TEMPORAL VARIATION OF METALAXYL RESIDUES IN FIELD-GROWN POTATO FOLIAGE. M.G. Milgroom and W.E. Fry, Cornell University, Ithaca, NY 14853

Metalaxyl was sprayed on field plots of potatoes, and leaflets were collected to monitor residues at various times after application. Residues were determined by bioassay separately for each of several thousand leaflets. Metalaxyl residues decreased exponentially from initial levels of 100-200 ng/cm^2 to 5-10 ng/cm^2 30 hours later. Subsequent decay rates were much slower. Residues were highly variable within the canopy at all times. Frequency distributions of residues resemble lognormal distributions. Overhead irrigation 5-6 hours after spraying reduced residues in the first day relative to non-irrigated plots. However, 3-5 days later there were no differences in residues in irrigated relative to non-irrigated plots.

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ROOT DISEASES AND YIELD DECLINE IN CONTINUOUS DOUBLE-CROP CORN. D. R. Sumner, G. J. Gascho, A. W. Johnson, and E. D. Threadgill, Coastal Plain Experiment Station, Tifton, GA 31793.

Yield of grain in the spring crop of continuous double-crop corn on Bonifay sand declined from 12 tonnes/ha (t/ha) in 1978

to 7 t/ha each year 1981-84. In 1985, soil treatments (fumigation with DD-MENCS, rye cover crop, control) were used to determine the causes of decline. Tillage, fertility, and rye treatments did not influence root disease severity (RDS). Soil fumigation suppressed populations of Rhizoctonia solani, R. zeae, Fusarium spp., Phoma spp., total soil fungi, and nematodes, and reduced RDS compared with the control. The greatest increase in silage yield was with soil fumigation compared with rye and the control (63, 28, and 25 t/ha). In the second crop of corn, the residual effect of soil fumigation reduced RDS and increased silage yield compared with the control (27 vs 18 t/ha). Pythium arrhenomanes, Phoma terristus, and Phoma sp. were isolated most frequently from root lesions.

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ASSESSMENT OF RESISTANCE TO PEANUT BLACK HULL. BL Jones and PJA van der Merwe, Grain Crops Research Institute, Private Bag X1251, Potchefstroom 2520, Republic of South Africa.

Black hull, incited by a highly virulent strain of Thielaviopsis basicola, is the most serious peanut desease in South Africa. It causes severe pod and root rot and has reduced peanut production drastically in most production areas under irrigation. Soon after its appearance in 1979 cultivars were assessed in field trials for resistance. Cultivars identified as having apparent resistance were subjected to additional testing in replicated field trials for 3 consecutive years. South African breeding line 52G25 and U.S. line 3286 were the most resistant. An intermediate resistant line, A10DPL, and 52G25 were crossed with commerical variety Sellie. Progeny from these crosses were assessed for resistance in a glasshouse by use of an attached pod inoculation technique. Results indicated that 52G25 resistance is polygenic.

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COMPARISON OF VECTORING POTENTIAL, AGGRESSIVENESS, AND PATHOGENICITY OF POLYMYXA BETAE ISOLATES. J. S. Gerik and J. E. Duffus, USDA-ARS-PBA, 1636 E. Alisal St., Salinas, CA 93905

Six non-viruliferous isolates of Polymyxa betae Keskin, from the U.S. and Canada, were all found to be able to vector beet necrotic yellow vein virus (BNYVV), the causal agent of the rhizomania disease of sugarbeet. The virus was vectored from systemically infected Beta macrocarpa, previously inoculated with BNYVV, to sugarbeet, in greenhouse pots. Two isolates of P. betae from the Sacramento and Imperial Valleys of California, consistently had higher infection index ratings, regardless of whether the fungi were viruliferous. All six isolates reduced root weights of infected plants, compared to the controls. Increased root branching, root tip mortality, and root necrosis were observed to be associated with fungal infection. These observations suggest that BNYVV could be rapidly spread throughout all sugarbeet growing areas by existing P. betae populations; they further suggest that non-viruliferous P. betae infections may be causing damage to sugarbeet.

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CONTROL OF RHIZOMANIA OF SUGAR BEET BY PREPLANT FUMIGATION, F.N. Martin and E.D. Whitney, Plant Pathology Dept. University of Florida, Gainesville, Fl 32611 and USDA-ARS, Salinas, CA 93905

Rhizomania is a devastating disease of sugar beet caused by Beet Necrotic Yellow Vein Virus which is vectored by the fungus Polymyxa betae. Crop rotation is ineffective in reducing disease and resistance is not available in commercial cultivars. Fumigation trials were conducted in a silty loam soil with a history of severe rhizomania. Fumigants were applied to the center of rows after bedding (70-cm centers) to a depth of 17cm and the soil surface sealed by compaction & light sprinkler irrigation. Trials were planted after 2 wks, sprinkler irrigated, subsamples taken at regular intervals to monitor virus infection, and yield data taken after 5 months. Telone II(32 & 64 L/ha), Vorlex (147 L/ha), Vorlex 201 (162 L/ha), and Pichlor 60 (38 & 74 L/ha) controlled disease with no or low levels of viral infection at harvest; compared to controls, root yield was increased by 67% & 75%, 76%, 83%, and 71% & 94%, respectively. Vapam and Chloropicrin at 74 and 23 & 50 L/ha, respectively, increased root yield (39% and 41% & 57%) but did not reduce viral infection.

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IDENTIFICATION AND CONTROL OF THE PATHOGEN CAUSING SESAME BLIGHT IN TEXAS. H. Myint, S. D. Lyda, and R. S. Halliwell. Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

A fungus causing blight of sesame in Texas was identified as Phytophthora nicotianae van Breda de Haan var. parasitica (Dastur) Waterhouse. Yellow to orange, ovoid to obpyriform sporangia (44.5 x 32.4 um), with prominent apical papillae, were produced terminally, and rarely laterally or intercalary. Length to breadth ratio of sporangia averaged 1.38:1. Yellow to brown, globose chlamydospores (32.4 um) formed in culture and oospores (18.5 um) formed in cultures more than two months old. Cardinal temperatures for growth were 15, 28-30, and 37C. The fungus grew over a pH range of 3.0 to 9.0, with optimum growth at 6.5. Metalaxyl, mancozeb and terrazole exhibited strong inhibition to mycelial growth at 0.1 to 1.0 ug/ml. In-furrow applications of metalaxyl (1.12 and 2.24 kg a.i./ha) significantly reduced disease under field conditions. Foliar applications of aluminum phosethyl (2.24 kg a.i. /ha) decreased disease incidence and increased yield.

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EFFECTS OF PRE-EMERGENCE FLOODING TEMPERATURE ON SOYBEAN SEEDLING DISEASE. R. S. Ferriss, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Soybean seeds planted in untreated soil were subjected to a flooding period at specific soil temperatures in 5 C increments, followed by a 3-day draining period and then 18 days at moderate moisture and 20-25 C. For 3-day flooding periods at 5 to 30 C followed by draining at 20 C, final emergence was 10-20 % at 5 C, maximum (35-45 %) at 15 C and near zero at 25 or 30 C. This response to flooding temperature was observed whether flooding started immediately after planting or after 2 days under conditions resulting in germination. For flooding and draining periods at 10 to 25 C started immediately after planting, emergence following a 3-day flooding was significantly greater than that following a 1-hr flooding at 20 or 25 C, but not at 10 or 15 C. These results indicate that mechanisms of flooding damage may differ at low (15 C and below) and high (20 C and above) temperatures.

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POSSIBLE ROLE FOR PYTHIUM ROOT ROT IN SUGARCANE STUBBLE DECLINE. J. W. Hoy and R. W. Schneider. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University, Agricultural Center, Baton Rouge, LA 70803.

A major constraint to sugarcane production in Louisiana is declining stubble crop (ratoon) yields which limit the crop cycle to three years. Significant increases in initial growth and stubble regrowth rates were exhibited by plants of cultivar CP 70-321 grown in pots containing field soil treated with fumigants or metalaxyl. Isolates of Pythium arrhenomanes and P. graminicola were found to be pathogenic to CP 70-321 in greenhouse tests. Feeder root necrosis was the main disease symptom under summer growing conditions, but under cool conditions or when plant stress was introduced by simulating harvest and stubble regrowth, extensive rotting of main roots occurred and significant decreases in plant root and top weights resulted.

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DISTRIBUTION AND INCIDENCE OF THE RATOON STUNTING DISEASE IN COSTA RICA. L. G. Jiménez A. and B. Fernández. Univ. of Costa Rica, Microbiological School, San José, Costa Rica.

A total of 260 cuttings of sugarcane were obtained from the following regions: Filadelfia, Cañas, Grecia, San Carlos, Juan Viñas and Pérez Zeledón. Sap extracts were obtained from samples by centrifugation of internodes. The presence or absence of the causal bacterium in samples was determined by phase contrast microscopy. The pathogen was found in cane samples from all regions. The incidence of RSD by cultivar ranged from 0 to 77%. The highest incidence was found in the cultivar B 47-44 from San Carlos.

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OCCURRENCE OF QUAIL PEA MOSAIC VIRUS (QPMV) INFECTING BEANS AND SOYBEANS IN VENEZUELA. E.A. Debrot and F. Centeno. CENIAP, Seccion Fitopatologia, Apartado 4653, Maracay 2101, Venezuela.

The comovirus QPMV has been detected commonly in beans and soybeans in Venezuela for the past four years. Affected plants showed stunting, mosaic, and distortion. In bean plantings, dis-

eased plants occurred predominantly towards the edges of the fields, were delayed in maturity, and showed considerable yield reduction: 44% in number of pods, 65% in dry weight of pods, 61% in number of seed, and 67% in dry weight of seed. Isolates of the virus were transmitted mechanically to hosts of QPMV, and by the beetles Andrector arcuatus and A. ruficornis to beans. In agar immunodiffusion tests they reacted with antiserum against the type strain of QPMV from Arkansas, but not with bean rugose mosaic virus antiserum. Partially purified preparations of the virus contained isometric particles of c. 27 nm in diameter, some of which were apparently empty. Host range behavior of the Venezuelan isolates differed somewhat from that reported for the type and curly dwarf mosaic strains of QPMV.

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THE STRUCTURE OF THE 3'ENDS OF PEA ENATION MOSAIC VIRUS GENOMIC RNA'S. T. L. German, S. Demler, and G. A. de Zoeten, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The 3'-termini of PEMV-RNAs 1 and 2 were examined by sequence analysis of a 380 bp cDNA clone prepared from viral RNA. Evidence based on Northern Blot analysis and on RNase T1 digestion patterns suggests that this sequence is conserved between both genomic RNA molecules. Although it is known that PEMV RNA cannot be aminoacylated in vitro, the genomic RNAs terminate with the 5' ACCA 3' tetranucleotide typical of many amino acid accepting species. We have also found, based on RNase T2 3'-terminal analysis, that both genomic RNAs exist as a heterogenous population terminating in either A or C residues. These latter two observations will be discussed with respect to the possible secondary structure of the 3'-termini of the genomic PEMV-RNAs.

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LACK OF RECOMBINATION BETWEEN TWO CAULIMOVIRUSES IN MIXED INFECTIONS. D. S. Warkentin and R. J. Shepherd, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Two caulimoviruses which show little nucleotide homology in hybridization tests, CaMV and figwort mosaic virus (FMV) were used to produce systemic mixed infections of Nicotiana edwardsonii. Each virus was shown to persist for more than one year post-infection in N. edwardsonii by immunosorbent tests and mechanical transmission to selective hosts, Brassica campestris for CaMV and Datura innoxia for FMV. DNA taken from N. edwardsonii was positive in dot blot hybridization assays on nitrocellulose using cloned viral DNAs as radioactive probes. Southern blot analyses with viral DNAs subjected to restriction endonucleose digestion revealed only those restriction fragments characteristic of each viral DNA. These results indicate that CaMV and FMV will multiply together for prolonged periods without interference or recombination.

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CLONING AND PHYSICAL MAPPING OF DNA COMPLEMENTARY TO POTATO LEAFROLL VIRUS RNA. O. P. Smith[1], K. F. Harris[1], R. W. Toler[2], and M. D. Summers[1], Department of Entomology[1] and Department of Plant Pathology and Microbiology[2], Texas A&M University, College Station, TX 77843.

Potato leafroll virus (PLRV) strain 4 (Virology 105:379) was aphid-transmitted from potato (Solanum tuberosum cultivar Russet Burbank) to Physalis floridana, where it was maintained by serial aphid transmission. The PLRV serotype was confirmed by J. E. Duffus (personal communication). Polyadenylation of RNA isolated from purified virus and first-strand complementary DNA (cDNA) synthesis followed standard protocols. Alkaline gel electrophoresis of ^{32}P-labeled first-strand cDNA indicated a major transcript of 3.4 kilobases (kb). A small percentage of transcripts were full length (6 kb). Following RNase H and DNA polymerase I-mediated second strand synthesis, double-stranded cDNA was dC-tailed and annealed to dG-tailed pUC9. Escherichia coli JM109 transformants were screened with first-strand ^{32}P-cDNA in colony hybridization experiments to confirm that recombinants contained PLRV-specific sequences. Three clones, pPLRV4-15, pPLRV4-28, and pPLRV4-53, containing inserts of 1.2, 1.8, and 1.5 kb pairs, respectively, were physically mapped with restriction endonucleases. Southern blot hybridization experiments indicated that these cDNAs formed an overlapping set, representing approximately 3.3 kb of the total viral genome (6 kb).

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HOST RESPONSE TO CAULIFLOWER MOSAIC VIRUS (CaMV) IN SOLANACEOUS PLANTS IS DETERMINED BY A 496 bp DNA SEQUENCE WITHIN GENE VI. J. E. Schoelz, R. J. Shepherd, and S. D. Daubert, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Most strains of CaMV are limited to the cruciferae in host range. However, some CaMV strains replicate and cause hypersensitive (local) reactions on a few solanaceous species. Recently an unusual strain, D4, has been isolated which causes systemic infections in various solanaceous plants, including Datura stramonium and Nicotiana bigelovii. In the present study, we have constructed recombinant virus genomes between D4 and ordinary CaMV strains to determine which portion of the genome is responsible for the expanded host range. A 496 bp fragment consisting of the first half of the coding region of gene VI was found to determine the type of host response (localized hypersensitivity versus systemic chlorotic mottle). Sequence comparisons between D4 and the incompatible strain CM1841 revealed that within the 496 bp DNA segment there were 20 nucleotide differences which resulted in amino acid exchanges.

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COMPLEMENTARY DNA CLONING OF CITRUS TRISTEZA VIRUS AND THE EXPRESSION OF VIRAL PROTEINS IN E. coli. L. A. Calvert,[1] R. F. Lee,[2] and E. Hiebert.[1] [1]Dept. Plant Path., Univ. of Fla., Gainesville, FL 32611 and [2]Univ. of Fla., IFAS, CREC, 700 Expt. Sta. Rd., Lake Alfred, FL 33850.

The RNA of citrus tristeza virus (CTV), a closterovirus with a 20 kb single-stranded RNA genome, has been used to make a library of over 500 cDNA clones. The clones were confirmed by hybridization analysis and over 50 clones have been sized by restriction digest analysis. Two clones, which overlap, represent approximately 40% of the CTV genome. The largest CTV cDNA clone, over 7 kb, was subcloned into the Weinstock et al. open reading frame expression vectors (ORF) and transformed into E. coli. The ORF clones, which were Lac$^+$, were identified and confirmed to contain CTV cDNA inserts by restriction and hybridization analysis. The longest insert is approximately 330 bases and has been mapped back to the 7 kb clone. At least four distinct trihybrid fusion proteins have been identified and these will be purified for use as immunogens.

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TRANSCRIPTION OF DOUBLE STRANDED RNA IN VIRIONS OF HELMINTHOSPORIUM VICTORIAE VIRUS. S. A. Ghabrial and W. M. Havens, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

The 190S virus of Helminthosporium victoriae was shown to possess virion-associated RNA polymerase activity. The activity was resistant to actinomycin D and required magnesium ions and all four ribonucleoside triphosphates. Addition of bentonite at a concentration of 800 µg/ml had no effect on tritiated UTP incorporation into acid-insoluble radioactivity. The products of the polymerase reaction were single stranded RNA (ssRNA) molecules, as determined by fractionation on a Cellex N-1 cellulose column; 98% of the tritiated UTP was incorporated in ssRNA. The rate of RNA synthesis was linear with time for 3-4 hr and was proportional to virus concentration in the range of 50 to 250 µg/ml. Electrophoretic analysis of the ssRNA product of a 24 hr standard reaction suggested that it was a full length transcript, thus the polymerase activity of the 190S virus is a transcriptase.

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cDNA CLONING OF RCNMV RNA AND STUDY OF RCNMV RNA-ENCODED PROTEINS BY HYBRID-ARRESTED IN VITRO TRANSLATION. Z. Xiong, and S. A. Lommel. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Red clover necrotic mosaic virus (RCNMV) genomic RNAs were cloned into pBR322 by a method described by Gubler and Hoffman. The method combined the first strand synthesis with RNase H; DNA polymerase I; and DNA ligase second strand synthesis. Clones representing 60% of the 4.1 kb RNA-1 and full length clones of the 1.4 kb RNA-2 were identified by Northorn blot hybridization analysis. For hybrid-arrested in vitro translation, fractionated RNA-1 and RNA-2 were annealed with complementary M13 subclones. Analysis of the translation products of the RNA-DNA hybrids revealed that the 1.5 kb RNA-1 clone (pBRR008) contained information for a 55kd polypeptide and the 1.0 kb clone (pBRR201) contained partial message for a 36kd polypeptide. No protein with a mobility similar to the 39kd RCNMV capsid protein was observed in the translation products. The possibility of the capsid protein not being translated in vitro by encapsidated genomic RNA is currently being studied.

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THE NUCLEOTIDE SEQUENCE OF TOBACCO VEIN MOTTLING VIRUS RNA. K. Franklin, L. L. Domier, M. Shahabuddin, G. M. Hellmann, S.

Hiremath, J. G. Shaw and R. E. Rhoads, Department of Plant Pathology and Department of Biochemistry, University of Kentucky, Lexington, KY 40546.

The nucleotide sequence of tobacco vein mottling virus (TVMV) RNA has been determined by dideoxy sequencing of cDNAs cloned into M13, and by direct RNA sequencing. The RNA contains 9470 nucleotide residues, excluding the poly(A) tail. There is an open reading frame, beginning at nucleotide 206 and ending at nucleotide 9239, which has the capacity to code for a protein of 340,000 daltons; no other reading frame in either the (+) or (-) sense of the TVMV RNA sequence can code for a protein of greater than 16,000 daltons. The 3' non-coding region is AU rich, but no polyadenylation signal (AAUAAA) has been identified. Further characterization of the TVMV nucleotide sequence and of the protein sequence deduced from the viral RNA is now in progress.

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TOBACCO RINGSPOT VIRUS INFECTIONS GENERATE HIGH MOLECULAR WEIGHT RNAs WITH NUCLEOTIDE SEQUENCES OF SATELLITE TOBACCO RINGSPOT VIRUS RNA. George Bruening+, M. Lynn Russell+, Irving R. Schneider+ and Robert M. Goodman*, +Department of Plant Pathology, University of California at Davis, and *Calgene, Inc., 1920 Fifth Street, Davis, California 95616.

The satellite RNA of tobacco ringspot virus (STobRV RNA) is encapsidated in tobacco ringspot virus (TobRV) coat protein and reduces the extent of TobRV accumulation in inoculated plants. We inoculated Red Russian tobacco with apparently satellite RNA-free TobRV from soybean and separately collected tissue from the inoculated lower leaves and from upper leaves. Extracts of the former induced a severe infection that killed Blackeye 5 cowpeas. In contrast, extracts from the upper leaves of the tobacco plant induced only mild symptoms, characteristic of infections by TobRV + STobRV RNA. RNA with the mobility of STobRV RNA was detected in extracts of the cowpea leaves by sequence-specific probes. Extracts of tobacco leaves showed more slowly migrating zones that we interpret as possible high molecular weight precursors of the satellite RNA.

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ANALYSIS OF ANTIGENIC SPECIFICITY OF MONOCLONAL ANTIBODIES TO SEVERAL POTYVIRUSES. Ramon Jordan and John Hammond. USDA-ARS, BARC-W, HSI, F&NCL, Beltsville, MD 20705.

Using a mixture of potyviruses [including six strains of bean yellow mosaic virus (BYMV)] as immunogen, forty-five potyvirus specific monoclonal antibody (McAb) secreting hybridoma cell lines were generated. Thirty-two of these McAbs react only with one or more of the BYMV isolates tested. The remaining thirteen McAbs reacted with BYMV as well as with at least one of the 15 other potyviruses tested. The McAbs gave higher A_{405} values in indirect ELISA with dissociated antigens than with intact virions. The reactivities of these McAbs indicates the presence of conserved coat protein antigenic determinants common to members of the potyvirus group previously characterized as serologically distinct or unrelated. These serological results will be discussed in relation to DNA:RNA hybridization analysis using a c.800 bp cDNA clone prepared from the 3' end of BYMV RNA.

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TWO VIRUSES OF VANILLA IN SOCIETY ISLANDS. G. C. Wisler, F. W. Zettler and L. Mu. Div. Plant Ind., P.O.Box 1269, Gainesville, FL 32602; Plant Path. Dept., Univ. FL, Gainesville 32611; and GERDAT-IRAT, B.P. 494, Papeete, Tahiti, Society Islands

Single infections of cymbidium mosaic virus (CyMV) and an unidentified potyvirus were detected in 2 and 7 of 43 Vanilla tahitensis plants, respectively, from Huahine, Raiatea, Tahaa, and Tahiti. Both viruses threaten vanilla production, which is projected to increase from 2.5 to 150 tons/year by 1990. Identity of CyMV was confirmed by SDS immunodiffusion serology. Potyviral infected plants had conspicuous foliar mosaic and distortion symptoms. Uranyl acetate-stained leaf extracts revealed flexuous rod-shaped particles (80% of 100 were 767 nm long). Leaf tissues stained in calcomine orange/Luxol brilliant green had potyvirus-like cytoplasmic inclusions. Thin sections stained in uranyl acetate/lead citrate had cylindrical inclusions like those of subdivision III potyviruses (Edwardson. 1974. Univ. FL Monogr. 4). The potyvirus was transmitted to 12 of 16 Vanilla pompona test plants, each inoculated with 10-25 Myzus persicae aphids allowed $\leq$1 min acquisition probes.

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OCCURRENCE OF CUCURBIT VIRUSES IN N.J. AND AFFECTS OF STORAGE CONDITIONS ON DETECTION BY ELISA. R. F. Davis and M. K. Mizuki, Dept. of Plant Pathology, Cook College, N.J. Agric. Exp. Sta., Rutgers Univ., New Brunswick, N.J. 08903, and Dept. Pl. Virol., Inst. Biologico-S. Paulo, Brazil, C.P. 7119, respectively.

A disease of cucurbits resulting in crop losses of up to 100% occurred in New Jersey during late summer, 1985. Zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus 2 (WMV-2), papaya ringspot virus W, and cucumber mosaic virus were detected by double sandwich indirect ELISA in 83, 57, 36, and 2 of 94 samples from zucchini foliage and fruits, yellow squash, and pumpkin (Cucurbita pepo), winter squash (C. moschata), and cucumber (Cucumis sativus), collected from fields in southern N.J. Many samples were infected with a mixture of two or more of these viruses, most frequently ZYMV and WMV-2. Conditions at which samples were stored affected the detection of viruses by ELISA in single and in mixed infections. Some viruses in mixed infections could not be detected in test plants mechanically inoculated with those samples.

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POTENTIALITY OF MILD VIRUS MUTANTS FOR CONTROL OF PAPAYA RINGSPOT VIRUS IN TAIWAN. Shyi-Dong Yeh and Hui-Liang Wang, Dept. Plant Pathology, Chung Hsing University, Taichung, Taiwan, ROC, and Dept. Plant Protection, TARI, Fengshan, Taiwan, ROC.

Two nitrous-acid induced mild mutants of papaya ringspot virus (PRV), PRV HA 5-1 and 6-1, were used to study the potentiality of using cross protection for control of PRV in Taiwan. Both mutants did not cause severe damage on the major commercial papaya varieties and induced symptomless infection in the test plants of Chenopodiaceae and Cucurbitaceae, indicating that the possibility of damaging the protected crop and other crops in its vacinity is minimal. Also, under greenhouse conditions, HA 5-1 and 6-1 provided a high degree of protection in papaya against the severe effect of two prevalent PRV strains of Taiwan. A very efficient method of mass inoculation was achieved by using a spraying gun, standard nozzle of 1.2 mm, with pressure of 4-8 kg/cm^2 at 10-20 cm distance. In general, both mutants meet the requirements as an useful protectant strain and have a great pontentiality for control of PRV in Taiwan.

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THE USE OF SEMI-SYNCHRONOUS REPLICATION IN CROSS PROTECTION STUDIES. M. G. Tiffany and J. A. Dodds, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Differential temperature inoculation (DTI) involves keeping inoculated plants at a non-permissive temperature (5°C) and then shifting them to a permissive temperature (25°C). This semi-synchronous replication technique was used in cross protection (CP) studies in tobacco plants infected with two strains of cucumber mosaic virus (CMV-S and CMV-P). CMV-S DTI inoculated plants were challenged with a severe strain, CMV-P, at various times after transfer to 25°C. CP was first detected in inoculated leaves when challenge inoculation was 3-4 days after temperature shift, which was 1-2 days after initial symptom expression of CMV-S. Greenhouse plants expressed CP 7-8 days post protection inoculation. The DTI technique seems to induce a rapid onset of CP by CMV-S.

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SCREENING PEANUT GENOTYPES (ARACHIS HYPOGAEA L.) FOR RESISTANCE TO ASPERGILLUS FLAVUS GROUP OF FUNGI AND AFLATOXIN PRODUCTION. H. A. Azaizeh and R. E. Pettit. Department of Plant Pathology and Micro., Texas Agric. Exp. Sta., College Station, Tx 77843.

Five peanut genotypes were evaluated for resistance to the A. flavus group of fungi in artificially infested soil in greenhouse and box plot experiments. Three moisture regimes were imposed 100 days after planting: 1, drought stress until harvest; 2, 15 days drought stress followed by optimum irrigations; and 3, no drought stress. To determine possible resistance mechanisms, tannin-related compounds were extracted from peanut pegs, seed coats, and cotyledons. Infection of kernels, pods, and pegs differed among genotypes. PI 337409, with seed coat resistance, was most highly infected; its seed contained up to 230 ppb aflatoxin. Genotype SN55-437 had less A. flavus infection and no detectable aflatoxin. Differences in tannin content were noted. SN55-437 tannins inhibited A. flavus in liquid culture and reduced aflatoxin production. Extracts from PI 337409 and PI 365553 failed to inhibit fungal growth or aflatoxin elaboration.

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A PARTIAL, POTENTIALLY RACE-NONSPECIFIC RESISTANCE TO ANTHRACNOSE IN BRAZILIAN LAND RACES OF DRY BEANS. M. J. Havey, EMBRAPA CNPAF, Caixa Postal 179, 74000 Goiânia-GO, Brazil.

Land races of dry beans (Phaseolus vulgaris L.) from five states of Brazil were evaluated in field nurseries for partial resistance to anthracnose (Colletotrichum lindemuthianum Scrib.). Nurseries (1.5x15m) were planted by alternating rows of the land race with one row of the susceptible check cultivar Carioca. Twenty-one days after planting, plants were sprayed until runoff with a conidial suspension of race Brasileiro I (BI). Evalutions of leaf and stem symptoms were made 8 and 11 days after inoculation. Resistant plants were selected based on significant differences from the mean disease severity index (DSI) of adjacent rows of Carioca at the 5% level. Selected plants were allowed to self. S_1 families were then evaluated in environmentally controlled chambers and again in the nurseries for reaction to races BI, delta, and kappa. Families partially resistant, relative to Carioca, to the three races of anthracnose under field conditions and in the chambers were retained.

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STRUCTURAL ASSESSMENT OF PEANUT CULTIVARS FOR ASPERGILLUS FLAVUS POD RESISTANCE. R. D. Henson, R. E. Pettit, and R. A. Taber. Department of Plant Pathology and Microbiology, Texas A&M University, Texas Agri. Exp. Sta., College Station, TX 77843.

Five peanut (Arachis hypogaea) cultivars were assessed for resistance to pod colonization by Aspergillus flavus. Full-sized, sound pods in 9 maturity classes were collected from field plots, dipped in an A. flavus conidial suspension, and incubated 7 days at 27 C and 95% R.H. The shells were subsequently softened in ethylene diamine and processed for microscopic examination. Rate and degree of lignin deposition in sclerenchyma tissues differed among cultivars and maturity classes. Sclerenchyma bands developed earlier and thickened more extensively in cultivars Toalson and PI 365553 compared to Florunner, TSN/UF 73-4022, and Starr. Inoculated immature pods of all cultivars were rapidly colonized by A. flavus. Shells stained to locate A. flavus hyphae in the tissues revealed heavily lignified sclerenchyma bands within the mesocarp that restricted the spread of A. flavus from the mesocarp into the endocarp.

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PARSNIP CULTIVAR RESISTANCE TO PHOMA CANKER. R.F. Cerkauskas, Agriculture Canada, Vineland Research Station, Vineland Station, Ontario, LOR 2E0

Resistance of parsnip (Pastinaca sativa) cultivars to Phoma complanata was evaluated at Bradford Muck Research Station and at Vineland using a randomized complete block design with 5 and 10 cultivars in the respective locations. Plants were inoculated with a suspension of 250,000 spores/ml and harvested 65 and 34 days later at the respective sites. Significant (P=0.05) differences between cultivars were found for percentage of roots with canker, plant stand and weight of harvestable parsnips. Harris Model, the most susceptible cultivar, had 90% and 74% of roots with canker at Bradford and Vineland respectively. Hollow Crown and All American were the most resistant cultivars with 3% and 6% Phoma canker respectively. The most resistant cultivars had the greatest stand and weight of harvestable parsnips. Disease development on foliage of resistant cultivars was significantly less than on susceptible cultivars and the final foliage ratings were highly correlated with percent incidence of Phoma canker on roots.

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REMOVAL OF DUVATRIENEDIOLS FROM THE SURFACE OF TOBACCO LEAVES INCREASES THEIR SUSCEPTIBILITY TO BLUE MOLD. M. Reuveni, S. Tuzun, J. S. Cole, M. R. Siegel and J. Kuć, Plant Pathology Department, University of Kentucky, Lexington 40546.

Dipping leaf strips of greenhouse or field-grown tobacco (Ky-14) plants into acetone for 1 s prior to inoculation with sporangia of Peronospora tabacina Adam markedly increased their susceptibility to blue mold, as determined by disease severity and sporangial production. TLC and GLC analyses of the acetone extracts indicated that 95% or more of the major cuticular diterpenoids α- and β-4,8,13-duvatriene-1,3-diols (DVT) were removed from the surface by dipping for 1 s. Aqueous suspensions of the acetone-soluble constituents, as well as authentic DVT, inhibited sporangial germination of P. tabacina (ED_{100} = 25 ppm). When DVT was removed from a leaf surface and added back to the same leaf strip, the resistance of the leaf tissue was restored. As tobacco plants aged, their susceptibility to blue mold decreased and the quantity of DVT on leaf surfaces increased. The data support a role for DVT in the resistance of tobacco against blue mold.

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REMOVAL OF ANTIFUNGAL FACTORS FROM LEAVES OF TOBACCO WITH ACETONE AND ITS EFFECT ON GENETIC RESISTANCE AND INDUCED SYSTEMIC RESISTANCE TO BLUE MOLD. S. Tuzun, M. Reuveni, M. R. Siegel and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Dipping leaf strips from genetically resistant and induced systemically resistant cultivars in acetone for 1 sec. removed >90% of the fungitoxic leaf surface compounds α- and β-4,8,13-duvatriene-1,3-diols (DVT) and increased their susceptibility to blue mold. The susceptibility of strips from genetically and induced resistant plants dipped in acetone remained lower, however, than that of dipped strips from susceptible burley Ky 14 of the same age. Approximately twice as much DVT was removed from the most resistant cultivar, Ovens 62, and systemically induced burley Ky 14 plants than from uninduced burley Ky 14 and the resistant cultivars Incekara and Izmir Ozbaz. DVT may contribute to resistance of Ovens 62 and systemically protected Ky 14; however, it is apparently not the sole determinant of resistance.

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PROTECTION OF TOBACCO AGAINST BLUE MOLD CAUSED BY METALAXYL-TOLERANT STRAINS OF PERONOSPORA TABACINA BY STEM INJECTION WITH THE FUNGUS. S. Tuzun, W. Nesmith, M. Wiglesworth, and J. Kuć, Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546 and J. Juárez, TABAMEX, San Andres, Tuxtla, Veracruz, Mexico.

Metalaxyl-treated burley and Mexican cigar tobaccos, grown on the Gulf Coast of Mexico, were stem-injected with metalaxyl-tolerant strains of P. tabacina. Highly significant protection against blue mold caused by metalaxyl-tolerant strains of P. tabacina was observed on stem-infected plants compared to non stem-infected controls, regardless of soil or foliar applications of metalaxyl. Protection against tolerant strains of P. tabacina also appeared to occur naturally in nearby commercial tobacco plantings where a ten-fold reduction in blue mold severity, without stunting, was observed on plants having stem necrosis as compared to plants without stem necrosis. The necrosis resembled that on stem-injected plants.

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AN ELECTRON MICROSCOPIC STUDY OF HOST RESPONSE AND DEVELOPMENT OF COLLETOTRICHUM LAGENARIUM IN SYSTEMICALLY IMMUNIZED CUCUMBER. X.-L. Xuei, Cornell Univ., Ithaca, NY 14853; U. Jarlfors and J. A. Kuc, Univ. of Kentucky, Lexington, KY 40546.

To learn more about the mechanisms of systemic immunization, cucumber plants were inoculated on the first true leaf with conidia of C. lagenarium (immunized) or treated with water (control) when leaf 2 was ca. expanded. Seven days later, leaf 2 was challenged with conidia of the fungus. Ultrastructural differences between the cell walls of immunized and control tissues of leaf 2 were not apparent before challenge. In controls, penetration was frequent 48 h after challenge and was followed by extensive ramification and host cell damage. In immunized tissues, penetration was rarely seen and started later. Buckling inward of penetration pegs was evident 24 h after challenge, and host walls were highly electron dense subjacent to appressoria. Where present, aborted penetration pegs were thin and usually surrounded by large papilla.

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IMAGE ANALYSIS OF XYLEM ALTERATIONS IN SCAFFOLD CITRUS ROOTS INFECTED WITH Fusarium solani. C. H. Blazquez, and G. J. Edwards, Univ. of Fla., IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, and Stan Nemec, USDA-ARS-SAA, 2120 Camden Rd., Orlando, FL 32803

Internal characteristics of Fusarium solani infected citrus scaffold roots were determined using an image analyzer (LMS), with a video camera positioned in the trinocular tube of a Nikon S-Ke II microscope. Fields of view were recorded on a VHS video tape recorded and transferred to a soft disk video motion analyzer run by an Apple II+ microcomputer. Roots containing sectors of healthy and infected tissue were sectioned on a cryostat and stained with Schiff's reagent. More paren-

chyma and fiber cell wall mass was observed in cross-sections of healthy than in diseased tissue. Total vessel area was greater in diseased sectors than healthy sections. These results would not have been easily obtainable with other analytical systems.

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LATE BLIGHT EARLY DETECTION IN TOMATO FIELDS WITH 35 MM COLOR INFRARED AERIAL PHOTOGRAPHY. C. H. Blazquez, and L. E. Hedley. Univ. of Fla., Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

Weekly photographs of ground tomato fields were taken with paired 35 mm cameras, loaded with color and color infrared film. Films were visually interpreted, and potential sites of infection were confirmed by ground inspection. Diseased plants were observed better with color infrared than with color film. Spectrophotometric measurements of films in wavelengths of 400 to 700 nm produced two intensity peaks, one at 480 nm and another at 610 nm. Dividing the first peak by the second produced a ratio greater than 1 and indicated a correlation with increased disease. These results agree with earlier observations and suggest than spectrophotometric measurements of tomato plant photographs taken with color infrared film are effective in detecting late blight from aerial color infrared photographs.

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THE USE OF THE INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY (I-ELISA) AND A FLUORESCENT ANTIBODY (FA) TECHNIQUE TO IDENTIFY MACROPHOMINA PHASEOLINA (TASSI) GOID. P. T. Rotkis, S. M. Alcorn, Dept. of Plant Pathology, Univ. of Az. Tucson, AZ. 85721

Rabbit antisera were made against cell wall and soluble proteins of hyphae and microsclerotia (MS) of M. phaseolina. Hyphal and MS antisera were used in I-ELISA and FA tests in attempts to locate M. phaseolina within tissues of Euphorbia lathyris. Using the I-ELISA, hyphal antiserum was specific in that it reacted uniformly to cultured hyphae of the homologous antigen and to hyphae of 32 additional isolates of M. phaseolina collected from different hosts and geographical locations. The same serum did not react to hyphae of Rhizoctonia solani, Fusarium compactum, F. oxysporum, and Verticillium dahliae. Reactivity of the MS antiserum will be discussed. M. phaseolina could not be detected in infected E. lathyris or Glycine max using the hyphal antiserum in the I-ELISA. However, hyphae fluoresced in fresh sections of infected E. lathyris when a fluorescein-conjugated secondary anti-rabbit antibody was used.

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PATHOGENIC VARIATION IN SINGLE-CONIDIAL ISOLATES OF PYRICULARIA ORYZAE. Bai-chai Wu and F. M. Latterell, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701

In a continuing study of the controversial subject of pathogenic variation in Pyricularia oryzae, the rice blast pathogen, two cultures representing races IB-1 and ID-13 were isolated from Texas specimens collected in 1985 and 1975, respectively. Among 120 single conidial isolates (SCI), 60 from each original culture, one pathogenic variant appeared (from ID-13) which gained pathogenicity to two of the eight international differential cultivars, and lost pathogenicity to one, becoming race IE-1. Among 60 more SCI from single lesions of the original specimen of ID-13, no variants appeared. From 60 more SCI from three lesions from these tests, one isolate mutated in both cultural and pathogenic characters, to race IB-5, a gain of pathogenicity to three cultivars. The frequency of pathogenic variation we observed is far lower than reported by some workers. Sources of error that have led to controversial results are suggested, and improved testing methods proposed.

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SAMPLING PROCEDURES FOR ASSAYING POPULATIONS OF PHYTOPHTHORA PARASITICA IN FLORIDA CITRUS SOILS. H. A. Sandler, L. W. Timmer, and J. H. Graham, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850.

Propagule recovery depended on the moisture and temperature treatment of soil samples and on sample site location. When the soil moisture of the samples was maintained at field capacity, population estimates of P. parasitica varied little for the first 8 days after collection. Air-drying soil reduced fungal populations drastically. Heat or cold treatment prior to assay did not increase propagule recovery. Sampling to a soil depth of 92 cm indicated that population and root density decreased logarithmically with depth. Sample site location studies indicated that P. parasitica populations and root densities were higher under the tree canopy than outside of the canopy between trees and between rows. In general, populations of P. parasitica were linearly correlated with root densities.

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EFFECT OF TILLAGE AND CROP ROTATION ON PREMATURE DYING OF SOYBEANS. R.H. von Qualen, T.S. Abney & D.M. Huber, Dept. Btny. & Pl. Path., Purdue Univ. and USDA-ARS, W. Laf., IN 47907.

Premature dying -"Sudden Death Syndrome"- occurred in 'Miami' soybeans in the USDA-ARS/Purdue Integrated-Pest-Management (IPM) systems established in 1980. This pattern of dying also occurred in southern Indiana fields. Premature dying was more extensive in continuous soybean (S-S) than the corn-soybean (C-S) rotation, and was lowest in wheat-corn-soybean (W-C-S) rotation (10,6, and 1%, respectively). The S-S, C-S, and W-C-S rotations yielded 2650, 3047, and 3262 kg/h, respectively. Premature dying varied in the three tillage systems, but appeared to be increased by no-till. The dying pattern paralleled yield reductions and was also consistent with observations of Diaporthe/Phomopsis on stems and pods. Although a definite causal agent has not been verified, 1985 IPM data indicate involvement of the Diaporthe/Phomopsis complex. Soil fumigation with metham (sodium methyldithiocarbamate) reduced the incidence of premature dying and increased yield but was less effective with no-till and chisel than in the conventional tillage plots.

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PATHOGENICITY OF FUSARIUM SPECIES ASSOCIATED WITH COTTON SEEDLING DISEASES IN NORTHERN LOUISIANA. P. D. Colyer, Louisiana State University Agricultural Center, Red River Research Station, Bossier City, LA 71113.

Fusarium spp. isolated from diseased cotton seedlings were single-spored and transferred to acidified potato-carrot agar for identification to species and for pathogenicity testing. Approximately 85% of the Fusarium spp. recovered were either oxysporum or solani. Other species isolated included acuminatum, moniliforme, and graminearum. The different species and isolates within a species were highly variable in their pathogenicity based on a visual evaluation system. Few isolates caused damping-off, but most caused root and hypocotyl discoloration and/or "root nubbing." The solani isolates were slightly more pathogenic than other species.

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REDUCTION IN APHANOMYCES ROOT ROT INDUCED BY REPEATED PEA CROPPING UNDER ARTIFICIAL CONDITIONS. J. L. Parke, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

Soils were collected from 14 Wisconsin pea fields naturally infested with Aphanomyces euteiches f. sp. pisi. In an attempt to enhance the development of soil microorganisms suppressive to Aphanomyces, each soil was sown with peas in six consecutive cropping cycles in a growth chamber experiment. Environmental conditions conducive to disease development were maintained during each of the 19-day growing cycles. In most soils, disease incidence increased during the initial cycles and remained high in subsequent cycles. In three soils, however, there was a decline in Aphanomyces root rot from cycle 1 to cycle 6; disease incidence was reduced from 98% to 36%, 45% to 9% and from 99% to 29%. Dilution plating on selective media with soil from each of the planting cycles indicated that changes in microbial populations were associated with the decline in disease incidence.

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SEASONAL GROWTH OF CITRUS FEEDER ROOTS AND SHOOTS AND RHIZOSPHERE POPULATION FLUCTUATIONS OF PHYTOPHTHORA PARASITICA. A. Lutz and J. Menge, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Shoot and feeder root growth of navel orange trees and populations of Phytophthora parasitica in the rhizosphere were monitored in the field. Feeder root flushes were examined using rhizotrons. The different kinds of P. parasitica propagules and relative abundance of each kind were assessed at different times of the year by microscopic examination of slides coated with a selective medium and layered with a soil slurry. The main shoot flush occurred during March and April. Feeder root

growth followed shoot growth and began in May, ceasing in November. Populations of P. parasitica increased concurrently with root growth from 3 prop/g soil in May to a maximum of 105 prop/g soil in July and August. Sporangia and zoospores accounted for 62% of the propagules that were recovered from samples taken in November, and dropped down to 5% in February. Chlamydospores increased from 33 to 68%, and oospores increased from 5 to 18% from November to February.

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FUSARIUM BASAL ROT OF GARLIC. F.J. CROWE, Oregon State University, Central Oregon Experiment Station, Redmond, OR 97756; S.C. SCARDACI and A.S. GREATHEAD, Farm Advisors, University of California Cooperative Extension, Colusa County, Colusa, CA 95932 and Monterey County, Salinas, CA 93901, respectively; D.H. HALL and P.A. SOMERVILLE, University of California, Davis, CA 95616; and E. KURTZ, Agricultural Consultant, P.O. Box 1763, Salinas, CA 93902.

A new disease of garlic incited by Fusarium roseum f. sp. culmorum (FRC) is described. Decay of stem plate and below-ground protective and storage leaves results in reduced emergence; stunting, chlorosis, withering and death of leaves; premature death of plants; reduced bulb size and bulb decay. Further loss at harvest occurs when cloves shatter from bulbs that have damaged stem plates and leaf sheaths. Additionally, for garlic grown as seed, decayed and infected bulbs carried through storage contribute to poor seed quality, and possibly serve as sources for soil infestation in subsequently planted fields. The garlic isolates of FRC also cause common root rot of wheat and barley, but have not infected onions or various other crops tested. In contrast, other cereal isolates of FRC are not pathogenic to garlic. This garlic disease has become a major factor in the California and Oregon garlic industry since its discovery in 1976.

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DIFFERENTIAL EFFECTS OF POTATO GENOTYPE AND METAM SODIUM ON THE POPULATION DYNAMICS OF VERTICILLIUM SPP. Davis, J.R. and L.H. Sorensen, Univ. of Idaho Res. & Ext. Center, Aberdeen ID 83210.

Continuous cropping with a Verticillium-resistant potato clone A66107-51 (-51) or soil fumigation with metam sodium (ms) may have differential effects on two soilborne Verticillium spp. Field experiments at two different locations during a period of several years provided evidence that continuous cropping with the -51 potato resulted in higher soil populations of Verticillium tricorpus than under continuous cropping with the Verticillium-susceptible cv Russet Burbank. Sprinkler-applied ms at 468 l/ha suppressed V. dahliae populations in soil while having no effect on V. tricorpus. Since both greenhouse and field studies have indicated positive responses with V. tricorpus (increased growth and wilt suppression), these differential effects are interpreted to have possible significance for the management of Verticillium wilt of potato.

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ASSOCIATION OF PYTHIUM SPP. WITH SEEDLING DISEASE AND FEEDER ROOT DECLINE IN RICE. M. Pillay, R. W. Schneider and M. C. Rush. Dept. of Plant Path. and Crop Phys., La. Agr. Exp. Sta. LSU Ag. Ctr., Baton Rouge, LA 70803.

Pythium spp. were the dominant fungi recovered from rice roots in a water-seeded field in Crowley, La., during the 1985 crop. Soil treatment with metam-sodium, under field and greenhouse conditions, gave highly significant increases in plant stand and growth over the untreated control. Despite severe reinfestation of treated field plots by Pythium spp. a marked increase in yield was achieved. Metalaxyl significantly delayed and reduced root colonization by Pythium spp., but had a depressive effect on plant growth and yield. P. dissotocum and P. spinosum which were previously implicated only in the water-mold disease of water-seeded rice, incited a root infection beyond the seedling stage in pot experiments. Some of the Pythium spp. isolated caused severe seedling mortality under water-seeded conditions and others caused yellowing and stunting of 4 to 5-leaf stage seedlings under non-flooded conditions in the greenhouse.

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INTENSIFIED SOIL SOLARIZATION WITH CLOSED GLASSHOUSES. J. Katan, I.Mahrer, R. Avissar, O. Naot and A. Gamliel, Dept. of Plant Pathology and Microbiology, and Dept. of Soil and Water Sciences, The Hebrew University, Faculty of Agriculture, Rehovot 76100, Israel.

Soil solarization or solar heating (SH) is effective in raising soil temperatures and killing soilborne pathogens during June-August in Israel but not during May or September which are cooler months with lower solar irradiation. Tarping the soil inside a closed glasshouse (CG) was tried as a means for intensifying SH for the improvement of its effectiveness. Both in May and Sept. 1985, SH in CG effectively reduced populations of Verticillium dahliae and Fusarium oxysporum f. sp. niveum at 10 and 30cm depths, while no pathogen control was obtained in a solarized soil outside CG. A partial pathogen control was obtained in a closed, but nontarped CG. Soil temp in the tarped CG were 2-5C higher than in the tarped soil outside. Air temp in the CG was about 20C higher than that outside but this was not reflected in a comparable increase in soil temp due to the reduced solar irradiation inside CG. A numerical model for predicting tarped and nontarped soil temp inside CG was developed, with satisfactory results.

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EFFECTS OF THE BIOLOGICAL SEED TREATMENT BACILLUS SUBTILIS ON THE RHIZOSPHERE ECOLOGY OF PEANUTS. J. T. Turner and P. A. Backman, Dept. of Plant Pathology, Auburn University, AL.

Ecological studies indicate that several factors may contribute to the varying yield responses of peanuts treated with B. subtilis. Colonization by B. subtilis was greatest on the basipetal portion of the taproot, and decreased toward the root tip. This distribution may explain why improvements in root quality have been detected only for the upper taproot region. Bacterized peanut plants have shown increased Rhizobium nodulation and increased levels of nitrogen. Mycorrhizal associations were unaffected. Bacterized plants grown in a rhizotron produced more secondary and feeder roots than did nontreated plants. Repeated use of B. subtilis treated seeds resulted in soil populations sufficient to bacterize roots of winter wheat, weeds, and subsequent crops of nontreated peanuts. Annual application of the bacterium as a seed treatment may offer diminished benefits once the organism has become established in the field.

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EPIDEMIOLOGY OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA AND STEM CANKER DEVELOPMENT IN SOUTHERN SOYBEANS. Elisa F. Smith, P. A. Backman, and M. A. Crawford, Dept. of Plant Pathology, Auburn University, AL.

Four factors related to soybean infection by Diaporthe phaseolorum var. caulivora (Dpc) and stem canker severity have been determined. Environmental factors, temperature, rainfall, and soil moisture were found to influence ascospore development and dispersal. Infections occurring shortly after emergence result in the greatest disease severity, while infections occurring during late vegetative or early reproductive stages remain asymptomatic. Data indicate that both resistant and susceptible cultivars are equally infected by Dpc and all produce fungal inoculum. However, disease severity and quantity of inoculum produced are related to the level of resistance. Plant stress brought on by drought, girdling insects or nematodes increase disease severity.

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CHANGES IN PAL, TOTAL PHENOLICS AND LIGNIN AND SUSCEPTIBILITY OF INJURED ORANGE RIND TO INFECTION BY PENICILLIUM DIGITATUM. G. Eldon Brown. Florida Dept. of Citrus, CREC, 700 Experiment Station Road, Lake Alfred, FL 33850.

Following injury to the exocarp at 30C near 100% R.H., maximum activity of PAL (phenylalanine ammonia-lyase) usually occurred within 48 hr, total phenolics decreased at 24 hr but reached original or higher levels by 48 to 72 hr., and lignin increased. Total phenolic content of injured rind decreased with maturity, but the ability to synthesize PAL, phenolics and lignin increased with maturity. Presence of P. digitatum in normally resistant injuries suppressed activity of PAL but increased levels of total phenolics and lignin. Activity of PAL and accumulation of total phenolics and lignin were suppressed by ethylene (300 or 1000 ppm) applied to injured fruit. However, levels were enhanced if ethylene was applied to intact fruit before injury. Resistance of fruit, treated with ethylene before injury and inoculation with P. digitatum, was significantly increased in some but not all trials.

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EFFECTS OF HV TOXIN ON SUSCEPTIBLE OAT PROTOPLASTS AS DETERMINED BY AN IMPROVED ASSAY. E. H. Gendloff, R. P. Scheffer, and S. C. Somerville. Michigan State University, East Lansing, MI 48824.

Leaf mesophyll protoplasts were used to assay for HV toxin (victorin). Protoplasts were placed in 250 µl of toxin or control solutions in wells of microtiter plates, incubated for 3 hr, and exposed to fluorescein diacetate to determine via-

bility. The assay was as sensitive and quantitative as was the standard root growth assay. Resistant and control cells were not affected. The assay was used to detect toxin in culture filtrates and extracts in the purification procedure. The effect of protoplast density on toxicity was determined, using 2000 to 20000 protoplasts per well; there were no differences in mortality. Protoplasts were exposed to a range of toxin concentrations for various times, then washed and incubated without toxin. Survival at 3 hr was high for 1 min exposures to toxin, intermediate for 10 min, and minimal for >30 min. The significance of these data in relation to hypothetical toxin receptors will be discussed.

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RAPID INHIBITION OF CHLOROPHYLL SYNTHESIS IN MAIZE LEAVES BY SELECTIVE TOXIN FROM HELMINTHOSPORIUM CARBONUM. J.B. Rasmussen and R.P. Scheffer. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Etiolated maize leaves were pretreated with HC toxin or water for 2 hr and incubated in the light for greening. Chlorophyll was extracted and analyzed spectrophotometrically after various incubation times. Toxin at 20 µg/ml reduced $O.D._{665}$ by 30-40% in susceptible but not in resistant leaves after 4 hr incubation. Using 5 hr incubation times, the selective inhibition of chlorophyll synthesis increased with increases in toxin concentration from 1.0 to 10 µg/ml; the effect leveled off with 40-50% inhibition at 50 µg/ml. There were comparable inhibitions in resistant tissues, provided toxin concentrations were 100-fold higher. This is the most rapid inhibitory response determined to date for HC toxin. The data may provide a basis for an improved bioassay, and may give clues to the mode of action.

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AGLYCONE OF THE SELECTIVE TOXIN FROM HELMINTHOSPORIUM SACCHARI: ISOLATION FROM MYCELIUM AND INCORPORATION INTO TOXIN. H. Nakajima and R.P. Scheffer. Michigan State University, East Lansing, Michigan 48824.

H. sacchari produces oligosaccharide-sesquiterpene toxins (HS toxins A, B, and C) that are required for infection and disease development. Two free sesquiterpenes were isolated from mycelium but not from culture fluids of the fungus. One sesquiterpene was identified by HPLC and MS as the aglycone of HS toxin C. This sesquiterpene was obtained also by enzymatic hydrolysis of HS toxin C. The other sesquiterpene was identified as the 2-keto form of the same compound. The aglycone of toxin C was labeled with ^{3}H by successive treatments with active manganese dioxide, sodium boro(^{3}H)hydride, and lithium aluminum hydride. The labeled compound was fed to cultures of H. sacchari; radioactivity was incorporated into HS toxin C and into its lower mol wt homologs. The results suggest a metabolic route for biosynthesis, which will be discussed.

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ATTRIBUTES OF ISOLATES IN VEGETATIVE COMPATIBILITY GROUPS OF SOUTHERN Diaporthe phaseolorum. R.C. Ploetz, and F.M. Shokes. NFREC, Route 3, Box 4370, Quincy, FL 32351.

The cultural morphology, in vitro growth rate, virulence, and sensitivity to Bayleton and Benlate fungicides of isolates in vegetative compatibility groups (vcgs) of southern Diaporthe phaseolorum (incitant of soybean stem canker in the southeastern United States) were studied. In general, a high degree of homogeneity was observed among isolates in the same vcg with regard to the characteristics above. Isolates in a given vcg were uniformly virulent (scored as lesion length) on seedlings of a given soybean cultivar; lesion length on 8 cultivars interacted significantly ($P<0.05$) with the 6 vcgs tested. These results support the hypothesis of a heterogeneous population of the pathogen with regard to virulence, and the possible existence of physiologic races.

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SEQUENCE VARIATION OF TMV. David L. Beck and William O. Dawson, Department of Plant Pathology, University of California, Riverside, CA 92521.

The sequence heterogeneity of tobacco mosaic virus (TMV) strain U1 was studied. Four independent cDNA clones of TMV were sequenced through the 5' terminal 250 nucleotides and all were identical. The sequence determined for the 5' terminus of TMV-U1 was 100% homologous to the shorter of two reported variants [Proc. Natl. Acad. Sci. USA (1982) 79, 5818]. In order to determine the effect of host passage upon sequence variation within a population of virus, in vitro transcripts of pTMV 204 (a plasmid which contains full-genomic cDNA of TMV-U1) were propagated in tomato. The sequence of the viral genome after passage in tomato is compared to that of the parental U1 and tomato strain of TMV.

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IDENTIFICATION OF TMV SEQUENCES INVOLVED IN DISEASE SYMPTOMS. D. A. Knorr, and W. O. Dawson, Dept. Plant Pathology, University of California, Riverside, CA 92521.

To identify sequences of tobacco mosaic virus (TMV) associated with symptom expression, a parental population of TMV, strain U1, was generated from RNA transcribed in vitro from pTMV 204, a plasmid which contains a full-length cDNA copy of the viral genome. After treatment of progeny virus with nitrous acid, independent mutants were selected that induced hypersensitivity in normally systemic hosts, or altered systemic symptoms. One class of mutants causes local lesions on N. sylvestris and systemic necrosis on N. tabacum, yet almost no symptoms in tomato. Another mutant class causes brilliant yellow mosaic symptoms in N. tabacum and N. sylvestris. To determine regions of the viral genome responsible for inducing different symptoms, fragments from cDNA clones of independent mutants are being substituted independently into pTMV 204 in place of the corresponding parental sequences. Effects of different regions of the TMV genome on symptom expression will be discussed.

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CHARACTERIZATION OF MUTANTS OF TMV WITH INSERTIONS OR DELETIONS IN THE COAT PROTEIN GENE. W. O. Dawson and G. L. Grantham. Department of Plant Pathology, University of California, Riverside, CA 92521.

To examine whether the coat protein of tobacco mosaic virus (TMV) is involved in functions other than assembly into virions, mutants were constructed by making insertions or deletions in the cDNA of the genome of TMV from which infectious transcripts can be produced. The cDNA was cleaved at a unique Acc I restriction site in the coat protein gene, digested with nuclease Bal 31, followed by insertion of a Xho I linker. Transcripts of selected cDNA constructions were used to infect tobacco plants. Most mutants spread within inoculated leaves and systemic infection throughout the plant occurred sporatically. Other biological characteristics of these mutants will be described.

307 Withdrawn

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IN VITRO TRANSLATION OF MAIZE RAYADO FINO VIRUS RNA. Ana M. Espinoza, Pilar Ramirez and Pedro Leon. Centro de Investigacion en Biologia Celular y Molecular, Univ. de Costa Rica, San Jose.

In a nuclease-treated reticulocyte system, maize rayado fino virus (MRFV) RNA strongly stimulated incorporation of radioactive amino-acids into TCA precipitable polypeptides. Fluorographic analysis revealed a complex of polypeptides (160K to 22K) directed by this RNA. Addition of exogenous tRNA or RNA precipitated with 2M LiCl from infected plants caused HMW polypeptides to predominate. The largest product represents the translation of almost the entire viral RNA. Limited proteolysis of translation products revealed a common peptide in all protein bands in the gels. Proteolytic maturation of the polypeptides is not the main strategy for genome expression of MRFV. Reactions with PMSF, DTT and amino-acid analogs produced only slight modifications. Serology indicates that coat proteins of MRFV are not synthesized in vitro when full length RNA is translated. Results suggest that the positive MRFV genome is functionally monocistronic, with subgenomic messengers which are not encapsidated but direct coat protein synthesis.

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MODE OF INHERITANCE OF RESISTANCE IN CORN TO MAIZE CHLOROTIC DWARF VIRUS (MCDV). Eugen Rosenkranz and G. E. Scott. USDA, ARS, Depts. of Plant Pathology and Weed Sci. and Agronomy, Miss. State Univ., Mississippi State 39762.

A 6-parent diallel cross, comprised of 3 MCDV-resistant and 3 MCDV-susceptible inbreds was used to estimate genetic variabi-

lity of host response to MCDV and gain information on the type of gene action involved in MCDV resistance in corn. The 15 possible crosses were grown, in 2 yr, in a screenhouse into which inoculative leafhoppers (Graminella nigrifrons) were released. Each year, ca. 125 plants of each cross were evaluated 6-7 times for presence of disease. In both years, the mean disease incidence of the 9 resistant(R) x susceptible(S) crosses equaled the mean disease incidence for the combined 3 RxR and 3 SxS crosses. Diallel analysis showed a relatively large and highly significant mean square for general combining ability and a relatively small and statistically nonsignificant mean square for specific combining ability. These results strongly indicate that the predominant gene action in the inheritance of resistance to MCDV in corn is of the additive variance type and that dominance variance is insignificant.

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RELATIONSHIP BETWEEN NUMBER OF RESISTANCE GENES TO MAIZE DWARF MOSAIC VIRUS, DISEASE INCIDENCE, AND INCUBATION PERIOD IN CORN. E. Rosenkranz and G. E. Scott. USDA, ARS, Depts. of Plant Pathology and Agronomy, Miss. State Univ., Miss. State 39762

Corn inbreds for which the number of resistance genes to MDMV is known were compared with inbreds of unknown genetic constitution. Earlier, we established that inbreds CI21, Va35, Mp339, T232, and Pa405 have 0, 1, 2, 3, and 5 genes for resistance to MDMV strain A. In the early planted test, CI21, Va35, Mp339, T232, and Pa405 had 100, 71, 45, 6 and 0% diseased plants, respectively, and the "unknown" inbreds Mp313, E199, E663, and SC54 had a disease incidence of 100, 69, 2, and 0%, respectively, 38 days after inoculation. In the late test, CI21, Va35, Mp339, T232 and Pa405 had 100, 62, 42, 16, and 0% diseased plants, while Mp313, E199, E663, and SC54 had a disease incidence of 99, 82, 2, and 0% 39 days postinoculation. We predict that Mp313, E199, E663, and SC54 have 0, 1, 4, and 5 resistance genes. Each additional resistance gene delayed symptom expression by 5-6 days. Thus, it took Va35, Mp339, and T232 19, 24, and 30 days, respectively, to reach the 10% disease incidence.

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REPLICATION OF BEAN RUGOSE MOSAIC VIRUS IN BEAN (Phaseolus vulgaris) PROTOPLASTS FROM SUSCEPTIBLE AND RESISTANT STRAINS. Ana L. Fuentes y Pedro Leon. Centro de Investigacion en Biologia Celular y Molecular. Universidad de Costa Rica, San Jose.

Leaf mesophyll protoplasts isolated from bean rugose mosaic virus (BRMV) susceptible and resistant bean (Phaseolus vulgaris) cultivars were inoculated with purified virus and replication monitored by ELISA. A typical virus growth curve resulted in protoplasts of the susceptible cultivar. Eclipse occurred 4 hrs post-inoculation; exponential increase was seen 12 hrs later and the curve plateaued after 25 hrs. No eclipse or exponential increase was observed in protoplasts from resistant cultivars. Both types of protoplasts also were inoculated with an unrelated virus, Maize Rayado Fino virus (MRFV). In susceptible protoplasts eclipse was observed 12 hrs after inoculation and exponential increase occurred 28 hrs later. In resistant protoplasts, MRFV antigen showed neither eclipse nor exponential increase. Thus resistance is retained in protoplasts, is not virus-specific and suggests a basic mechanism operating at cellular levels, possibly during or after decapsidation.

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RESPONSE OF RHIZOSPHERE MICROORGANISMS TO OZONE AND SIMULATED ACIDIC RAIN. S.R. Shafer, USDA/ARS, N.C. State Univ., Dept. of Plant Pathology, Raleigh, NC 27695-7616.

Seedlings (3-wk-old) of a sorghum x sudangrass hybrid ('Sweet Sioux IV') in pots of non-sterile soil-sand mix were exposed to ozone (O_3) at 0, 0.15, or 0.30 ppm (7 hr/da, 3 da/wk) and simulated rain (SR) adjusted with H_2SO_4 + HNO_3 to pH 5.5, 4.0, or 2.5 (2 cm in 90 min per event; 2 events/wk) over 3 wk in a greenhouse. Effects induced by 0.30 ppm O_3 (compared to 0 ppm) included a 47% suppression of shoot dry weight and a 58% increase in numbers of phosphatase-positive bacteria in the rhizosphere. Effects induced by SR at pH 2.5 (vs pH 5.5) included a 27% stimulation of shoot dry weight, a 100% stimulation of the total bacterial population in the rhizosphere, but a 52% suppression of starch-hydrolyzing bacteria. Numbers of fungi and casein-hydrolyzing bacteria were unaffected by pollutants. Intermediate pollutant levels induced effects intermediate to those specified. Results demonstrate ecological changes in the rhizosphere of plants exposed to air pollutants.

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INFLUENCE OF ACIDIC FOG ON PLANT INJURY AND DISEASE DEVELOPMENT. P. M. McCool and R. C. Musselman. Statewide Air Pollution Research Center, University of California, Riverside, CA 92521

Greenhouse plants were exposed to acidic fog simulating ambient exposures via a portable fogging system. Celery, radish, bean, tomato, strawberry, azalea, zinnia, onion and citrus were measured each week for injury and growth following twice-weekly, 2-hr nighttime fog exposures for 6 weeks. Fog pH ranged from 1.6 to 5.6. Typical plant injury following fog exposures was irregular, bifacial, tan to rust-colored lesions on the exposed foliage. Tissue injury was limited primarily to plants exposed to fogs of pH 3 or below, although degree of injury varied with plant species. Severity of Septoria graveolentis leaf spot of celery was evaluated for plants exposed to acidic fog of pH 1.6, 2.4, 3.2 or 5.6. Septoria infection increased most rapidly in pH 1.6 and 2.4 fog treatments. At 6 weeks, all treatments had similar levels of Septoria infection, except for pH 3.2 (38% lower). Observations indicated that Septoria colonized areas previously injured by acidic fog, which may explain more rapid disease development at the lower pH treatments.

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EFFECT OF OZONE ON BURLEY TOBACCO IN PRESENCE AND ABSENCE OF TOBACCO ETCH OR TOBACCO VEIN MOTTLING VIRUSES. R.C. Rufty and R. A. Reinert, Departments of Crop Science, Plant Pathology, and USDA-ARS, North Carolina State University, Raleigh, 27695

"Weather fleck," caused by atmospheric ozone (O_3) appears to be more severe on burley tobacco infected with tobacco etch virus (TEV) or tobacco vein mottling virus (TVMV). To test the hypothesis of an O_3-virus-cultivar interaction, three cultivars differentially sensitive to O_3 and the viruses were grown in a charcoal-filtered greenhouse air and inoculated with TEV or TVMV. Virus-free plants were used as controls. Plants were exposed to increasing concentrations of O_3 (0.0, 0.05, 0.1, 0.2 and 0.4 ppm) 3 hrs/day, 5 days/wk for 3 wks after virus symptoms were expressed. Symptoms on TEV infected plants resembled weather fleck in all cultivars. Additive or interactive effects between O_3 and TEV were not detected. TVMV infection enhanced damage caused by O_3 on Burley 21 and Greeneville 131, but not on Burley 49. Thus, the effect of O_3 was dependent on specific virus-cultivar combinations.

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IMPACT OF O_3 AND/OR SO_2 ON A FIELD-GROWN POTATO CROP. E. J. Pell, N. S. Pearson, and G. A. McGruer, The Pennsylvania State University, University Park, PA 16802.

Solanum tuberosum L. cv. Norchip were grown in open top chambers. Plants were treated with: charcoal filtered air, nonfiltered air, nonfiltered air and 33, 66 or 99% supplemental O_3, 140, 280 or 560 ppb SO_2, 33, 66 or 99% add on O_3 plus 280 ppb SO_2, and 140 or 280 ppb SO_2 plus 66% add on O_3. O_3 treatments were delivered from 1000-2000 h, 7 days/week from June 14-August 22, 1985. Plants were exposed to SO_2 from 0900-1200 h once every second week for a total of four treatments. On days when SO_2 treatments were applied O_3 exposures began at 1200 h. There was a linear relationship between O_3 dose and number and weight of grade one (>6.35 cm) tubers and weight of all tubers; SO_2 effects were apparent only at the 560 ppb treatment. O_3 reduced total solid content of tubers; SO_2 did not. Effects on total glycoalkaloid and sugar content of tubers were not detected in response to either gas. SO_2 did not enhance the O_3 response. O_3 suppressed the differences between weights of grade one tubers treated with SO2.

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FOLIAR SENSITIVITY OF SHORT-SEASON SOYBEANS TO OZONE AND INHERITANCE OF INJURY RESPONSE. J.P. Damicone, W.J. Manning, S.J. Herbert, and W.A. Feder. Department of Plant Pathology, University of Massachusetts, Amherst, MA 01003.

Soybean genotypes of maturity classes 00,0, and I were evaluated 2 yr for foliar sensitivity to ambient ozone (O_3). PI 153.283 and PI 153.284 were most sensitive exhibiting 25%-50% leaf stippling and defoliation. Nineteen of 35 genotypes, including PI 189.907 and PI 153.317, were tolerant showing little or no visible injury. Crosses were made between 2 O_3-tolerant lines (PI 189.907 and PI 153.317) and 1 O_3-sensitive line (PI 153.283). Parental, F_1,F_2, and backcross populations were exposed to 0.30 ppm O_3 for 4 hr. Injury response distributions were not significantly different among O_3-tolerant parents, reciprocal F_1 popu-

lations, and F_2 populations. F_1 plants were intermediate in injury response compared to parental types. Injury response distribution in the F_2 exhibited 3 peaks and fit a ratio of 3 tolerant: 6 intermediate: 7 sensitive. Foliar sensitivity to O_3 appears to be inherited by two genes with complete dominance at one locus and partial dominance with epistasis at the second.

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EFFECTS OF CHRONIC DOSES OF OZONE ON FIELD-GROWN LOBLOLLY PINE SEEDLINGS. 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) from four full-sib families of loblolly pine (Pinus taeda) were planted in a field and exposed daily (27 May to 25 October) to charcoal-filtered (CF) air, non-filtered (NF) air, or NF air supplemented (12 hr/da) with O_3 to produce O_3 concentrations of 1.33, 1.53, 1.70, or 1.98 x NF in open-top chambers (128 seedlings/chamber). One-fourth of the plants was harvested in late October. Compared to plants exposed to CF air, plants exposed to 1.98 x NF averaged a 10% shorter main stem; a 6% smaller diameter at the root collar; 19% shorter total branch length; 12% less shoot dry weight; and 10% shorter secondary needles at mid-stem. Intermediate O_3 doses caused smaller growth suppressions than the 1.98 x NF. One family exhibited foliar symptoms of O_3 injury at ambient O_3 levels. Remaining plants will be harvested after additional exposures during 1986 and 1987.

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APPLICATION OF GEOSTATISTICS TO SPATIAL STUDIES IN PLANT PATHOLOGY. D.O. Chellemi, K.G. Rohrbach, R.S. Yost, and R.M. Sonoda. University of Hawaii, 3190 Maile Way, Honolulu, HI 96822 and University of Florida, IFAS, AREC, Ft. Pierce, Fl 33454.

Levels of copper placed in plots of silica sand were used to simulate random or aggregated patterns of inoculum. The variance/mean ratio and Moran I statistic for the random and aggregated plots were (1.01, .017) and (2.37, .915) respectively. Semi-variograms from the random pattern showed a "nugget effect" indicating no spatial dependence. Semi-variograms from the aggregated pattern resembled the linear or spherical models associated with spatial dependence. Semi-variograms from copper induced mortality of pepper seedlings transplanted into the random and aggregated plots also resembled a "nugget effect" and the linear or spherical models respectively. Models from geostatistics can be used to describe the spatial dependence of inoculum or diseased plants.

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A RAPID SCREENING TECHNIQUE FOR DETERMINING TIPBURN TOLERANCE IN LETTUCE CULTIVARS AND LACTUCA P. I. LINES. C. L. Patterson and R. G. Grogan, Department of Plant Pathology, University of California, Davis, CA 95616.

Tipburn (TB) is the most important abiotic disease of lettuce. The disease occurs on rapidly growing calcium (Ca) deficient internal lettuce tissue subjected to periods of warm daytime temperatures (> 30 C). Lettuce was planted in 100 ml of quartz sand in 500 ml plastic freezer crispers in a growth chamber. The day/night temperatures were 30 and 15 C, respectively, in 12 h durations. The lettuce seedlings were irrigated with Hoagland's nutrient solution containing 0.0, 1.0, or 100.0 ppm Ca. About twenty-eight days after planting TB symptoms occurred on seedlings irrigated with nutrient solution containing 0.0 and 1.0 ppm Ca. No TB occurred on lettuce irrigated with nutrient solution containing 100.0 pm Ca. This TB screening technique is sensitive, reliable, and requires less time and space than other screening methods.

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EFFECTS OF ACIDIC MIST ON INFECTION EFFICIENCY AND SPORULATION OF PHYTOPHTHORA INFESTANS ON POTATO. A.H.C. van Bruggen, J.F. Osmeloski, and J.S. Jacobson. Boyce Thompson Institute, at Cornell University, Ithaca, N.Y. 14853.

Rooted potato cuttings, cv. Norchip, Monona, and Katahdin, were exposed to simulated acidic mist (pH 2.8-4.6) for 24 hr before and after inoculation with Phytophthora infestans. The mist solutions contained sulfuric and nitric acids in a 2:1 mass ratio, and background ions common in ambient mist. After appearance of symptoms the cuttings were again exposed to acidic mist to induce sporulation. In one series of experiments, in which mist was applied at the same pH before and after inoculation, infection efficiency and sporangium production increased curvilinearly with increasing pH of mist, with maxima at pH 4.0. In another series of experiments exposure of potato leaves to mist at pH 2.8 before inoculation and at pH 4.0 after inoculation resulted in higher infection levels than exposure at pH 4.0 before and after inoculation. However, exposure to mist at pH 2.8 after inoculation reduced infection levels compared to pH 4.0. The post-inoculation pH effect was more important than the pre-inoculation pH effect.

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USING POLLEN TO PREDICT SPECIES RESPONSE TO OZONE STRESS. William A. Feder, Suburban Experiment Station, 240 Beaver St. Waltham, MA 02254

85% of the genes expressed in pollen of some species are also expressed in sporophytic tissues of those species indicating that only 15% of the pollen genes are unique. The genetic program expressed during pollen development is extensive and there seems to be a substantial overlap between genes active in gametophytic and sporophytic tissues, indicating a positive effect of the haploid phase on the success of the sporophyte. Further, pollen growth from ozone-sensitive cultivars of several plant species is adversely affected when that pollen is exposed to ozone stress. Pollen growth from ozone-tolerant cultivars of the same species is significantly less affected by ozone stress. These effects are quantifiable and suggest that pollen response in the presence of pollutant stress can be used to predict the effect of that pollutant (ozone) stress on the mature plant species or cultivar which produced the pollen. Thus, it may be possible to predict long-term plant response using a very simple, short-term response.

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STUDENT GRADES CORRELATED WITH RATINGS OF A COLLEGE PROFESSOR. D. A. Roberts, Department of Plant Pathology, University of Florida, Gainesville, Florida 32611.

Equivocal results of studies involving different courses and instructors have prompted the consensus that student grades have little or no relationship with student evaluations of their instructors (Doyle, K. O., Jr. 1975. Student evaluation of instruction. D. C. Heath and Co., Lexington, MA). But student ratings of the same professor of one course, "Fundamentals of Plant Pathology," were highly correlated with student grades. Of the 523 upper-division students in 12 classes taught over six years, 393 anonymously rated the same instructor during the last week of every term. Evaluation questionnaires were not available to the instructor until at least a month after final student grades had been awarded. Average grades and "overall rating of the instructor," both weighted to account for different class sizes, were significantly correlated (r = 0.9386). Thus, good students gave the professor high ratings.

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THE EFFECT OF 2-DEOXY-D-GLUCOSE ON ml-o RESISTANCE TO BARLEY POWDERY MILDEW. C. J. Luczka and J. R. Aist, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

The ml-o barley mutant (R) contains a single gene for resistance to powdery mildew and has an enhanced papilla response compared to the susceptible isoline (S). Papillae contain callose and may be involved in ml-o resistance. We tested the effect of 2-deoxy-D-glucose (DDG), an inhibitor of callose formation in plants, on papilla frequency (PF) and penetration efficiency (PE). When partially dissected coleoptiles were floated on DDG (10^{-3} M) and inoculated with conidia of Erysiphe graminis f. sp. hordei, PF dropped from 88% to 30% in R and from 48% to 34% in S. Moreover, papillae formed in the presence of DDG were much smaller than usual. The DDG treatment increased PE in R from 2% to 72%, while in S the PE was unchanged (86%). The results with S show that there are no relevant deleterious effects of DDG on either the host or the parasite and those with R support the hypothesis that papilla formation is the ml-o resistance mechanism.

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RELATIONSHIPS BETWEEN PLANTING DATES, INCIDENCE OF MAIZE DWARF MOSAIC VIRUS STRAIN A AND YIELD OF SORGHUM IN SOUTH CAROLINA. Graydon C. Kingsland, Dept. of Plant Pathology and Physiology,

and Eugene McClain, Dept. of Agronomy and Soils, Clemson University, Clemson, SC 29634-0377.

Yields of maize dwarf mosaic virus-infected sorghum [Sorghum bicolor (Linn.) Moench] plants (avg 39 g/plant) and adjacent symptomless plants (avg 49 g/plant) of four varieties seeded 17 May were not different. Yields were different (P=0.05) when seeded 31 May (35 g/plant and 46 g/plant, respectively) and 14 June (avg 20 g/plant and 38 g/plant, respectively). Numbers of diseased plants increased (P=0.05) to the 12 July rating for all four varieties seeded on the three dates. Incidence averaged 13 and 8% on 12 July in plots seeded 17 and 31 May, respectively. Incidence did not increase in these plots subsequent to 12 July. Incidence increased to an averaged of 42% on 13 August in the plots seeded 14 June. Infection (or symptom expression) may have been limited by stage of host maturity for plants in plots seeded 17 and 31 May in this experiment.

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AMELIORATION OF TAN SPOT OF WHEAT WITH NITROGEN. D.M. Huber, T.S. Lee, M.A. Ross, and T.S. Abney. Botany and Plant Pathology Department and USDA, ARS, Purdue University, West Lafayette, IN 47907.

The effect of N rate and form on tan spot were evaluated with wheat cultivars Auburn, Caldwell, and Blazer (resistant, moderately susceptible, and susceptible to Pyrenophora tritici-repentis, respectively) planted in a randomized, complete block design field experiment. The severity of tan spot decreased and yields increased as the rate of N increased. Disease severity was reduced further by inhibiting nitrification with nitrapyrin which prevented overwinter loss of N and increased the proportion of ammonium N taken up by plants. The rate of lesion development was markedly reduced as the N rate increased, and the pin-point lesions developing on Blazer at the highest rate of stabilized N were similar to those on the resistant Auburn. This research indicates that both the rate and form of N influence the severity of tan spot of winter wheat, and that N management may be an important cultural control of this disease.

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THE ROLE OF WATER STRESS IN THE DEVELOPMENT OF FUSARIUM FOOT ROT OF BARLEY. T. R. Gordon, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Barley (Hordeum vulgare L. cv. CM 72) was grown in a soil mix infested with macroconidia of Fusarium graminearum. After four weeks under greenhouse conditions, plants were transferred to a growth chamber where they were subjected to an episode of water stress lasting 30 days. This was accomplished by using a two phase rooting medium. The upper phase was soil, and the lower phase was a nutrient solution into which the root system grew from the soil. Water stress was imposed by amending the liquid phase with progressively higher concentrations of PEG-3350. Infected plants subjected to water stress developed substantial discoloration of the crown and died before reaching maturity. Healthy plants given the same stress treatment showed some firing of the leaf tips but otherwise appeared comparable to non-stressed plants. Disease symptoms on infected plants not subjected to water stress were restricted to, at most, discoloration of the lower leaf sheaths.

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AN EXPLANATION FOR THE PERPETUATION OF YIELD LOSS IN MOROCCAN BARLEYS FROM PYRENOPHORA TERES. J. R. Burleigh, M. Tajani, & B. Ezzahiri. Institut Agronomique et Veterinaire, Rabat, Morocco and Dept. of Pl. Path., Univ. of Minn., St. Paul, MN 55108.

In Morocco selection of barley lines for resistance to Pyrenophora teres is done when high temperatures and lack of moisture may arrest the disease even though, early in the season, it has been severe on senesced lower leaves. Studies of yield components linearly regressed on areas under the disease progress curve (AUDPC) for growth stage intervals corresponding to when tillers, ears, spikelets, kernels and kernel weight are formed revealed that both numbers of ears and kernel weight were inversely related to AUDPC for growth intervals when ears are formed. This indicates that our cultivars, selected at adult stages which show little infection, may be damaged severely by early infections that reduce ear number and kernel weight.

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COMPONENTS CONTRIBUTING TO DILATORY RESISTANCE TO PUCCINIA POLYSORA UNDERW. IN CORN. Natale Zummo, USDA-ARS, Dept. of Plant Pathology and Weed Science, Miss. State, MS 39762

Four characters of southern corn rust, pustule incidence, size, tumescence, and rupture were evaluated in the field and greenhouse to determine relative consistency on individual corn genotypes and their contribution to dilatory resistance. Inoculated plants of a susceptible genotype consistently had greatest pustule incidence. Pustules on this genotype were significantly larger, more tumid, and ruptured earlier than pustules produced on more resistant corn genotypes. A genotype from a cross of susceptible parents followed a similar pattern of susceptibility when compared to genotypes from crosses of parents with dilatory resistance.

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THE INHERITANCE OF RESISTANCE IN SPRING AND WINTER BREAD WHEATS TO TWO ISOLATES OF MYCOSPHAERELLA GRAMINICOLA. T. Danon and Z. Eyal, Dept. of Botany, Tel Aviv Univ., Tel Aviv 69978, Israel.

The inheritance of low pycnidial coverage was investigated in parental F_1, F_2, F_3 and backcrossed populations derived from crosses among spring and winter bread wheats crossed in a complete diallel scheme. Field grown populations were replicated four times within two blocks, each block inoculated with a different isolate of Mycosphaerella graminicola (anamorph, Septoria tritici). The semidwarf winter wheat cultivars Aurora, Bezostaya I, Kavkaz and Trakia were susceptible to isolate ISR8036 but not to isolate ISR398 (ATCC48507). The semidwarf spring wheats Hazera 2230 and Lakhish were susceptible to both isolates. Resistance of the four winter wheats to isolate ISR398 is controlled by one or two dominant genes. There was no indication for maternal effect on the expression of disease coverage. The two isolates of M. graminicola possess at least two different genes for virulence. Low correlations were expressed between heading date, plant height and pycnidial coverage of septoria tritici blotch.

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Improved "Single tillers" method for loss assessment in wheat.
Shtienberg D., D. Marani and A. Dinoor
Faculty of Agriculture, Rehovot, Israel

Common methods for loss assessment include (a) establishment of experimentally derived damage functions expressing the relationships between disease level and yield. (b) estimates, based on the function, of losses in other fields. There are drawbacks to that, like (1) the cost, preplanning and prospects for success of the field trials, (2) validy of the function for other fields. In the "Single tillers" method the function is based on hundreds of tillers. The main drawback to it, is the validity of estimates derived from single tillers to a whole crop. the advantages of this method are: (1) it can be applied simply in any desired situation without preplanning and without allocation of field experiments, (2) the function is calculated for each field evaluated, (3) it is very non-expensive in operation. Attempts at standartization of procedures for improving the validy of the method were tested in field trials. The losses determined by the improved method did not differ significantly from the values calculated from field trials.

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IMPORTANCE OF SOILBORNE PLANT PATHOGENS ON WHEAT AS INFLUENCED BY TILLAGE AND CROPPING SYSTEM. C. S. Rothrock, Department of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

The importance of soilborne plant pathogens on wheat yields as influenced by no tillage and soybean/wheat doublecropping were examined. Tillage and cropping treatments were split into fumigated (methyl bromide) and nonfumigated subplots. Yields under no tillage were significantly lower than conventional tillage in 1984, but were similar for 1985, a year with a very dry spring. In 1984 yields were increased 62% and 12% by fumigation for no tillage and conventional tillage, respectively. In 1985 yields were increased 15% and 11% for these same treatments. Yields were not affected by cropping system (wheat/fallow vs. wheat/soybean). The growth parameter most affected by fumigation was tillering. The most common group of pathogens isolated from roots were Pythium spp., primarily P. irregulare. Isolation frequency of Pythium spp. was lower under conventional tillage.

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THE EFFECT OF SAND PARTICLES SIZE ON INFECTION OF WHEAT ROOTS BY GAEUMANNOMYCES GRAMINIS VAR TRITICI. H.M. El-Nashaar, R.A.

George and L.W. Moore. Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR 97331.

The effect of blending three sizes of silica sand with oat seed inoculum of Gaeumannomyces graminis var tritici (10 mg inoculum/g sand) on development of take-all disease on 14-day-old wheat seedlings was examined. Oat seed inoculum was milled to 0.25 , 0.335, or 0.5 mm sizes. Take-all sevirity was evaluated by visual rating (scale of 1 = no disease to 5 = lesions on roots, crown and basal stem) and quantitative ELISA (El-Nashaar et al 1985, Phytopathology). Comparative disease evaluations indicated slight effect of inoculum particle size, and significant increase in disease with increases in sand size. For coarse, medium, and fine sand, respectively, visual ratings were 3.29, 2.65, and 1.36, and ELISA ratings were 0.351, 0.342, and 0.193. Enhanced take-all in coarse sand may reflect different physical properties for the growth medium or increase wounding from root abrasion.

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The effect of foliar diseases on growth processes of spring wheat

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The relationship between the severity of foliar diseases and wheat yield in Israel was found to change during the growing season. A linear relationship existed early in the season, and a parabolic relationship was observed towards the end of the wheat growing season. Disease-free plants had lower yields than slightly diseased plants.
The effect of septoria leaf blotch on yield accumulation was a reduction in rate of dry weight accumulation, but on the other hand the duration of dry weight increased.
The effect of yellow rust severity on the photosynthesis rate was changing during the day throughout the growing season, and in some cases slightly diseased leaves had the maximum rate of photosynthesis. Transpiration rate decreased as disease severity increased, but not at the same rate.
The change during the season in relationships between foliar diseases and yield might be explained by the effect of the pathogens on photosynthesis and transpiration).

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OCCURRENCE OF ASPERGILLUS FLAVUS FROM TWO SOIL TYPES AND FROM GRAIN OF CORN FROM THESE SOILS IN SOUTH CAROLINA. Graydon Kingsland, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Significantly more (P=0.01) colony-forming units (cfu/g) of Aspergillus flavus grew on malt-salt agar soil dilution plates from composite samples of an unirrigated (avg moisture 4.8%) Grady loam (avg 1,870 cfu/g dry wt) than from an irrigated (avg moisture 8.8%) Norfolk loamy sand (avg 22 cfu/g) on 8 dates. Soil moisture was determined with an Ohaus 1610 balance. More kernels (P=0.05) from plants on Grady soil were contaminated with A. flavus (avg 85%) than from Norfolk soil (avg 45%). The same relationships were true of A. terreus from soil, but A. terreus was not identified on any kernels. Predisposition of corn on the Grady soil, by low soil moisture, may have contributed to the higher level of kernel contamination by A. flavus.

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DETECTION OF CORYNEBACTERIUM MICHIGANESE SUBSP. TESSELLARIUS IN SEEDS AND WHEAT PLANTS. J. H. McBeath* and M. Adelman, Agricultural & Forestry Experiment Station, University of Alaska, Fairbanks, AK 99701

Corynebacterium michiganese subsp. tessellarius has been detected in high frequencies in seeds of spring wheat plants displaying typical symptoms of bacterial mosaic disease by using a selective medium. Chlorox surface sterilization of the contaminated seeds was found to be an ineffective seed treatment. Scanning electron microscopy (SEM) of the seeds revealed the presence of clusters of bacteria in the seed coat - endosperm interface near the embryo. C. michiganese subsp. tessellarius has been consistently detected from the root, sheath, and leaf tissues of seedlings and plants germinated from the contaminated seeds, although frequently these plants have not yet expressed symptoms. Results from SEM studies of the host tissues showed that the bacterium exists mostly in the sieve elements. Its low populations in the cells may contribute to the delayed symptom expression.

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RYTHMIC VARIATION IN THE INFECTIBILITY OF SORGHUM BY THE SUGARCANE MOSAIC VIRUS. Jack L. Dean, USDA, ARS, SAA, Sugarcane Field Station, Star Route Box 8, Canal Point, FL 33438.

To study changes in the infectibility of a highly susceptible sorghum cultivar by the sugarcane mosaic virus as a function of time of day of inoculation, plants were inoculated at 3-hr intervals by a standardized procedure. The most likely sources of variability in the amount of infection other than changes in the test plants were studied and ruled out. Regression analysis showed that the infectivity of the freeze-dried inoculum did not change over a 4-yr period beginning 2 yr before and ending 2 yr after the inoculations reported here. A series of six uniformity trials showed that no significant sampling error was associated with reconstitution and preparation of inoculum. Significant quadratic regression of percent infected plants on hr of the day showed a low point in infectibility about dawn and a high in the afternoon or evening.

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CHARACTERIZATION OF CLONED AVIRULENCE GENES FROM XANTHOMONAS CAMPESTRIS PV. MALVACEARUM. P.C.Turner and D.W.Gabriel, Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611.

Congenic cotton lines containing resistance (R) genes recognise avirulence (A) genes of the pathogen Xanthomonas campestris pv. malvacearum (Xcm) in a specific, gene-for-gene manner. Six A genes were isolated from a gene bank of a multiply-avirulent strain of Xcm by screening for clones which conferred specific avirulence on a normally virulent Xcm strain. One such clone, pUFA704, carried a 5.7 kb DNA fragment containing the avirulence genes $avrB_{5a}$ and $avrB_{In}$ which are recognised by the host R genes B_5 and B_{In} respectively. The 5.7 kb fragment has been physically mapped. Work is in progress to position the A gene coding regions by the use of vectors which allow positive selection of DNA fragments containing open reading frames (ORFs). The vectors create translational gene fusions of ORFs to the E.coli lacZ gene. Two ORFs have been identified to date.

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ANALYSIS OF AN AVIRULENCE GENE FROM PSEUDOMONAS SYRINGAE PV. TOMATO WHICH ELICITS THE HYPERSENSITIVE RESPONSE ON SOYBEAN. D. Y Kobayashi and N. T. Keen, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Three distinct classes of clones from a cosmid library of Pseudomonas syringae pv. tomato DNA elicited the hypersensitive response (HR) on different cultivars of soybean. One class of clones, represented by pPt2404, produced an incompatible phenotype on soybean cultivars which is similar to that elicited by race 6 (R6) of P. syringae pv. glycinea. Southern blot analysis indicated that pPt2404 and the avrA gene cloned from P. syringae pv. glycinea R6 (Proc. Natl. Acad. Sci. 81:6024-6028) shared homologous sequences. pPt2404 hybridized to genomic DNA from seven other isolates of P. syringae pv. tomato. However, pPt2404 did not show homology to other screened pathovars of P. syringae. Conjugal transfer of pPt2404 into P. syringae pv. tabaci resulted in an HR on Nicotiana benthamiana. However, other tested pathovars of P. syringae containing pPt2404 failed to elicit an HR on their respective host plants.

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SEQUENCE ANALYSIS OF TWO AVIRULENCE GENES FROM PSEUDOMONAS SYRINGAE PV. GLYCINEA. S. Tamaki and N. T. Keen, Dept. of Plant Pathol., Univ. of California, Riverside, CA 92521.

Two avirulence genes cloned from race 0 of P. syringae pv. glycinea (Staskawicz and Dahlbeck, unpublished) were subcloned as 2.18 and 2.60 kb DNA fragments, respectively, and called avrB and avrC. The two genes do not appear to be tightly linked. DNA sequence analysis of the subcloned genes disclosed that the avrB gene contained a single open reading frame (ORF) coding for 321 amino acids. However, the avrC gene fragment contained two tandem ORFs, the first encoding 352 amino acids. The second avrC ORF codes for 240 amino acids and is in frame with the first ORF, being displaced by 27 nucleotides from the TAA of the first ORF. The first ORF of avrC sub-cloned in pUC19 directed production of a 39 kD protein in E. coli that could be readily observed on SDS gels of whole cell proteins. The first ORF of the avrC gene also shared considerable sequence homology with the ORF of avrB.

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IDENTIFICATION OF A COSMID CLONE CONTAINING THE COPPER RESISTANCE GENES FROM PSEUDOMONAS SYRINGAE PV. TOMATO. C. L. Bender and D. A. Cooksey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

We previously found that copper resistance (Cu^r) in Pseudomonas syringae pv. tomato (Pst) strain PT23 is mediated by two conjugative plasmids, pPT23A (101 kb) and pPT23C (67 kb). Our strategy for cloning the copper resistance gene(s) from PT23 was to construct a cosmid library of PT23 plasmid DNA in the cosmid vector pLAFR3. Copper resistance was not expressed in E. coli; therefore, recombinants were shuttled into the Cu^s strain PS61 of P. syringae pv. syringae. Using this approach, the cosmid clone pCOP1 was identified; PS61 transconjugants containing this clone were able to grow on copper-containing media at levels inhibitory to the wild type. Tn5 mutagenesis and subcloning methods are being used to define the extent of the Cu^r gene(s) within the cosmid clone. One subclone showed strong homology to the indigenous plasmid pPT23D (37 kb) but not to pPT23A or pPT23C. Therefore, the cosmid clone probably originated from pPT23D, suggesting that strain PT23 contains three Cu^r plasmids.

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ISOLATION AND ANALYSIS OF Tn5 MUTANTS OF PSEUDOMONAS SYRINGAE PV. TOMATO ALTERED IN TOXIN PRODUCTION. C. L. Bender, H. E. Stone, J. J. Sims, and D. A. Cooksey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Some strains of Pseudomonas syringae pv. tomato (Pst) produce the chlorosis-producing phytotoxin, coronatine. Pst strain PT23 produces necrotic lesions on tomato which are surrounded by a diffuse yellow chlorosis. 1000 Tn5 mutants of PT23 were screened for loss of chlorosis induction on tomato leaves; ten mutants were recovered which produced little or no chlorosis. Two mutants were further tested and found to be defective in coronatine production by organic acid extraction and HPLC analysis. The necrotic lesions induced by the mutants reached a maximum diameter (0.22 mm) 4 days after inoculation, but wild-type lesions continued to expand until the eighth day and were significantly larger (0.62 mm) than those induced by the mutants. The increase in bacterial populations was correlated with the increase in lesion size. Therefore, coronatine synthesis appears to play an important role in lesion expansion and consequent multiplication of Pst on tomato leaves.

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A KAN,SAC CARTRIDGE FOR GENERATING DIRECTED, UNMARKED MUTATIONS IN GRAM-NEGATIVE BACTERIA. J.L. Ried and A. Collmer, Dept. of Botany, Univ. of Maryland, College Park, MD 20742.

The sac region from Bacillus subtilis, which confers sensitivity to sucrose in Gram-negative bacteria, was subcloned from pUCD800 (Gay et al., J. Bacteriol. 164:918) into one of the PstI sites flanking the kan gene in pUC4K. The resulting 3.8 kb DNA fragment can be excised with BamHI and inserted into a BamHI or Sau3A site in a target gene. Genes inactivated by this insertion can be marker-exchanged into the bacterial chromosome by selecting for kanamycin resistance. Subsequent PstI digestion of the plasmid containing the insertionally inactivated gene, followed by religation, leaves an out-of-frame 28bp insert and results in the generation of an unmarked mutation. Such mutations should be useful for inactivating the multiple pel genes in the soft-rot erwinias by exchange recombination of the unmarked for the marked mutation followed by selection for tolerance to sucrose.

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MULTIPLICATION AND VIRULENCE IN POTATO TUBERS OF ESCHERICHIA COLI CARRYING A HIGHLY EXPRESSED CLONED PECTATE LYASE GENE. J.H. Payne[1], N.T. Keen[2], and A. Collmer[1], [1]Dept. of Botany, Univ. of Maryland, College Park, MD 20742 and [2]Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

Escherichia coli JA221(pPL748) produces large amounts of pectate lyase from Erwinia chrysanthemi EC16 pelE cloned in the expression vector pINIII[113] (Tamaki and Keen, Phytopathology 75:1326). EC16 was compared to JA221(pPL748) and JA221 (pINIII[113]) for ability to macerate and grow in whole potato tubers under aerobic and anaerobic conditions after inoculation with 50-100 bacteria per site and incubation for 48 hr at 30 C. Tubers were macerated only under anaerobic conditions. Mean fresh weights of macerated tissue per inoculation were 122±52 mg (EC16), 553±25 mg (JA221[pPL748]), and 0 mg (JA221 [pINIII[113]]). The final mean bacteria per site were 6.4 x 10^4, 4.0 x 10^4, and 1.2 x 10^9, respectively in aerobically incubated tubers, and 2.7 x 10^8, 2.3 x 10^8, and 1.7 x 10^7 in anaerobically incubated tubers. Thus, maceration ability is not required for enterobacterial growth in potato tubers.

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PARTIAL CHARACTERIZATION OF THE ERWINIA HERBICOLA ICE NUCLEATION GENE. Douglas Gurian-Sherman, Steven E. Lindow, and Nicholas J. Panopoulos, University of California, Berkeley, CA 94720.

Transposon mutagenesis of a ~6 kb subclone of the Erwinia herbicola 26SR6-2 ice nucleation gene (iceE) was conducted in Escherichia coli using the Tn3lac transposon, Tn3-HoHol. Clones with reduced or undetectable ice nucleation activity (INA) span a ~4 kb region. Orientation of Tn3-HoHol leading to blue or white color after growth on X-Gal media suggests transcription proceeds toward the EcoRI site. Two insertions ~1.0-1.5 kb from the EcoRI site, with transcription in the opposite direction compared to iceE, exhibit INA equal to subclones with insertions only in the vector. Insertions in the putative promoter proximal half of iceE retain residual low temperature INA, while INA is not detectable in promoter distal insertions. Similar results have been observed with ice nucleation genes from two other species.

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DETECTION AND CLONING OF AN INSERTION SEQUENCE COMMON TO CHROMOSOMAL DNA AND PLASMID pCS1 OF CORYNEBACTERIUM SEPEDONICUM. B.D. Mogen and A.E. Oleson, Depts. of Plant Pathology and Biochemistry, North Dakota State University, Fargo, ND 58105.

We have recently characterized a 50.6-kb plasmid found in a substantial percentage of Corynebacterium sepedonicum isolates obtained from diverse geographic regions. Fragments of this plasmid, designated pCS1, have been cloned into M13mp11. The resulting library was screened against chromosomal DNA to detect clones containing insertion sequences which are present in the bacterial nucleoid. One of the clones containing a 2.9-kb insert was found to be present in multiple copies within the chromosomal DNA of all C. sepedonicum isolates tested and may be present in more than one copy on the plasmid. This multicopy

insertion sequence is currently being characterized and has already proven to be useful for restriction fragment length polymorphism (RFLP) analysis. In addition, this cloned fragment is being used to evaluate the feasibility of developing a dot-blot hybridization assay for the detection of low levels of the ring rot pathogen.

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GENETIC VARIATION IN CORYNEBACTERIUM SEPEDONICUM REVEALED BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS. B.D. Mogen and A.E. Oleson, Depts. of Plant Pathology and Biochemistry, North Dakota State University, Fargo, ND 58105.

A cloned 2.9-kb fragment of Corynebacterium sepedonicum plasmid pCS1 has been shown to contain an insertion sequence that is present in the chromosome of all tested strains of this species. This clone was used as a probe in the Southern blotting procedure for RFLP analysis of 12 isolates of C. sepedonicum. Five restriction enzymes previously shown to give complete digests were used for the initial analysis. All enzymes were able to distinguish plasmid-positive and -negative strains of the pathogen. Three enzymes were also able to detect genetic microheterogeneity in the chromosomal DNA. Based on the data from the limited number of restriction enzymes used, it was possible to distinguish at least 8 different RFLP patterns among the tested isolates. These results suggest that substantial genomic variation exists within the C. sepedonicum population and that RFLP analysis will be useful in distinguishing specific isolates in the absence of other morphological or biochemical markers.

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CHANGES IN FATTY ACID PROFILES OF ERWINIA AMYLOVORA DUE TO GROWTH MEDIUM AND PHYSIOLOGICAL AGE. T. van der Zwet, USDA, ARS, Kearneysville, WV 25430; F. J. Casano, Istit. Sperimentale Patologia Vegetale, Rome, Italy 00156; and J. M. Wells, USDA, ARS, Rutgers Univ., New Brunswick, NJ 08903.

Three strains of Erwinia amylovora were grown for 1, 3 and 6 days on GYCA, KB, NA and TSA medium and analyzed for total cellular fatty acids by gas-liquid chromatography. A microcomputer was used to convert data on 31 identified components into a fatty acid class analysis by strain, medium and age. Percentage of saturated even-cargon straight chains were significantly higher on GYCA than on other media. On NA and TSA saturated, odd-carbon straight chains were 3 to 4 times higher than on GYCA and KB. As a consequences, the physiologically-important ratio of saturated/unsaturated acids was significantly lower on KB. As cells aged, concentrations of saturated acids increased and unsaturated acids decreased, resulting in a shift of the saturated/unsaturated ratios from 1.2 to 1.7.

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DETECTION OF Xanthomonas campestris FROM CITRUS BY MEMBRANE ENTRAPMENT AND IMMUNOFLUORESCENCE. R. H. Brlansky and R. F. Lee, Univ. of Florida, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850 and E. L. Civerolo, USDA, Beltsville, MD 20705.

The Xanthomonas campestris (X.c.) pathovar, first discovered in Florida in 1984, causing a bacterial spot disease in Florida citrus nurseries, was easily detected and identified from culture and from leaf extracts of symptomatic and asymptomatic leaves by membrane entrapment and subsequent immunofluorescent staining. Bacteria were trapped on either 0.2 um or 0.4 um black polycarbonate membranes and detected with fluorescence microscopy after incubation in tetramethylrhodamine isothiocyanate-labeled immunoglobulin (IgG) specific for the bacterium. Bacteria were detected at concentrations as low as 10^2/ml. X. campestris pv. vesicatoria was not detected with labeled IgG specific for the Florida X.c. pathovar.

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ACTIVATION OF A HOST PLASMA MEMBRANE K+ EFFLUX/H+ INFLUX EXCHANGE PROMOTES BACTERIAL MULTIPLICATION IN BEAN LEAVES. Merelee M. Atkinson and C. Jacyn Baker, USDA-ARS, Microbiology and Plant Pathology Laboratory, Beltsville, MD, 20705

Bacterial induction of host plasma membrane K+ efflux/H+ influx has been shown to be highly correlated with pathogenicity of Pseudomonas syringae pv. syringae on bean. Low rates of K+/H+ exchange preceeded rapid and simultaneous increases in both the exchange rate and bacterial populations in leaf tissue. K+/H+ exchange led to an alkalization of the host cell wall environment from approximately 5.7 to 7.5. Infiltration of leaf intercellular spaces with pH 7 or 8 buffers promoted rapid growth of nonpathogenic bacterial mutants. These results suggest that alkalization of host cell wall spaces by the K+/H+ exchange promotes bacterial multiplication. In view of the apparent role of plasmalemma H+ gradients in the active transport of sucrose, amino acids and other nutrients in plant cells it is hypothesized that alkalization leads to increased nutrient levels in host intercellular spaces where bacteria reside.

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IN VITRO AUXIN PRODUCTION BY XANTHOMONAS CAMPESTRIS PV.GLYCINES William F. Fett, Stanley F. Osman and Michael F. Dunn, Eastern Regional Research Center, USDA, ARS, 600 E. Mermaid Lane, Phila. PA. 19118.

Xanthomonas campestris pv. glycines (Xcg)causes a disease on soybean called bacterial pustule. Symptoms consist of small lesions on leaves with raised centers. Pustule formation is due primarily to enlargement of leaf mesophyll cells indicating the possible involvement of elevated auxin levels in symptom development. The ability of pathogenic and nonpathogenic strains of Xcg to produce auxins in vitro was assessed. Culture filtrates were examined for auxins both by colorimetric assay and by solvent extraction, thin-layer chromatography, high-performance liquid chromatography and GC-MS analysis. All strains produced indole-3-acetic acid when tryptophan was added to the liquid medium. The presence of the additional indole compounds indole-3-lactic acid, indole-3-aldehyde and N-acetyl tryptophan was also confirmed. Presumptive evidence for the presence of very low levels of indole-3-acetamide has been obtained.

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RECOGNITION OF PECTATE LYASE IN WESTERN BLOTS BY MONOCLONAL ANTIBODIES. E. A. Maher, R. S. Livingston, and A. Kelman. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Pectate lyase (PL) in transblotted proteins from different sources was recognized by different monoclonal antibodies (MAbs) directed against purified extracellular PLs from Erwinia carotovora subsp. carotovora (Ecc). Purified PLs, whole cell suspensions of Ecc and of Escherichia coli harboring pBR325 plasmids expressing PL activity, and protein extracts from potatoes decayed by Ecc were subjected to discontinuous SDS-PAGE prior to electrophoretic transfer to nitrocellulose. Most MAbs recognized bands corresponding to PL I (42 kD) and PL II (40 kD) in purified enzyme fractions. Of these, some MAbs recognized the PL I band in Ecc, in E. coli harboring the PL+ plasmid, and in decayed potato extracts; other MAbs recognized the PL I band in E. coli, but not in Ecc or decayed potato extracts. Different MAbs recognize at least two distinct sites on PL; in Ecc cells and potato extracts one of these sites may be absent, modified or unavailable to MAbs after transblotting.

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SENSITIVITY OF RHIZOCTONIA SOLANI ISOLATES FROM OKLAHOMA PEANUT FIELDS TO TOLCLOFOS-METHYL. P. M. Inskeep and A. B. Filonow, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Radial growth of 18 Rhizoctonia solani (Rs) AG4 isolates from peanut fields in Oklahoma was measured after 72 h on a defined solid medium(Phytopathology 59:1601-1605) amended with 0, .01, .1, .5 and 10 µg ai/ml Tolclofos-methyl (Rizolex,50 WP). Forty four percent were sensitive(ED50=<0.1 µg ai/ml), 50% were moderately sensitive(ED50=>0.1 to <5 µg ai/ml) and 6% showed little sensitivity(ED50=>5 µg ai/ml). In greenhouse tests, no emergence of peanut seedlings(cv. Spanco and Florunner) occurred in nontreated soil infested with a Rizolex-sensitive isolate. However, Rizolex applied preplant at 5.6 kg ai/ha resulted in 80-100% emergence. Peanut grown in field microplots containing soils infested with a mixture of Rs isolates varying in sensitivity to Rizolex were treated at flowering with 11.2 kg ai/ha Rizolex. Inoculum densities of Rs in Rizolex-treated soils were reduced; however, severity of pod rot was not reduced significantly (P=0.05).

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OBSERVATIONS ON ANATOMICAL AND MORPHOLOGICAL CHANGES IN SCLEROTIA OF RHIZOCTONIA SOLANI. G.F. Joye and G.T. Berggren, 302 Life Sciences Bldg., Louisiana State University, Baton Rouge, La., 70803

Rhizoctonia solani Kuhn (AG-1), the causal agent of Aerial Blight of soybeans, produces sclerotia that are reported to be the primary propagules for survival and dispersal. Sclerotia were buried in soil at four depths ranging from 0 to 25 cm. Viability was determined bimonthly. Numbers of viable sclerotia were reduced more than 70 percent over the test period. Observations using SEM and TEM will be presented on the changes in the size, shape, and amount of living and non-living material from initial formation to maturity and after being buried in soil for extended periods of time.

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ANALYSIS OF GENES PREVENTING SEXUAL COMPATIBILITY BETWEEN OPPOSITE MATING TYPE ISOLATES OF SCLEROTINIA MINOR. C. L. Patterson, R. K. Webster, and R. G. Grogan, Department of Plant Pathology, University of California, Davis, CA 95616.

Sexual compatibility (SC) between heterothallic isolates of Sclerotinia minor (SM) is controlled by a major gene locus with two alleles, A/a. Pairings between some opposite mating type isolates, however, resulted in sterile sclerotia that did not produce apothecia. In addition to the major gene locus there were five other loci (B_1 - B_5) conditioning SC by preventing fertility between opposite mating type isolates of SM. Analysis of the B loci indicated that the genes were probably located on the same chromosome as the major compatibility locus. SC between opposite mating type isolates of SM was prevented when the individual B loci occurred in a homozygous recessive condition in the fusion nucleus, regardless of the condition of the major gene locus (i.e. Ab_n X ab_n). Thus, opposite mating type groups that express infertility were recessive (b_n) for the B locus conditioning incompatibility.

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USE OF ISOZYMES FOR THE ANALYSIS OF UNEXPECTED SEGREGATION RATIOS IN F2 AND BACKCROSS PROGENY OF PHYTOPHTHORA INFESTANS. L.J. Spielman, J.A. Sweigard, R.C. Shattock, and W.E. Fry, Cornell University, Ithaca, NY 14853.

In previous studies, crosses between field isolates of Phytophthora infestans produced normal segregation ratios for genotypes of glucose phosphate isomerase (GPI) and peptidase in F1 progeny. In our current studies with subsequent F2 and backcross generations, we have observed abnormal segregation ratios. Two F2 matings and 3 out of 4 backcrosses, producing a total of 523 single-oospore progeny, showed deficiencies of the slow homozygote of GPI. Two backcrosses not involving the slow allele of GPI gave normal segregation ratios. We have developed additional enzyme systems for use as genetic markers in P. infestans. Six of these (fumarase, mannose phosphate isomerase, hexokinase, fructose diphosphatase, alkaline phosphatase, and malate dehydrogenase) are polymorphic in the crosses described above, and can be used to determine the cause of the observed segregation distortion.

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ISOZYME AND RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN RHYNCHOSPORIUM SECALIS. S.B. Goodwin, M.A. Saghai-Maroof, R.W. Allard, and R.K. Webster*. Dept. of Genetics and Dept. of Plant Pathology*, University of California, Davis, CA 95616

Electrophoretic methods have been developed to differentiate isolates of the barley scald organism, Rhynchosporium secalis. Among ten isozyme systems studied, four (PGI, PGM, LAP and CAT) have been variable for California isolates of R. secalis. There are two different phenotypes for each of the four variable enzyme systems, giving a possibility of distinguishing 16 different phenotypes. Among these 16 phenotypes, 8 have been found in California. Restriction fragment length polymorphisms (RFLP's) have been visualized by probing restricted DNA with a ribosomal gene from yeast; four phenotypes have been found using restriction enzymes Eco RI and Pst I. The pathogenicities of the different isolates have been determined by inoculating 14 differential cultivars of barley. The objective is to permit rigorous testing of the hypothesis that correlations exist between pathogenicity and the various electrophoretic phenotypes.

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VARIABILITY IN CULTURAL TYPE, OOSPORE FORMATION AND VIRULENCE ON ALFALFA OF SINGLE ZOOSPORE AND OOSPORE ISOLATES OF PHYTOPHTHORA MEGASPERMA F. SP. MEDICAGINIS (PMM). Donald C. Erwin, Dept. of Plant Pathology, University of California, Riverside, CA 92521 and Xinghan Li, Dandong Agr. Res. Inst., Feng Cheng, Lianoning, People's Republic of China.

Variability from single oospores (SO) of Pmm (self fertile) appears to be greater than from single zoospores (SZ). All SZ colonies from P1057 (appressed mycelial colony) were appressed with fast growth rates (GR) (18-20mm/day; ex. SZ63). SO isolates from SZ63 produced both appressed (ex. SO69) and fluffy (ex. SO79) colony types; GR varied from 10 to 24 mm/day. SO isolates from 069 (GR 16 mm/day) yielded both appressed and fluffy colonies; GR varied from 10-20 mm/day. SO isolates from 079 (GR 11 mm/day) yielded both appressed and fluffy colonies; GR varied from 7-16 mm/day. GR of most SZ isolates from 069 and 079 was like the parent. Root disease indices (DI) caused by SO isolates varied from low to severe on a resistant alfalfa (A77-10B), but DI caused by SZ isolates was uniformly low on A77-10B.

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DETECTION OF DOUBLE-STRANDED RNA IN PHYTOPHTHORA INFESTANS. P. W. Tooley and A. D. Hewings, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD and K. F. Falkenstein, Dept. of Biology, Hood College, Frederick, MD 21701

Cellular nucleic acids were isolated by phenol extraction from vacuum-dried mycelial mats (5 g) of P. infestans grown in 20% V8-juice broth at 18 C in darkness. Double-stranded RNA (dsRNA) was selectively purified from other nucleic acids with Whatman CF-11 non-ionic cellulose in 16.5% ethanol. The product was analyzed by gel electrophoresis, and then by ribonuclease digestion in high and low salt, to confirm the dsRNA nature of the bands. We detected dsRNA in eight of 15 Mexican isolates tested, and three distinct banding patterns were observed. No dsRNA was detected in nine U.S., two British, or two European isolates. Attempts to purify mycoviruses from isolates which contain dsRNA are now in progress. The presence of dsRNA in P. infestans provides a valuable cytoplasmic marker for genetic and epidemiological studies.

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EFFECT OF TEMPERATURE ON THE PRODUCTION OF PRIMARY SPORIDIA AFTER PROMYCELIAL FORMATION BY *TILLETIA CONTROVERSA*. H. S. Fenwick, S. V. Jones, and D. J. Eschen. Dept. Pl., Soil, & Ent. Sci., Univ. of Idaho, Moscow, Idaho 83843

Teliospores of *Tilletia controversa* were seeded onto 2% soil extract agar in plastic Petri plates (1.4 x 10^5 CFU/plate). After 14 days at 5 C in a growth chamber, all plates were examined daily for promycelial development. Between days 18 and 19, 773 teliospores with promycelia were identified by marking the undersides of the plates. Groups of 20 plates were then incubated at 1, 5, 10, or 22 C. The numbers of identified teliospores/20 plates/temperature were 104, 326, 187, and 155, respectively. The teliospores were examined daily for eight days for primary sporidia. The daily progressive % primary sporidial production was: 1 C - 27, 34, 49, 81, 100; 5 C - 21, 30, 50, 66, 81, 88, 90, 92; 10 C - 29, 52, 70, 79, 87, not read, 95, 100; 22 C - 24, 32, 38, 43, 45, 46, 46, 46. These data suggest that most primary sporidial production requires more than one day, and may be inhibited above 10 C.

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MORPHOLOGICAL ASPECTS OF THE ONTOGENETIC AND THE Vf-RESISTANCE OF APPLE LEAVES AGAINST VENTURIA INAEQUALIS. Gessler, C. Institute of Phytomedicine, 8092 ETH-Zürich, Switzerland.

Development of the scab fungus was compared on susceptible leaves (young leaves from Golden Delicious) and on resistant leaves (old leaves from G. D. and leaves from the Vf-resistant variety Liberty). Percentage of germination of conidia and appressoria formation was equal in all cases. Penetration through the cuticular membrane was always observed. Percentage of appressoria forming stroma visible in the light microscope diminished proportionally to the increasing age of the leaves in both varieties. In young leaves from Liberty stroma seamed to collapse and disappear again in 30% of all cases and the remaining stroma grew less, running hyphae and secondary stroma were formed less and latter and the stage of sporulation was never reached compared to young leaves from G. D. Similar delay in pathogenesis was noted in older ontogenetic resistant leaves from G. D. In older leaves from the Vf-resistant variety Liberty the delay was even more pronounced.

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HISTOPATHOLOGY OF PEANUT BLACK HULL. B.L. Jones, Grain Crops Research Institute, Private Bag X1251, Potchefstroom 2520, Republic of South Africa.

A strain of Thielaviopsis basicola, which causes severe peanut black hull, was used to infect peanut roots and pods. Mode of penetration into roots and pods was observed and the advance of T. basicola was followed through the shells of infected attached pods. Conidia germinated on surface of host within 2 hr at 25 C. Penetration was direct through cell walls and was completed within 8-12 hr at 25-28 C. Hyphae on surface of invaded cells formed phialoconidia within 21-30 hr and chlamydospores within 4-5 days after inoculation. The endocarp of pods usually remained uninvaded for 12-15 days after inoculation. Eleven additional fungi were isolated from infected pods. Gliocladium catenulatum, Fusarium oxysporum and a binucleate Rhizoctonia solani - like fungus were isolated most frequently. Once the endocarp was invaded, rot developed rapidly into interior of pod.

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QUANTITATIVE EFFECTS OF PARASITE:HOST:ENVIRONMENT SPECIFICITY IN Puccinia recondita:Triticum. **L. E. Browder and M. G. Eversmeyer, USDA-ARS, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.**

Replicated tests of three near-isogenic lines of Triticum aestivum and their recurrent parent inoculated with an avirulent or a virulent culture of Puccinia recondita and exposed to 16 environments were conducted. The environments consisted of 12 hr increments of time at 26 C or 19 C before transfer to 5 C. Sporulation estimates resulting from the different treatments were difficult to place into discrete classes. Mean sporulation estimates indicated that reduction of sporulation was dependent on presence of corresponding genes in parasite and host and time at 26 C or 19 C prior to transfer to 5 C. Although specificity that results in resistance and susceptibility is commonly portrayed as discrete class differences, this system contains parasite, host, and environmental variation adequate to provide continuous phenotypic variation.

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HOST-SPECIFIC TOXIN PRODUCED BY ALTERNARIA BRASSICAE. P.S. Bains and J.P. Tewari, Dept. of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.
A chromatographic procedure was developed to purify the toxin produced in culture by Alternaria brassicae, the causal organism of black spot of rapeseed. High resolution mass spectrometry of the purified toxin gave an exact mass of 593.3991 with a molecular formula $C_{30}H_{51}N_5O_7$. Negative ninhydrin reaction of HPLC-purified toxin in conjunction with its positive ninhydrin reaction after acid hydrolysis and the presence of amino acids in the acid hydrolysates, indicated the toxin to be a cyclic peptide. Molecular weight, ninhydrin reactions, amino acid composition, proton nuclear magnetic resonance spectrum, and HPLC retention time of the toxin were identical to those of Destruxin B. The order of sensitivity of Brassicas to the toxin was similar to the order of their susceptibility to the fungus. The toxin did not cause any chlorosis or necrosis on eight non-hosts of A. brassicae. The results indicate that the toxin is host-specific.

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GLIOTOXIN METHYLATION BY GLIOCLADIUM VIRENS. R. W. Jones and J. G. Hancock, Department of Plant Pathology, University of California, Berkeley, CA 94720.

During a study of fungal resistance to the antibiotic gliotoxin (m.w. 326) it was discovered that gliotoxin was converted rapidly to a non-toxic form by methylation of the reactive disulfide bridge by the producer organism, Gliocladium virens. Gliotoxin was inhibitory to G. virens until it had produced gliotoxin endogenously, at which time it expressed methylating capabilities. Addition of methionine (^{14}C methyl) at the onset of gliotoxin production resulted in labelling of the methylated form, suggesting methyltransferase activity. Methylated gliotoxin (m.w. 356) remained stable in culture filtrates after removal of mycelium and was non-toxic to a number of gliotoxin-sensitive organisms. Five isolates of G. virens as well as an isolate of G. deliquescens followed identical patterns of gliotoxin production and methylation during the mid- and late-log phase of growth. This suggests that residual gliotoxin would not accumulate in the soil. Methyltransferases may play a role in detoxification of other diketo-epithiapiperazines.

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DECOMPOSITION OF RICE STRAW BY FOUR SPECIES OF TRICHODERMA IN NATURAL SOIL. A. M. ROSALES & T. W. MEW, The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Application of Trichoderma species to natural soil enhanced decomposition of Rhizoctonia solani-infected rice straw and reduced the recovery rate of R. solani. Trichoderma aureoviride and T. harzianum were more effective than T. glaucum and T. pseudokoningii in the decomposition of rice straw in the soil. The cellulolysis adequacy index of these Trichoderma spp. was directly correlated with their ability to decompose R. solani-infected straw. T. harzianum and T. aureoviride were more effective in colonizing dry, mature rice straw, but were less effective in colonizing wet, green rice straw than R. solani. Rice straw embedded in Trichoderma-infested soil decomposed faster than when placed on the soil surface. T. aureoviride was able to decompose rice straw even in relatively dry soil.

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EFFECT OF STREPTOMYCIN ON DEVELOPMENT OF SEPTORIA TRITICI BLOTCH. F. J. Gough, F. Mehdizadegan, and E. G. Krenzer. USDA-ARS Plant Science and Water Conservation Laboratory, P.O. Box 1029, Plant Pathology Dept., and Agronomy Dept., Oklahoma State University, Stillwater, OK 74078-0285.

Streptomycin HCl (125 ppm) in distilled water was sprayed on flag leaves of winter wheat cultivar TAM W-101 in field plots. Sprays were applied four times at about weekly intervals in May of 1984 and in April and May of 1985. Application of streptomycin resulted in a 51% increase in lesion numbers caused by Mycosphaerella gramminicola (anamorph: Septoria tritici) per gram of dry flag leaf tissue from plants grown in 1984, and in a 14% and 22% increase in lesion numbers from plants grown in no-till and clean-till plots, respectively, in 1985. These results indicated that the microbial community of the phylloplane had been altered to favor the pathogen. From flag leaves of adjacent unsprayed plants, cultures of Bacillus subtilis and Pseudomonas fluorescens were isolated that were inhibitory to growth and infectivity of S. tritici.

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BIOCONTROL OF RHIZOCTONIA CROWN ROT OF SUGAR BEETS BY BINUCLEATE RHIZOCTONIA SPP. AND LAETISARIA ARVALIS. L. J. Herr, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Greenhouse tests included use of sterile, nonsterile or recolonized-sterilized soil; one beet/15-cm-diam. pot. Laetisaria arvalis (L.a.#7), two binucleate Rhizoctonia spp. (BN-1, BN-2) and Rhizoctonia solani AG-2 (R) were cultured on sterile barley. After 6-10 wk growth of beets, 6-12 BN-1-, BN-2- or L.a.#7-colonized seed were applied/pot. R-colonized seed were added 0-7 d later. After 4 wk beets were rated on 0=healthy to 5=dead disease rating (DR) scale. BN-1 consistently gave control; L.a.#7 and BN-2 did not. In a field test of the effects of L.a.#7, BN-1 and chlorothalonil 75% WP on crown rot, antagonists were broadcast (112 kg/ha) and chlorothalonil was spray-banded (2.5 kg/ha) in June. In July chlorothalonil was again applied and plots were side-dressed with R inoculum (45 kg/ha). In October, the BN-1 + R treatment plant loss=10.4%; yield=56.4 kg/plot; DR=0.6 compared to 28.1%, 45.2 kg/plot, 2.7 DR in the no antagonists + R treatment. Whereas, chlorothalonil + R values were: 13.7%, 55 kg/plot, 1.0 DR.

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APPLE SCAB BIOCONTROL BY CHAETOMIUM GLOBOSUM FAILS UNDER CONTROLLED CONDITIONS WHEN PATHOGEN APPLICATION IS DELAYED.

M. A. Boudreau and J. H. Andrews, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Previous growth chamber studies demonstrate up to 90% reduction in apple scab severity, ascribed to antibiosis, when ascospores of Chaetomium globosum and conidia of Venturia inaequalis are applied simultaneously to McIntosh seedlings. However, control was eliminated when V. inaequalis application was delayed by 7 days to seedlings previously treated with either spores or water extracts from cultures of C. globosum. C. globosum extract spread on sterile agarose-coated slides significantly ($P<0.05$) reduced germination of V. inaequalis conidia, but this effect was lost if conidia were added 3 days after the extract. Trends were similar using various concentrations of C. globosum ascospores in lieu of extract. These results suggest that the antibiotics produced by C. globosum are degraded abiotically and independently of the leaf, thus accounting for the loss of biocontrol.

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CONTROL OF PHYTHOPHTHORA INFESTANS ON TOMATO LEAVES AND POTATO TUBERS THROUGH WATER EXTRACTS OF COMPOSTED ORGANIC WASTES.

We have previously studied the effect of water extracts from composted organic wastes on downy mildew of grapes. As the disease outbreak was suppressed by spray applications, we tried the same approach with Phythophthora infestans on tomato leaves and potato tubers under laboratory and greenhouse conditions. In both cases there was a strong control effect. Composted wastes of different raw materials were equally effective. Various extraction times and modes of application were studied. The extracts had no direct effect on zoospore release. Steril filtration or heat treatment left the extracts ineffective. Studies to use the effect with in biological or integrated control programs were initiated.

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IMMUNOHISTOCHEMICAL STAINS FOR VENTURIA INAEQUALIS AND THE ANTAGONIST ATHELIA BOMBACINA. C. S. Young and J. H. Andrews, Dept. of Plant Pathology, Univ. of Wisconsin, Madison 53706.

Polyclonal antibodies were produced against Athelia bombacina in rabbits and Venturia inaequalis in chickens, using cell wall and soluble protein preparations from hyphae of each fungus. Antibody specificity was tested by ELISA and hyphal staining. In an ELISA, the titer of antibodies produced against A. bombacina was higher with A. bombacina than with other species of Athelia, related basidiomycetes, or V. inaequalis. The titer of antibodies produced against V. inaequalis was higher with V. inaequalis than for other ascomycetes or A. bombacina. Hyphae were stained with alkaline-phosphatase conjugated IgG and an insoluble substrate, naphthol AS phosphate, that was coupled to a red or blue diazonium salt. Some cross reactions occurred among fungi that were taxonomically similar to A. bombacina. Hyphae of A. bombacina were stained and localized in apple leaf litter. Similar studies to localize V. inaequalis are in progress.

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IMPLICATIONS OF RHIZOSPHERE COMPETENCE IN TRICHODERMA HARZIANUM. Jaleed S. Ahmad and R. Baker, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO, 80523.

Trichoderma harzianum (T-95) was rhizosphere competent in soil of pH 5.0, 6.0, and 7.0, at temperatures 19, 26, and 33 C, with or without the addition of 10 ug/g soil of benomyl. Cucumber seeds, treated with freshly harvested T-95 conidia, were sown in raw soil kept under constant matric potential with no additional water added. The roots grew 8 cm in 8 days. Untreated seeds produced roots 7 cm long. Fewer cfu of Pythium ultimum per mg were isolated from rhizosphere soil of the treated seedlings than in the untreated controls. P. ultimum was not detected in the 8th (furthest from the seed) cm root segment of treated seeds, whereas the last cm of root segment from untreated seeds yielded 3000 cfu/g rhizosphere soil. Seeds treated with T-95 showed higher emergence than those treated with a rhizosphere-incompetent T. harzianum isolate (WT), at 19 C.

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COMPETITIVE SAPROPHYTIC ABILITY AND CELLULOLYTIC ACTIVITY OF RHIZOSPHERE COMPETENT MUTANTS OF TRICHODERMA HARZIANUM. Jaleed S. Ahmad and R. Baker, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO, 80523.

Competitive saprophytic ability (CSA) of 6 spp. and strains of Trichoderma was determined by the modified Cambridge Method (sensu Garrett). Rhizosphere competent mutants (T-95 and T-12B) had higher CSA index than the rhizosphere-incompetent Trichoderma spp. and strains (WT, T-12, T-8, and T-S-1). The isolates were grown for 6 days on Czapek Dox broth, with cotton linters, carboxy methyl cellulose, or cellobiose as sole sources of carbon. Cellulase (E.C. 3.2.1.4) was determined quantitatively by following the release of free glucose. Mutants produced more units of cellulase than the wild types. Cellulase production of these isolates was directly correlated with CSA and rhizosphere competence.

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PREPARATION OF NONPARENTAL STRAINS OF TRICHODERMA HARZIANUM BY PROTOPLAST FUSION. T. E. Stasz and G. E. Harman, Dept. of Hort, Sciences, NYSAES, Cornell University, Geneva NY 14456.

Auxotrophic mutants of two biocontrol strains of T. harzianum, T12 and T95, were fused, and nonparental progeny were obtained. The parents differed in four isozyme phenotypes. Also, T95 was resistant to two fungicides and required lysine for growth, and T12 required histidine. Protoplasts were released from young hyphae using Novozyme 234, fused using polyethylene glycol and $CaCl_2$, and plated on a minimal salts medium containing 0.6M sucrose. Colonies appeared 2 to 6 weeks after plating, grew very slowly, contained hyphae with abnormal swellings, and were similar to T12 in isozyme phenotype. Subsequently, more rapidly growing sectors formed, and some resembled T95 in isozyme phenotype and fungicide resistance. Progeny included types similar to T12 but not requiring histidine; types similar to T12 in isozyme phenotype but that grew slowly, sporulated infrequently, and did not require histidine; and types that resembled the T95 and T12 parents.

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COMPARED BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI BY FUNGAL AGENTS AND MYCOPHAGOUS COLLEMBOLA. Robert Lartey, E. A. Curl, and Curt M. Peterson, Dept. of Botany, Plant Pathology and Microbiology, Alabama Agricultural Experiment Station, Auburn University, AL 36849.

Fungal biocontrol agents (Laetisaria arvalis, Gliocladium virens, and Trichoderma harzianum) and mycophagous collembolan insects (Proisotoma minuta and Onychiurus encarpatus) were compared for biological control potential. Both insects were more attracted in vitro to Rhizoctonia solani than to either of the fungal agents and rapidly consumed the pathogen mycelium. The fungal agents, which severely inhibited Rhizoctonia on agar plates, were consumed by the insects in the absence of alternative food sources. In Rhizoctonia-infested soil, fungal agents and collembolan species, when tested separately, significantly increased the rate of seed germination and reduced disease incidence and severity on cotton seedlings. When fungal and insect agents were combined, seedling emergence was further enhanced and disease severity further reduced by the interaction.

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INTERACTIONS OF A PEAT CARRIER AND POTENTIAL BIOLOGICAL CONTROL AGENTS. D.M. Huber, J.E. Wagner, H.E.L. Nashaar, and

L.W. Moore. Purdue University, W. Lafayette, IN 47907; Allied Corporation, Syracuse, N.Y.; and Oregon State University, Corvallis, OR.

Five isolates of fluorescent Pseudomonads reported to reduce take-all root, crown, and foot rot were cultured in neutral peat and evaluated as seed treatments in a sandy loam field soil naturally infested with Gaeumannomyces graminis. Seed of the take-all susceptible winter wheat cultivar "Caldwell" treated with finely ground sterile peat at pH 5.2 or 7.0, and non-treated seed served as controls. The % white heads from take-all were 7, 18, and 20%; and grain yields were 3560, 2840, and 2240 kg/ha for the untreated control, pH 5.2 peat control, and pH 7.0 peat control, respectively. All bacterial isolates partially or completely nullified the predisposing effects of peat on take-all. This study demonstrated significant predisposing effects of peat as a carrier for potential biological control agents for take-all.

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RELATION OF CARBOHYDRATE CONCENTRATIONS TO PYTHIUM DAMPING-OFF IN CONTAINER MEDIA AMENDED WITH COMPOSTED HARDWOOD BARK. W. D. Chen, H. A. J. Hoitink, A. F. Schmitthenner, and O. H. Tuovinen, Depts. of Plant Pathology and Microbiology, The Ohio State University, OARDC, Wooster 44691.

Container media amended with low temperature compost were suppressive to Pythium damping-off, whereas those amended with high temperature (>60 C) compost were conducive but became suppressive after 4-5 days incubation at room temperature. In conducive media, concentrations of glucose, reducing substances and total carbohydrates were higher than in suppressive media until 24 hr, 4 and 6 days after planting, respectively. A positive correlation was established between soluble carbohydrate concentrations and disease severity. It is postulated that the suppressive effect was due to nutrient competition.

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BIOLOGICAL CONTROL OF PYTHIUM DAMPING-OFF OF CUCUMBERS WITH PYTHIUM NUNN.

T. Paulitz and R. Baker. Dept. of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523

Addition of Pythium nunn to aerated steamed soil infested with Pythium ultimum and planted with cucumbers resulted in disease and pathogen suppression. Inoculum density/disease incidence (ID/DI) curves were constructed for treatments with and without P. nunn. The effect of initial inoculum density of P. nunn on disease incidence and population density of P. ultimum was also investigated. In greenhouse experiments, P. nunn reduced root pruning caused by P. ultimum, as evidenced by greater shoot and root dry weights. Percent emergence of P. nunn-treated cucumber seeds was significantly greater than non-treated seeds when planted in aerated steamed soil infested with P. ultimum.

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PREPLANT ROOT DIPS FOR APPLE ROOTSTOCKS NATURALLY INFESTED WITH PHYTOPHTHORA SPECIES. **S. N. Jeffers and W. F. Wilcox, Cornell University, NY State Agric. Exp. Sta., Geneva 14456**

To eradicate naturally occurring Phytophthora spp., roots of dormant MM.106 apple rootstocks were dipped for 10 or 60 min in 1000-ppm aqueous mixtures of metalaxyl, captafol, mancozeb, or $Cu(OH)_2$, in 1.05% NaOCl, or in water before each was planted in autoclaved vermiculite. Plants were grown for 10-13 wks and periodically flooded to enhance disease development. No treatment eradicated Phytophthora spp. from all plants, although rootstocks that had been dipped in metalaxyl for either duration, in NaOCl for 10 min, or in $Cu(OH)_2$ for 60 min produced healthy-appearing plants. All rootstocks dipped only in water developed severe Phytophthora crown rot symptoms. In a separate experiment, phytotoxic effects were not observed after 2 yrs on nursery-grown trees that had received preplant root dips in metalaxyl at 500 or 1000 ppm for 30 or 60 min but were observed on trees that had been dipped in 1.05% NaOCl for 60 min. Preplant root dips may be useful in managing Phytophthora crown rot in the orchard.

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SPECIFIC AND SLOW RUSTING RESISTANCE OF Hordeum spontaneum INFECTED WITH THREE RACES OF Puccinia hordei. L.M. Treeful and R.D. Wilcoxson. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Thirty-two lines of H. spontaneum and H. vulgare cultivars Larker, Robust, Vada and line MN 9062 were infected in the field with three races of P. hordei. Twenty-four lines of H. spontaneum had hypersensitive resistance to all three races. One line was susceptible to races 8 and 19 but resistant to race 13. Seven lines were susceptible to all three races. Rust severity was recorded on the susceptible lines at 7-day intervals for 3 weeks and areas under disease progress curve (AUDPC) were calculated. Four lines had AUDPCs similar to those of Vada and MN 9062 (slow rusting checks) and three lines had AUDPCs similar to those of Larker and Robust (fast rusting checks). H. spontaneum was concluded to possess resistance recognized by infection types as well as by slow development of rust.

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CORRELATIONS BETWEEN LEAF AGE AND SUSCEPTIBILITY OF PHASEOLUS VULGARIS L. TO UROMYCES APPENDICULATUS (PERS.) UNGER VAR. APPENDICULATUS. Meher Shaik and James R. Steadman. Dept. of Plant Pathology, Univ. of Nebraska, Lincoln NE 68583-0722.

The relationship between leaf age or size at the time of inoculation on the size of pustules has been mentioned in the literature, however, it has never been quantified. This relationship was investigated in susceptible and resistant reactions. In a susceptible reaction, characterized by large pustules, the area of pustules was negatively correlated with leaf-age ($r = -0.926$, $P<0.01$) or leaf-size ($r=-0.808$, $P<0.01$). The secondary sporulating area was also negatively correlated with leaf-age ($r=-0.860$, $P<0.01$) or leaf-size ($r=-0.852$, $P<0.01$). In the resistant reaction, characterized by minute pustules often surrounded by necrotic areas, the pustule area was not correlated with leaf-age ($r=0.175$, $P>0.05$) or leaf-size ($r=-0.130$, $P>0.05$).

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RESISTANCE TO CLUBROOT (PLASMODIOPHORA BRASSICAE) IN BRASSICA OLERACEA UNDER DIFFERENT INOCULUM POTENTIALS AND SOIL TEMPERATURES. R.L. Gabrielson, and J. Robak. Western Washington Research and Extension Center, Puyallup, WA 98371, and Research Institute of Vegetable Crops, Skierniewice, Poland.

Clubroot resistance to Race 7 was affected by resting spore density, frozen storage of inoculum and growth media. Percentage clubbed plants with Badger Shipper resistance increased from 9 at 10^2 to 70 at 10^7 spores/ml. Frozen storage of clubs reduced inoculum potential. Maximum percentage clubbed plants occurred using commercial greenhouse mixes, minimum using muck soil. Resistance source and temperature differentially affected club formation. Badger Shipper and Oregon CR-1 broccoli resistance was strong against Race 6, but weak and temperature sensitive to Race 7 above 20 C. In contrast Oregon cabbage #123 resistance was strong against Race 7, but weak and temperature sensitive to Race 6 above 20 C. As both inoculum potential and temperature affect disease reaction they should be closely controlled when determining races. Also, OR # 123 can be used to differentiate Race 6 in a mixture of Races 6 and 7.

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ASSESSMENT OF PARTIAL RESISTANCE TO BLAST IN LOWLAND RICE CULTIVARS. J.M. Bonman, W.H. Yeh, and J.M. Bandong, International Rice Research Institute, P. O. Box 933, Manila, Philippines.

The partial resistance to blast of six lowland rice cultivars was assessed in greenhouse experiments using specific isolates of Pyricularia oryzae and in upland nursery experiments using Marchetti's miniplot technique. IR36, Milyang 30, and Milyang 42, which have shown relatively little disease in farmers' fields for several years, had lower relative disease efficiency and smaller lesions with lower sporulation capacity than did IR50, Milyang 57, and Suweon 264. No isolate by cultivar interaction was detected in the greenhouse experiments. Relative disease progress in the miniplot experiments at several sites indicated a close correspondence with the greenhouse results for the six cultivars. Using IR36 and IR50 as agricultural checks in miniplot trials, 59 lines were screened to select those with partial resistance similar to IR36. The miniplot technique may be useful for identifying lines with acceptable levels of partial resistance.

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ASSESSMENT OF LEAFSPOT RESISTANCE AMONG ALFALFA CULTIVARS IN NORTH CAROLINA FIELDS. W. M. Thal, and C. L. Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Experimental plots were sampled destructively and evaluated visually for leaf spot severity to assess differences in disease level among 16 alfalfa cultivars in Rowan and Washington Co. in 1983 and in Rowan, Sampson, Wake, and Washington Co. in 1984. Disease severity was estimated for each leaf on a stem. Total length, length of defoliation up the stem, number of intact and defoliated nodes, and number of abscised main stem leaves were measured for each stem. High correlations ($r>0.70$) were seen among several of the variables measured. Differences were detected among cultivars in disease severity, maximum disease on a stem, and percent of nodes with abscised leaves. No cultivar by location interaction was detected. Florida 77 was more susceptible than most other cultivars and Raidor had a higher level of resistance to leafspot diseases than other cultivars. Stability analysis indicated that Raidor was the most stable cultivar.

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EFFECTS OF INDUCED SYSTEMIC RESISTANCE AGAINST BLUE MOLD ON SOLUBLE CARBOHYDRATES IN TOBACCO. S. D. Salt, S.-Q. Pan and J. Kuć, Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY 40546-0091.

After stem-inoculation of Kentucky 14 Burley tobacco plants with sporangia of Peronospora tabacina Adam for induction of resistance against blue mold, an increase in total soluble carbohydrates relative to control plants was found in stem and leaf tissues. By 3 weeks post-inoculation soluble carbohydrates in tissues of inoculated plants were 1.5- to 8-fold those of controls. Free glucose accounted for more than 80% of the increase in leaves, more than 60% in stems; free fructose increased to a lesser degree. No increase was found in sucrose or other mono- or disaccharides. The increase in sugars does not seem due to blockage of vascular transport from the leaves to the roots, elevated amylase or invertase activities, or hydrolysis of starch reserves. We are investigating whether changes in sugar levels directly influence induced resistance or whether they may serve as "markers" of efficacy of "immunization".

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EFFECT OF FUNGICIDES ON IN VITRO GROWTH OF ISOLATES OF THIELAVIOPSIS BASICOLA. Paul R. Bachi, University of Kentucky Research and Education Center, Princeton, KY 42445 and John R. Hartman, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Benomyl, benodanil, CGA 449, imazalil and iprodione were compared using agar plate assays for efficacy to three isolates [from tobacco (t), American holly (a), and blue holly (b)] of Thielaviopsis basicola. The three isolates varied in pathogenicity to 'Blue Maid' meserve holly, causing 0, 17, and 94 percent root infection following a root dip inoculation. All fungicides were incorporated into potato dextrose agar at concentrations of 0, 0.01, 0.1, 1.0, and 10.0 ppm. The relative mean ED 50 for the three isolates was between 0 and 0.01, 0.1 and 1.0, and 1.0 and 10.0, for imazalil, benomyl, and CGA 449 respectively and >10.0 for iprodione and benodanil. The t isolate had significantly greater mycelial growth than the a and b isolates over all concentrations and fungicides. This in vitro assay allows selection of the more active fungicides for field testing.

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EFFICACY OF NEW STEROL-INHIBITING FUNGICIDES FOR THE CONTROL OF EUROPEAN BROWN ROT. B.A. Latorre and M.A. Lolas, Departamento de Ciencias Vegetales, Facultad de Agronomía, Pontificia Universidad Católica, Casilla 6177, Santiago, Chile.

Studies conducted to determine the effectiveness of DPX-H6573 ('Nustar') and RH-3866 (Systhane') against the European brown rot fungus (Monilia laxa) have shown DPX-H-6573 and RH-3866 to be effective to control M. Laxa. However DPX-H6573 was more active than RH-3866. The ED50 values in poison agar tests were 1.58×10^{-2} and 25.10×10^{-2} ug a.i./ml for DPX-H6573 and RH-3866, respectively. Penconazole 7.5×10^{-2} ug a.i./ml and vinclozolin 7.98×10^{-2} ug a.i./ml. The ED50 values calculated from data obtained in sweet cherry tests, perfomed with fruits cv. 'Napoleon', inoculated with 2×10^6 conidia/ml and incubated in humid chambers at 20 C for 72 h, were 3.63 and 32.35 ug a.i./ml for DPX-H6573 and RH-3866, respectively. A complete control of European brown rot on sweet cherry fruits was obtained after 10 days at 0 C and 3 day at 20°C, with 20 mg a.i./l of DPX-H6573 and mg 120 a.i./L of RH-3866.

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EFFECT OF FUNGICIDES ON EAR AND STALK ROT OF MAIZE. H.L. Warren and S.K. von Qualen. USDA, ARS. Purdue University, West Lafayette, IN 47907.

Severity of stalk and ear rot of maize decreased following application of Benlate and Dithane M22 two weeks prior to anthesis. Maize inbreds B73, C103, and Pr were inoculated by the whorl inoculation technique one week prior to anthesis with a spore suspension of Stenocarpella maydis, Fusarium moniliforme, and Gibberella zeae, respectively. The percentage of ear rot and stalk rot severity were evaluated at plant maturity. Stalk and ear rot decreased significantly when compared to the control following fungicide treatment except for F. moniforme ear rot. The study helps to substantiate that ear and stalk rot pathogens are infecting the host prior to anthesis, while symptoms appear at plant maturity.

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EPIDEMIOLOGY AND CONTROL OF BOTRYOSPHAERIA PANICLE AND SHOOT BLIGHT OF PISTACHIO. T. J. Michailides and J. M. Ogawa, Dept. of Plant Pathology, University of California, Davis, CA 95616.

Inocula of Botryosphaeria dothidea reside in the pistachio orchards as pycnidia borne in rachises, blighted shoots, petioles, dead buds, and mummified fruit. In addition, newly formed pycnidia on current-season rachises, fruit, and shoots provide inoculum for late summer and fall infections. The fungus is also active as mycelium in perennial cankers. Rain and sprinkler irrigation spread fungal conidia which cause new infections throughout the growing season. Blight symptoms developed rapidly in late spring and summer when max air temperatures ranged from 21-40 C. Optimum temperature for growth of B. dothidea was 27-30 C and artifically inoculated fruit clusters became severely infected at these temperatures. Optimum temperature for pycnidia development was 30 C. Under California conditions, disease control was achieved by reducing body trajectory angle of sprinklers to deliver water beneath the tree canopy. Chemical control using chlorothalonil, benomyl, and captafol sprays significantly reduced cluster infection with captafol being the most effective.

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A MICROCOMPUTER-BASED MODEL FOR THE MANAGEMENT OF MIDSEASON FOLIAR DISEASES OF SOYBEAN. P. A. Backman, M. A. Crawford, and M. Schwartz, Dept. of Plant Pathology, Auburn University, AL.

A management model linking weather, plant growth, disease prediction and losses, and economics has been developed for the two midseason foliar pathogens Colletotrichum truncatum and Septoria glycines. Given early season prediction choices, e.g. cultivar, pest populations, and treatments, the model calculates a yield potential at the end of the vegetative period. For each rainfall event during early reproductive periods, the model decrements yield based on disease that will develop from that event. Fungicides are given a control efficiency rating (% of loss preventable) that is further adjusted based on method of application. Based on previous weather and weather forecasts, a dollar return above cost is calculated for the application. Subsequent spray events may also be suggested when damaging levels of disease occur outside of the window of activity described for the initial fungicide.

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DISEASE SEVERITY AND YIELD REDUCTION OF SOYBEANS CAUSED BY PYRENOCHAETA GLYCINES. G. L. Hartman and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana, IL 61801.

To evaluate the control of red leaf blotch of soybeans caused by P. glycines, treatments within four-replicated blocks in Zambia were either unsprayed or sprayed with fentin acetate at 0.6 kg a.i./ha (one, two, three or 11 times) during the 1984-85 growing season. Defoliation and disease severity were recorded five times between the R1 and R7 growth stages. Every leaf on five plants in each plot was evaluated at each rating time. Between 61 and 95 days after planting, defoliation increased from 45 to 83% in unsprayed plots and 41 to 52% in plots sprayed 11 times. At 96 days after planting, the disease was observed on all the leaves of plants in unsprayed plots and on 89% of those in the plots sprayed 11 times. Total seed yields were 22, 42, 43, and 43% greater for plots sprayed one, two, three or 11 times, respectively, than those of unsprayed plots. Seed size was 27% and 1000-seed weight 35% greater for plants sprayed 11 times than unsprayed plants.

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CELLULAR DIFFERENTIATION OF VENTURIA INAEQUALIS ASCOSPORES DURING PENETRATION OF APPLE LEAVES. Karen J. Smereka and W.E. MacHardy, Department of Botany and Plant Pathology, University of New Hampshire, Durham, NH 03824.

Mature apple leaves were inoculated with purified ascospore suspensions, and development was observed using various microscopic techniques. Spore attachment to the apple epicuticular wax was by a carbohydrate-positive mucilage. Direct penetration occurs from the spore proper or by germ tube and appressorium formation. Lipid and glycogen reserves were depleted during germination, and microbody and mitochondrial profiles became more numerous. Formation of a penetration pore in the spore wall was succeeded by an apparent invagination of the plasmalemma and subsequent development of a membrane-bound infection sac. High voltage electron microscopic stereo imaging and cytochemistry revealed junction-like structures where the infection sac membrane was appressed to the plasmalemma. We suggest a possible role of the infection sac to be compartmentalization of enzymes necessary for subcuticular penetration.

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WOUND-INDUCED LIGNIFICATION IN ALMOND (PRUNUS DULCIS (MILL.) WEBB) BARK. M. A. Doster and R. M. Bostock, Department of Plant Pathology, University of California, Davis, CA 95616.

Bark wounds in almond trees allow entry of canker forming fungi, but aged wounds become immune to infection. To investigate the relationship of lignification to resistance, a method of isolating lignin using thioglycolic acid was adapted for quantifying lignin in almond bark tissue. In orchard trees wounded in September and October, 193% more lignin was detected in 2-day-old than in fresh wounds and the amount of lignin increased linearly (r^2=0.95) as the wounds aged from 0 to 21 days. In potted almond trees kept at 25 C, 71% more lignin was detected in 1-day-old than in fresh wounds and lignin levels increased linearly (r^2=0.98) as the wounds aged from 0 to 9 days. Cupric oxide oxidation of the lignin thioglycolate adduct of aged wounds yielded more vanillin, p-hydroxybenzaldehyde, and syringaldehyde, supporting the claim that more lignin was present. Chitosan applied to fresh wounds further increased the lignin detected in 3-day-old wounds. Lignification may thus contribute to immunity to infection.

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LIGNIN DEPOSITION IN APIUM ROOTS AS A RESPONSE TO INFECTION BY FUSARIUM OXYSPORUM F. SP. APII, RACE 2. C. M. JORDAN and R. M. Endo, Department of Plant Pathology, University of California, Riverside, CA 92521.

Seedlings roots of three Apium lines either resistant or susceptible to Fusarium yellows were inoculated with conidia of Fusarium oxysporum f. sp. apii, race 2. The time of penetration of the roots of all three lines was similar, but the number of germ tubes that penetrated the roots was significantly higher in the two susceptible hosts. Lignin deposits were first detected using thioglycolic acid (TGA) in the resistant and susceptible hosts 3 hr and 6 hr after inoculation, respectively. The resistant host also gave greater yields (7-fold or more) of lignin-TGA than either the inoculated roots of the susceptible lines or the healthy uninoculated roots. The cell walls and tonoplasts of the inoculated resistant host stained intensely with diaminobenzidine (DAB) indicating the presence of peroxidase, but only slight staining was observed in the inoculated susceptible lines at 24 h after inoculation.

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NEMATODE REPRODUCTION AND COTTON YIELD RESPONSE IN PLOTS INFESTED WITH MELOIDOGYNE INCOGNITA AND FUSARIUM OXYSPORUM F.SP. VASINFECTUM. J. L. Starr, M. J. Jeger, R. D. Martyn, and K. Schilling. Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843

The ratio of final to initial nematode populations (Pf/Pi) and seed cotton (TAMCOT SP37) yields were determined in microplots infested with Meloidogyne incognita (MI) and Fusarium oxysporum f. sp. vasinfectum (FOV). There was a linear relationship between Pf/Pi and ln(Pi); the slopes of the regressions were negative and estimates of the slopes indicated that there was lower nematode reproduction in the presence of FOV. A curvilinear relationship was observed between seed cotton yields and ln(Pi). Linear regression analysis indicated that there was no effect of FOV on the slope of the yield response but the intercepts differed significantly. Effects of FOV on nematode reproduction and seed cotton yield were consistent with observed effects on plant mortality.

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EFFECTS OF FUSARIUM OXYSPORUM F.SP. VASINFECTUM AND MELOIDOGYNE INCOGNITA ON PLANT MORTALITY IN COTTON. R. D. Martyn, M. J. Jeger, J. L. Starr, and K. Schilling. Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

The effects of Fusarium oxysporum f.sp. vasinfectum (FOV) and Meloidogyne incognita (MI) on plant mortality in cotton (TAMCOT SP37) was assessed in microplots. The soil was uniformly infested with 2 levels of FOV (13,000 and 20,000 cfu/g soil) and 4 levels of MI (0.1, 1, 10, and 50 nematodes/100 cm^3 soil). Plant mortality was assessed at 2-wk intervals. A main effect of FOV was apparent early and it remained constant at both levels of FOV for the remainder of the season. There was also a significant and increasing effect of MI on plant mortality 16-22 wk after planting at the highest MI level, but not at lower levels. There was no significant MI x FOV interaction at any single assessment time. The different timings and slopes of the main effects represented an interaction with time and stressed the importance of sequential disease assessments in such studies.

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GENE ACTION AND INHERITANCE OF RESISTANCE TO RHIZOCTONIA SOLANI AND PYTHIUM ULTIMUM IN COTTON SEEDLINGS. M.A.T. Poswal, K.M. El-Zik, and L.S. Bird. Dept. of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

Data were obtained from seedlings of a six-parent diallel cross (F_1 and F_2, no reciprocals) of Upland cotton cultivars that were grown in controlled temperature water bath tanks. Soil was infested with R. solani and P. ultimum separately at two inoculum densities. Traits measured were emergence, pre- and post-emergence damping-off, final stand, and hypocotyl and root damage. Dominance effects were the most predominant for traits associated with resistance to both pathogens. The additive and epistatic effects were smaller in magnitude than the dominance effects. Heritabilities in the narrow sense were low ranging from 0.1% to 20.7%. General combining ability effects were important for the expression of resistance to R. solani and specific combining ability effects for P. ultimum. Resistance in cotton seedlings to both pathogens was polygenically inherited and conditioned by a complex of minor genes.

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DIFFERENTIATION OF VERTICILLIUM DAHLIAE PATHOTYPES AND COTTON TOLERANCE TO WILT AS AFFECTED BY STEM-PUNCTURE INOCULUM CONCENTRATION. J. J. Stapleton and J. E. DeVay, Department of Plant Pathology, University of California, Davis, CA 95616.

Stem-puncture inoculum concentrations (IC) of 10^1, 10^2, 10^4 and 10^6 conidia/ml of T9 (defoliating) and SS4 (nondefoliating) pathotypes of Verticillium dahliae were evaluated for foliar symptom progression and severity. Mainstems of Acala cotton cultivars differing in wilt tolerance were inoculated with 60 µl of each IC. Symptoms developed with all IC used on wilt-susceptible cotton seedlings. Inoculation of 60 µl at 10 conidia/ml approximated one conidium/plant. Regression analyses of foliar symptom development were highly significant ($P < 0.01$) and gave slopes that were similar for all IC within pathotypes and for the same IC between pathotypes. Earliness of symptom development increased with increasing IC. Stem inoculation at 10^1-10^2 conidia/ml reflected pathotype differences and wilt tolerance of cotton cultivars as well as those at higher IC.

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GROWTH RESPONSES OF SUDANGRASS CULTIVARS TO MAIZE DWARF MOSAIC VIRUS-STRAIN A. Stephen R. Vann, Robert W. Toler, Dept. of Plant Pathology and Microbiology, and Frederick R. Miller, Dept. of Soil and Crop Sciences, Texas Agricultural Experiment Station, College Station, TX 77843

Plant height, tiller number, and dry weight were evaluated for four resistant and four susceptible sudangrass (Sorghum sudanense Piper stapf.) cultivars following inoculation with MDMV-A in the field. Cultivars representing foreign and domestic sources were inoculated at the 3-6 leaf stage with a DeVilbiss spray gun at 6.33 kg/cm^2. Symptom expression, which varied from mild to severe mosaic with redleaf development, was rated after 3 weeks. Plant height declined as much as 32%, tiller number decreased up to 51%, and dry weight was reduced by as much as 38%.

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EVALUATION OF THE STEROL INHIBITOR FUNGICIDE XE-779 AS A SEED TREATMENT FOR CONTROL OF ACREMONIUM COENOPHIALUM, THE ENDOPHYTE OF TALL FESCUE. R. A. Shelby and L. W. Dalrymple, Alabama Agricultural Experiment Station, Auburn University, AL 36849.

In a two year study involving both greenhouse and field experiments, XE-779 was applied at rates of 0.3-4.8 g a.i./kg seed. Viable endophyte was determined by microscopic examination of leaf sheath tissue of 8-week-old seedlings. A. coenophialum was reduced to less than 5% in plants from infested seed in all treatments. Phytotoxicity, measured by percent germination in vitro, was significant at all rates, but appeared to vary with different seed lots. In the field, phytotoxicity was measured by stand percent, plant fresh weight and subjective rating of appearance. By these parameters, phytotoxicity was measurable only at rates greater than 0.6 g/kg.

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ESTIMATION OF CROP LOSS FOR THE UROMYCES-MEDICAGO PATHOSYSTEM. L. H. Jacome and R. D. Berger. Department of Plant Pathology, University of Florida, Gainesville, Florida 32611.

Phytopathometric data from three field trials of spatial and temporal epidemic development were used to estimate yield loss of alfalfa cv Florida 77 due to rust (Uromyces striatus). Multiple levels of disease were achieved by applications of mancozeb; epidemic rate and initial disease were lowered. Models based on critical point, multiple points, and AUDPC were evaluated. The power function of a simple predictor (Loss=11.9 x $AUDPC^{0.6}$) was statistically better (least sum of squares, and higher R^2 values) than the monomolecular or linear models. Both leaf area and plant dry weight loss could be predicted by the AUDPC, R^2=0.92 and R^2=0.75, respectively. Loss was also predicted by using disease incidence as a critical-point predictor. Limitations of critical-point models in predicting yield loss were overcome by use of disease incidence of the first two or three stages of growth as a multiple-point model.

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VERTICILLIUM WILT OF ALFALFA: INFECTION PROCESS OF VERTICILLIUM ALBO-ATRUM IN ALFALFA SEED. R. G. Gilbert, USDA-ARS, P. O. Box 30, Prosser, WA 99350.

An alfalfa cultivar susceptible to Verticillium wilt was planted and grown under greenhouse conditions. Spore suspensions of a Verticillium albo-atrum (Vaa) isolate pathogenic to alfalfa were inoculated into alfalfa stems below the flowers, which were pollinated 10 days earlier. Mature alfalfa curls (pods) were hand harvested and prepared for histological microscopic examination. Vaa was easily detected in the connecting vascular tissue from the spike of the flower influorescence to the placental pod tissue. But Vaa rarely invaded the pod cavity and colonized the adjacent immature seeds. As these colonized seeds matured and dried, Vaa was entrapped within the seedcoat, where the infection was confined. Due to the discontinuous nature of Vaa in diseased alfalfa and the short time after pollination that seed infection can occur, the incidence of internal seed infection in commercial seed is extremely low.

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DISCRIMINATION OF POTATO YIELDS USING PREPLANT LEVELS OF PATHOGENS INVOLVED IN POTATO EARLY DYING. L. J. Francl, L. V. Madden, R. C. Rowe, and R. M. Riedel. Dept. of Plant Pathol., The Ohio State Univ., Ohio Agr. Res. Dev. Cntr., Wooster, 44691

A discriminant model was developed using data from 3 yr of a microplot study in which Solanum tuberosum cv. Superior was inoculated with Verticillium dahliae (Vd) and Pratylenchus penetrans (Pp), pathogens involved in potato early dying. The model classified tuber yields as more or less than 90% of uninfected controls based on the natural logs of the preplant populations of Vd and Vd x Pp. The model correctly grouped 86% of the yields for the 3-yr data set and 82% of the yields from 5 yr in another location. It was 56% correct when tested on an additional 3 yr of data where Vd and Pp had only a slight effect on yield. For disease management, the more serious classification error is to predict an acceptable yield when, in fact, low yield is obtained. The percentage of such misclassifications over all data was reduced from 20% to 6% when a third category of 80-90% yield relative to the control was added to the analysis as an 'uncertain' result.

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THE INFLUENCE OF CROP ROTATION AND TILLAGE PRACTICES ON PLANT-PARASITIC NEMATODE POPULATIONS IN A COSTA RICAN SMALL FARMING SYSTEM. S. H. Thomas, Dept. Entomology and Plant Pathology, Box 3BE, New Mexico State University, Las Cruces, NM 88003 and J. L. Saunders, IPM Project Coordinator, Centro Agronomico Tropical de Investigacion y Ensenanza, Turrialba, Costa Rica.

Numbers of nematodes associated with maize, bean, and maize-bean intercropping alternated with maize monoculture under four tillage systems were monitored during 2 cropping cycles in Costa Rica. All plots received the same tillage and crop rotation sequences for a 6-year period prior to and during this study. Soil populations of Helicotylenchus dihystera, Meloidogyne incognita, Pratylenchus scribneri, and Paratrichodorus minor all differed among tillage treatments. Conventionally tilled plots were associated with the highest populations of all nematodes except H. dihystera. Cropping sequence generally had less effect than tillage on nematode populations.

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EFFECT OF GIGASPORA MARGARITA, MELOIDOGYNE INCOGNITA, AND PHOSPHORUS FERTILITY ON PEACH GROWTH AND YIELD. R. W. Roncadori and R. S. Hussey, Dept. of Plant Pathology, Univ. of Georgia, Athens 30602.

The influence of Gigaspora margarita (GM), Meloidogyne incognita (MI), and phosphorus (P) fertilization on peach growth, fruit yield, and microorganism reproduction was determined in fumigated microplots maintained from April 1981 through December 1984. Six treatments were established in a soil containing 23 µg P/g (LP): no inoculation, GM, MI, and GM+MI added at planting, and two treatments (MI-D and GM+MI-D) where MI inoculation was delayed 6 wks after planting. In a 71 µg P/g (HP) soil, treatments were MI at planting and no inoculation. Root collar diam, shoot wt, and fruit yield were not improved by GM or HP compared with LP non-inoculated plants but were suppressed by MI only when added at planting in LP soil. HP or GM combined with MI improved the growth and/or yield over MI LP plants. MI juvenile population densities were not consistently affected by GM or P rates. GM sporulation was suppressed only by MI added at planting.

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CONTROL OF MELOIDOGYNE INCOGNITA IN PEACH WITH PRE- AND POST-PLANT NEMATICIDE TREATMENT. P.F. Bertrand and D.R. Evert. The University of Georgia, Tifton, GA 31793

Previous studies indicated that peach trees established without initial stunting or other visible damage by root-knot nematode would tolerate subsequent buildup of infection in the root system. A study was set up to relate these findings to control practices. Peaches (cv 'Dixiland'/Lovell) were planted into a site inhabited by M. incognita in January, 1983. Treatments were: 1)check; 2) preplant fumigation with 134.7 l/ha EDB; 3) postplant treatment with 11.21 kg/ha fenamiphos applied in the spring and fall of each year beginning the fall of 1983; and 4) preplant fumigation with EDB followed by biannual applications of fenamiphos. At the end of the first two growing seasons, trees planted in fumigated soil were larger than trees in non-fumigated soil. At the end of the third growing season (1985) a growth response to fenamiphos was apparent in some trees. Soil counts of M. incognita juveniles followed inversely the same trends as tree growth in response to the treatments.

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FUNGAL FLORA OF WHEAT SEED IN EAST AZARBAIJAN, IRAN. M. Babadoost, Department of Plant Pathology, College of Agriculture, University of Tabriz, Tabriz 51664, Iran.

Seventy seed samples from various locations of East Azarbaijan province, harvested in 1985, were examined for associated fungi. Seed, either surface sterilized in 0.5% NaClO solution or not sterilized, were plated on four media namely: PDA, Oxgall Agar, Nash and Snyder P-PCNB.Agar, and Czapeck Dox Agar+6% NaCl. The growing fungi were isolated and identified. Fusarium, Alternaria, Stemphylium, Aspergillus, Penicillium, Trichothecium, and Rhizopus species were present in 47%, 91%, 17%, 100%, 74%, 7%, and 96% of the samples respectively, when the seed were not surface-sterilized. In surface-sterilized seed, Fusarium spp. were detected in only 13% of the samples. Degree of association of the fungi with seed differed with the source of samples and kind of the media. To study seedling infection, seed were sown 2.5 cm deep in soil in pots and the seedlings were examined for disease occurrence.

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COMPARISON OF MD5 AND XCS MEDIA AND DEVELOPMENT OF MD5A MEDIUM FOR DETECTING *XANTHOMONAS CAMPESTRIS PV. CAROTAE* IN CARROT SEED.
M.A. Cubeta and T.-L. Kuan, Asst. Plant Pathologist and Research Manager, Plant Pathology, Asgrow Seed Company, San Juan Bautista, CA 95045

Xanthomonas campestris pv. carotae (Xcc) causing carrot bacterial blight, can be seed-borne and seed transmitted. MD5 medium (Kuan, T.-L. et al., Phytopathology 74:796) and XCS medium (Williford, R.E. et al., Phytopathology 74:1142) are selective and have been used to detect Xcc in carrot seed. Two hundred and thirty-two commercial carrot seed lots were tested using both MD5 and XCS media. Both media detected Xcc in 30 seed lots and not in 187 seed lots. Fifteen seed lots gave differing results. Xcc was detected in 9 seed lots on XCS and not on MD5 and in 6 seed lots on MD5 and not XCS. These results demonstrate that both media have similar efficacy in detecting Xcc. Colonies of Xcc are detectable after 5 and 7 days incubation on XCS and MD5, respectively. The test time with MD5 has been shortened from 7 to 5 days and selectivity improved by adding L-glutamic acid (5 ug/ml), L-methionione (lug/ml), cephalexin (10 ug/ml) and bacitracin (10ug/ml) (MD5A).

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USE OF ALCIDE^{R} TO REDUCE OR ERADICATE XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS IN CRUCIFER SEEDS. G. E. Harman, T. E. Stasz and H. S. Humaydan, Department of Horticultural Sciences, Cornell Univ., NYSAES, Geneva, NY 14456; and Harris-Moran Seed Co., Rochester, NY 14624.

Soaking seeds in Alcide^{R} may eradicate seed-borne bacteria. Brassica seeds infected with X. c. pv. campestris were soaked for 30 min in an Alcide solution. A total of 21 samples of 5,000 - 10,000 seeds each, representing 10 seed lots, were treated. The pathogen was not detected in 19 samples, while in the remaining two samples from two different seed lots, very low levels of the pathogen were detected. High levels of X. c. pv. campestris were detected in all nontreated seeds. Alcide^{R} treatment reduced emergence slightly to moderately in 8 of 21 lots tested. Alcide seed treatment may be useful as an alternative to hot water seed treatments.

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EFFECT OF OSMORPRIMING OF SUGAR BEET SEED ON SUSCEPTIBILITY TO PYTHIUM DAMPING-OFF. R. M. Osburn and M. N. Schroth, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Sodium chloride and polyethylene glycol (PEG)-osmoprimed seed treatments significantly reduced damping-off by P. ultimum in greenhouse experiments at 16 C in naturally infested field soil. Reduction in disease was due to control of preemergence but not postemergence damping-off. Disease control resulted from a significant reduction in seed coat infection during germination. Since seedborne bacteria grow in high populations (up to 10^{8}/ml) in both priming solutions, the role of these bacteria in disease control was investigated. Comparison was made between seeds primed under normal conditions and seeds which were surface sterilized, then primed in the presence of antibiotics to prevent bacterial growth. No significant difference was found between the two treatments, indicating that the priming solution bacteria were not important in disease control. This supports our previous conclusion that reduced exudation by primed seeds is the critical factor in reducing susceptibility to P. ultimum damping-off.

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INVESTIGATION OF THE CAUSAL AGENT OF CASSAVA MOSAIC DISEASE IN SOUTHERN AFRICA. M.E.C.Rey, M.Sibara and H.M.Garnett, Department of Microbiology, University of the Witwatersrand, 1 Jan Smuts Avenue, Johannesburg, 2001.

Cassava mosaic disease (CMD) exists throughout Africa. Cassava latent virus (CLV) has been implicated as the etiological agent of CMD in Kenya and West Africa. Investigation of the causal agent of CMD in Southern Africa was undertaken. Isolation of a rod-shaped flexuous particle resembling cassava mosaic virus (CCMV) and presence of inclusion bodies in infected material suggested the possibility of CCMV. However serological tests employing antiserum against CCMV proved negative. Attempts to isolate CLV by virus and double-stranded DNA extractions failed. Recently antiserum against CLV was used in ELISA and protein A-I^{125} radioimmunoassays. Both tests were positive for CLV. Further evidence was provided by immunocytochemical TEM demonstrating gold particle staining in cells of infected leaves. The possibility of a double infection has not been overruled, particularly as inclusion whorls, not labelled with anti-CLV were seen. Hybridization tests using CLV probes are currently in progress.

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PATCHOULI (POGOSTEMON PATCHOULI) MOSAIC CAUSED BY A POTY VIRUS IN INDIA. B.L. SUBBA RAO; CIMAP, POST BAG NO.1, P.O.: R.S.M. NAGAR, LUCKNOW 226 016, INDIA.

A mechanically transmissible virus with a narrow host range was isolated from mosaic infected patchouli (Pogostemon patchouli) plants. The virus was also transmitted by grafting and Myzus persicae. The thermal inactivation point of the virus was 55C, dilution end point was 10^{-4} and the longivity in vitro (at 25C) was 10 days. The normal length of the virus particles was 710 nm. The infected cells consisted of pinwheels with scrolls and laminated aggregate inclusion bodies resembling those induced by viruses of potato Y group. The virus was serologically related to turnip mosaic virus reported on radish. The virus has not reacted with the antisera of BYMV, CYVV, CeMV, PVY and papaya mosaic virus. Based on this data the virus isolated was considered as a member of poty virus group and tentatively designated as PaMV.

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MOTTLING DISEASE OF ATROPA BELLADONNA L. IN INDIA. B.L.SUBBA RAO, Md. ZAIM and AKHTAR HUSAIN; CIMAP, POST BAG NO.1, P.O.: R.S.M. NAGAR, LUCKNOW 226 016, INDIA.

Leaf mottling, puckering some times distortion and severe stunting of Atropa belladonna plants grown in CIMAP farm at Lucknow, were caused by belladonna mottle virus (BdMV). The virus was readily transmitted mechanically to belladonna plants and 8 plant species belonging to Apocyanaceae, Chenopodiaceae and Solanaceae. The virus was not seed transmitted. Myzus persicae failed to transmit the disease. The virus was having longivity in vitro (at 25C) od 18-20 days, TIP was 80C and DEP between 10^{-5} to 10^{-6}. The virus was serologically related to BdMV reported on A. belladonna from West Germany and BdMV-physalis strain. Immunodiffusion tests were negative against CMV, tobacco and tomato ring spot viruses. The virus was polyhedral having a diameter of 30 nm and the 260/280 ratio was 1.76. Datura stramonium showed severe systemic symptoms and gave high yields of infectious virus.

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FIELD LOSSES IN SUGARCANE FROM SUGARCANE MOSAIC VIRUS (SCMV) INFECTION. M.P. Grisham, G.T.A. Benda, and H. Koike, USDA/ARS, U.S. Sugarcane Field Laboratory, P.O. Box 470, Houma, LA 70361

Mixtures of sugarcane mosaic virus(SCMV)-infected and healthy seed cane of five cultivars of sugarcane (Saccharum interspecific hybrids) were planted in field plots in randomized blocks with four replicates. Mixtures contained 0, 50, or 75% SCMV-infected stalks. Sugarcane in Louisiana is normally harvested annually for three years from a single planting. Total yields (sugar/ha) over three years in five or six successive 3-year crop cycles per cultivar were 5 to 19% less for treatments planted with 75% diseased stalks compared to plantings of mosaic-free cane. Cultivar CP 70-321, known to recover from infection, showed the least decrease in sugar/ha and CP 52-68, an intolerant cultivar, the greatest. Cultivars CP 72-356, CP 65-357, and CP 72-370 lost 7 to 10% sugar/ha. Although all cultivars were exposed to heavy additional SCMV infection by natural spread, initial levels of mosaic infection remained important in the amount of yield loss.

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A NEWLY RECOGNIZED VIRUS DISEASE OF SWEET POTATOES. J. W. Moyer, and J. A. Foster, Department of Plant Pathology, North Carolina State University, Raleigh, 27695-7616. US Plant Introduction Sta., Glenn Dale, MD 20769.

A sweet potato (Ipomoea batatas) clone exhibiting distinct chlorotic patterns on the foliage and severe stunting was received at the US Plant Introduction Station, Glenndale, MD. The causal agent was transmitted to Nicotiana benthamiana and biologically purified by repeated local lesion transfer in Chenopodium quinoa. Necrotic local lesions were observed and systemic invasion followed in several Ipomoea spp. and N. benthamiana. These plants died 14-21 da following inoculation. Only systemic symptoms were observed in I. purpurea and I. coccinea. The virus was mechanically transmissible with difficulty, was not transmitted by Myzus persicae, and was not serologically related to sweet potato feathery mottle virus.

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EFFECTIVENESS OF REDUCED RATES OF THREE DIFFERENT COPPER FUNGICIDES IN CONTROLLING COFFEE RUST IN EL SALVADOR. G. C. Gálvez and Z. U. Javed. Dept. Plant Pathology, ISIC, Santa Tecla and IICA, Apartado Postal (01) 78, San Salvador, El Salvador.

During the season 1983/84, reduced rates of applications of three different 50% copper fungicides formulated as cuprous oxide (Copper Sandoz and Copper Nordox), Cupric Hydroxide (Kocide 101) and Cupric Chloride (Cobox, Vitigran, Cupravit and Recop) controlled Coffee Leaf Rust (Hemileia vastatrix) effectively. Sprays of cuprous oxides and cupric hydroxide were applied at the concentration of 0.35% and of Cupric Chloride at 0.50% to control Rust. Three applications per treatment were made using motorised knapsack sprayers calibrated to deliver 350 litres of water per hectare. The cost of controlling Coffee Rust can be reduced significantly in El Salvador by using the reduced rates of some 50% copper fungicides.

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A NEW FUNGAL ERADICANT DISEASE MANAGEMENT TOOL -- THE ISOTHIAZOLIN GROUP. J. Cruz, R. H. Fulton and C. F. Robert. TELA RAILROAD CO., La Lima, Honduras and ROHM AND HAAS CO., Central America, Apartado 3908, San Jose, Costa Rica.

The basic key of chemical disease management is to interrupt the waves of massive primary inoculum sources so that subsequent fungicidal treatments could be applied at reduced dosages or spray number with equal or improved control. The demise of usual eradicant tools common of the 50's and 60's was in part due to EPA regulations and the birth of systemics. However, for example, the need of eradicant tools to reduce early season inoculum was needed to manage build-up common to hot spots within banana plantations. Evaluations of potential perithecial eradicant products against Mycosphaerella species verified that only 2-n-Octyl-4-isothiazolin-3-one was highly effective. A single application eradicated these species as evidenced by time lapse ascospore discharge studies.

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FORMATION OF AMORPHOUS PLUGS IN CITRUS TREES WITH BLIGHT. R. H. Brlansky, M. A. Peterson, L. W. Timmer, and J. H. Graham, Univ. of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850.

Citrus blight, a disease of unknown etiology, has a characteristic amorphous plug which occurs in xylem vessels and is associated with a decrease in water conductivity. The formation of amorphous plugs was studied in predeclining (high wood Zn, normal water conductivity) and declining (high wood Zn, low water conductivity) citrus trees. In a 2 year study, plugs in trunk wood often developed within 3 months followed by low water uptake and canopy decline. In ultrastructural studies of trees in early stages of plug formation, small amounts of dark-staining globules of unknown composition appeared in the xylem vessels. These materials often accumulated along or near vessel walls and are presumed to be precursors of amorphous plugs.

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THE EFFECT OF TWO ECTOMYCORRHIZAL FUNGI ON GROWTH AND CYLINDROCLADIUM ROOT ROT SUSCEPTIBILITY OF RED PINE SEEDLINGS IN A WISCONSIN NURSERY. MacFall, J.S., Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706

In the fall of 1983, following a methyl bromide fumigation, inoculum of Hebeloma arenosa, Pisolithus tinctorius (both Wisconsin isolates) or a growing medium without fungus was incorporated into 1 m^2 nursery plots and sown with red pine seeds. Trees were sampled at 3 wk intervals during 1984 and in the fall of 1985. In 1984 the rate of increase of shoot growth was significantly greater for P. tinctorius inoculated trees than for non-inoculated trees. In 1985 both P. tinctorius and H. arenosa inoculated trees showed greater shoot growth than non-inoculated trees. Each year a subsample of trees was removed, inoculated with Cylindrocladium floridanum, and grown for 6 wk in the greenhouse. P. tinctorius inoculation significantly reduced susceptibility to Cylindrocladium root rot. H. arenosa inoculation had no effect on decreasing Cylindrocladium root rot.

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EFFECTS OF SOIL MOISTURE, DRAINAGE, AND MICROBIAL INTERACTIONS ON FORMATION OF ENDO- AND ECTOMYCORRHIZAE IN EASTERN COTTONWOOD
D.J. Lodge, Center for Energy & Environment Research, G.P.O. Box 3682, San Juan, P.R., 00936.

Cottonwood cuttings were pre-grown with VAM and ectomcorrhizal fungi, sampled for initial infection levels, and transplanted to soil moisture gradient tubes (4 lengths x 6 reps). After three months the soil columns were divided into 15 cm sections and the percentages of VAM and ectomycorrhizal root lengths were determined. ANOVAs on normalized data showed that moisture (section) significantly affected the percentages of root length infected by ectomycorrhizal and VAM fungi. Ectomycorrhizae were most abundant in moist soil immediately above the water line but were almost absent from waterlogged and moderate to dry soils. These results agree with field data. Infection by endomycorrhizal fungi significantly fit a quadratic model for soil moisture, with greatest infection under moderate conditions. In contrast, VAM-infection in the field was least under moderate soil moisture conditions. These and other data suggest ectomycorrhizal fungi can interfere with VAM-infection.

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LOCALIZED, NON-PHOSPHATE MEDIATED EFFECTS OF A VA MYCORRHIZAL FUNGUS ON NODULE ACTIVITY IN COWPEA. Robert N. Ames and Gabor J. Bethlenfalvay, USDA-ARS, Western Regional Research Center, Albany, CA 94710.

Cowpea (Vigna unguiculata) plants were grown in a split-root system with side 1 receiving the vesicular-arbuscular mycorrhizal (VAM) fungus Glomus macrocarpum and 100 mg hydroxyapatite (HAP, P-source), or 100, 200, or 400 mg HAP without G. macrocarpum. Side 2 received only 100 mg HAP. Before adding HAP, soil P was 34 µg P/g. A mixture of four cowpea Rhizobium strains was applied to all pots at 30 d, and plants were harvested at 60 d. Nodule activity (acetylene reduction) and root dry weight were greater ($P<0.05$) on side 1 of VAM plants than side 1 of the P-only treatments. Side 2 was not affected by any side 1 treatment. Shoot growth was greater for VAM plants, but there were no differences within (side 1 vs. side 2) or between treatments in root or shoot N and P concentrations. The VAM fungus did not affect rhizobial competitive interaction, and the localized enhancement of nodule activity cannot be explained by plant P nutrition.

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CHITIN-DECOMPOSING MICROORGANISMS ASSOCIATED WITH SPORES OF A VA MYCORRHIZAL FUNGUS. Robert N. Ames, USDA-ARS, Western Regional Research Center, Albany, CA 94710.

Balcom soil (calcareous silty clay loam, pH 7.9) was collected from the field and wet sieved to retrieve spores of Glomus macrocarpum Tul. & Tul., a vesicular-arbuscular mycorrhizal (VAM) fungus. After washing in sterile, distilled water, 190 spores were placed on solidified chitin water-agar in petri plates and examined at 14 d and 28 d for the presence of chitin-decomposing organisms. One hundred spores (53%) produced colonies of chitinase-positive microorganisms. Of these, 82% were actinomycetes, 17% bacteria, and 1% fungi. Our data show that chitin-decomposing actinomycetes are commonly associated with spores of G. macrocarpum in the Balcom soil. We are investigating the significance of this association with VAM fungi from a variety of soil types.

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Interactions between Glomus diaphanum and two Fusarium roseum isolates in red clover grown in a soil at two P levels. Jin Xixuan and J. B. Morton. Plant Pathology and Ag. Micro., Box 6057, West Virginia University, Morgantown, WV 26506-6057.

Red clover was grown for 8 weeks in a sterilized pasture soil amended with 50 and 300 ppm P. Treatments included addition of autoclaved oat kernels (2% v/v) infested with F. roseum 'Acuminatum' Isol. #927 or F. roseum 'Avenaceum' Isol. #959 to the soil with or without pot culture inoculum of G. diaphanum (10% v/v). Red clover dry matter increased in mycorrhizal plants when soil P was low, but not high. Mycorrhizal infection significantly inhibited root colonization by both Fusarium isolates at both P levels. Fusarium infection was greatest in nonmycorrhizal red clover in the high P soil. Isolate #959 more aggressively colonized roots than #927, particularly after wounding. Neither Fusarium isolate suppressed red clover growth: #959 did not effect any change and #927 stimulated dry weight accumulation in both mycorrhizal and nonmycorrhizal plants.

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SPATIAL DISTRIBUTION OF GLOMUS INTRARADICES SPORULATION IN ONION ROOT SYSTEMS. A. Jarstfer and N.C. Schenck, Plant Pathology Dept University of Florida, Gainesville, Florida 32611

Eleven colonized root systems of 14-week-old, chamber-grown onions (cv. 'Evergreen Long White') were assessed for internal sporulation of Glomus intraradices. Root systems were dissected by age sequence into separate roots and subsequently into 30-mm root segments (40 per system). Mean root length per root system was 1500 mm. Sporulation intensity was graded for each segment according to a scale of 0-5 (0=no spores, 5=filled with spores). Sporulation occurred over 46 percent of the root length. Segments graded 4-5 comprised 9.4 percent of the root length while those graded 2-3 constituted 25 percent of the root length. Those segments graded 0-1 comprised the remaining 65.6 percent of the root length. The most intense sporulation (grades 4-5) was always associated with the area of inoculation (10 mm below the crown). Sporulation occurred in primary and secondary roots with intensity dependent on proximity to original inoculum. There was no evidence that sporulation was induced by non-host or host-mediated environmental stresses.

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ISOLATION AND STRUCTURAL CHARACTERIZATION OF SOIL-BORNE AUXILLARY CELLS OF *GIGASPORA MARGARITA* BECKER & HALL, A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS. Jabaji-Hare, S. H., Y. Piche* & J. A. Fortin. Forestry Dept., Laval University, Québec, G1K 7P4, Canada. * Biology Dept., Sherbrooke University, Sherbrooke, Québec, Canada.

Soil-borne auxillary cells of *Gigaspora margarita* Becker & Hall have been investigated with light and electron microscopy. The cell wall is composed of four prominent layers and autofluoresces intense yellow under blue light. Histochemically, it contains polysaccharides and lipids. The cytoplasm is composed of amorphous material, nuclei and a variety of osmiophilic granules. In old and moribund auxillary cells and subtending hyphae, gram-negative bacterium-like structures (BLS), sometimes dividing, were colonizing the senescent cytoplasm. These BLS were surrounded by peripheral membranes and their cytoplasm was clearly differentiated into electron-dense and transparent areas.

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INFECTION RATES AND EXTENT OF COLONIZATION BY CYLINDROCLADIUM CROTALARIAE OF CBR-RESISTANT AND CBR-SUSCEPTIBLE PEANUT ROOTS. G. S. Tomimatsu and G. J. Griffin, Dept. Pl. Pathol., Physiol., & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Using cultural methods, the observed infection rate, I_r^o (number of observed infections per m root per day per microsclerotium per g of soil), was calculated for peanut cultivars Florigiant and Spancross, susceptible and resistant to Cylindrocladium black rot (CBR), respectively, following 3 wk growth in greenhouse soil temperature tanks (25 C). In naturally infested soil, I_r^o was significantly higher (P=0.001) for Florigiant than for Spancross plants. Not all C. crotalariae infections observed on the root surfaces of either cultivar resulted in colonization of the cortex nor in subsequent colonization of the stele. A significantly greater (P=0.05) number of segments from the steles of Florigiant taproots were colonized by C. crotalariae than those from Spancross. Fusarium spp. was recovered from significantly higher percentages of cortex and stele segments of Spancross taproots than from those segments of Florigiant.

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THE DEVELOPMENT OF THE MULTINUCLEATE CONDITION OF PERONOSPORA TABACINA SPORANGIA. R. N. Trigiano and H. W. Spurr, Jr., USDA-ARS Tobacco Research Lab., Oxford, NC 27565, and Dept. of Plant Pathology, N.C. State University, Raleigh, NC 27695

The development of the multinucleate condition of Peronospora tabacina sporangia was studied using the fluorescent stain mithramycin. Sporangiophores and sporangia from infected leaf disks were collected 7 hr after induction of sporulation and thereafter at 30 min intervals for 5 hr. Since the developmental age of sporangia varied among sporangiophores within samples, length of sporangia was used as a proxy class variable for time. Initially one nucleus migrated into each small (<3 µm), spherical sporangium. A predominant number of sporangia in the 4 to 10 µm classes had either 2, 4 or 8 (2^n) nuclei. Chi square analyses of nuclei number per sporangium supported the hypothesis that nuclei were derived from 1 or 2 synchronous divisions of the daughter nuclei from the initial nucleus and not from random divisions or nuclear migration from the sporangiophore. Subsequent divisions occurred randomly. The average number of nuclei per mature sporangium was 15.

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LEAF SPOT OF JOHNSONGRASS CAUSED BY AN UNIDENTIFIED BIPOLARIS SPECIES. Mou-Yen Chiang, C. G. Van Dyke, and K. J. Leonard, Depts. of Bot. and Plant Path., NC State Univ., Raleigh, NC.

A distinctive leaf spot disease was observed on johnsongrass in NC. Lesions on mature leaves are ovate to oblong, up to 2x10 mm, with straw-colored centers and coffee-colored margins. A Bipolaris sp. isolated from lesions sporulated abundantly on sugar-proline agar, but poorly on V-8 juice agar or PDA. Johnsongrass seedlings inoculated with conidia developed lesions characteristic of the disease. Conidiophores on leaves are up to 280 um long with 3-5 pseudopleurogenous conidia. Conidia are fusoid, slightly curved, 8- to 9-septate and average 84(43-148) um long by 15(12-18) um diameter; the hilum is not conspicuous. Although morphologically similar to B. maydis, this species was not compatible with it in mating tests. Cultural characteristics and disease symptoms on johnsongrass are distinct from those of B. sorgicola and this species does not produce secondary conidiophores and conidia. Research is in progress to establish the identity and significance of this fungus for biocontrol of johnsongrass.

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HOST SPECIFICITY AND VEGETATIVE COMPATIBILITY IN VERTICILLIUM ALBO-ATRUM. T. R. Gordon, J. C. Correll, and A. H. McCain, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Verticillium albo-atrum has recently been identified as a pathogen of alfalfa, pelargonium, and ceanothus in California. Isolates were tested for host specificity and vegetative compatibility both within and between the various isolates. All isolates tested were pathogenic on cotton (cv. 'SJ-2') and cantaloupe (cv. 'Spartan Rock'), but not tomato (cvs. 'Bonny Best' and 'Peto 95') in root-dip greenhouse inoculations. In addition, the alfalfa isolate was pathogenic on pelargonium, but only the alfalfa isolate was pathogenic on alfalfa. Complementary nitrate non-utilizing mutants were generated within each isolate using cornmeal-dextrose agar (Difco) amended with potassium chlorate (15g/L). All isolates originating from alfalfa were vegetatively compatible and apparently represent a single vegetative compatibility group. The ceonothus and pelargonium isolates were not vegetatively compatible with isolates from alfalfa.

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BOTRYTIS CINEREA ANTIGENS IN CYTOPLASM OF INFECTED VICIA FABA. A.M. Svircev, R.B. Gardiner, W.E. McKeen, A.W. Day and R.J. Smith, Plant Sciences Dept., University of Western Ontario, London, Canada N6A 5B7

The results establish that antigens of the type found on the surface of Botrytis cinerea are present inside Vicia faba cells some distance from the nearest fungal hypha 8 and 12 hours after inoculation or 1 and 5 hours after fungal penetration and that no such antigens can be detected in uninfected plant tissue. Antisera and protein A-gold were used for the detection of Botrytis cinerea antigens in infected V. faba. The TEM immunogold technique used, provided a powerful tool for the localization of macromolecules in host-parasite interaction. Thin sections of uninfected V. faba leaves treated with anti-Botrytis and Ustilago violacea antifimbrial antisera then protein A-gold showed

little or no gold labeling. In contrast thin sections of host cells 8 and 12 hours after inoculation were strongly labeled whether the fungus was present in the cell or not. Gold labeling in the sections showed a high amount of labeling in chloroplasts, host cytoplasm and less in vacuoles and walls.

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BOTRYOSPHAERIA CANKER OF TABLE GRAPES. B.A. Latorre, X. Besoaín and V. Flores, Departamento de Ciencias Vegetales, Facultad de Agronomía, Pontificia Universidad Católica, Casilla 6177, Santiago, Chile.

Botryosphaeria dothidea (Moug. ex Fr.) Ces. et de Not. was consistently isolated on potato dextrose agar from grapes severely attacked by a canker disease in a 2-yr old trellis cv. 'Flame Seedless' near Rancagua, Chile. Diseased plants were characterized by a mild to moderate chlorosis and leaf necrosis by a poor seasonal growth, and eventually the entire plant collapse. Extensive cankers were always found along the trunk. All B. dothidea isolates were reisolated from lesions produced on inoculated fruits. Symptoms consisted initially of necrotic lesions, and later the entire fruit dried out and became mummified . Black pycnidia appeared on the infected fruits after 20 days of the incubation. Pathogenicity was also established on 1-yr old peach and plum trees inoculated on the trunk. Gum exudations and cankered tissues appeared after 15-20 days of incubation. In conclusion, this is the first report of B. dothidea infecting grapevines in Chile.

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EVALUATION OF BIOLOGICAL ASSAYS FOR THE ELICITATION OF GLYCEOLLIN IN SOYBEANS. L.J. Vaillancourt, J.D. Paxton; University of Illinois; Dept. of Plant Pathology; Urbana, IL, 61801

Phytoalexin production by soybeans in response to plant pathogens is important in disease resistance. A reproducible biological assay to quantify phytoalexin accumulation is needed to understand this phenomenon better. One common assay used presently consists of placing elicitor solutions on wound surfaces of sliced cotyledons. Several new techniques to elicit glyceollin production in soybean tissue were compared with this method. New techniques included treating wounds of a specific size, treating unwounded surfaces, and vacuum infiltrating intact soybean tissues, with elicitor solutions. Abiotic as well as biotic elicitors were used in the assays. Vacuum infiltration of etiolated seedlings (.01 MPa, repeated 5 times for 5 minutes)has given more reproducible results than the other methods tested.

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RESPONSE OF POTATO GENOTYPES TO CRUDE TOXIN PREPARATION FROM CULTURE FILTRATE OF VERTICILLIUM DAHLIAE. S.K. Mohan, J.R. Davis, D.L. Corsini and J.J. Pavek. Univ. of Idaho and USDA-ARS, Aberdeen, ID 83210.

Potato cultivars and clones with different field reactions to Verticillium dahliae were evaluated for their sensitivity to extracellular toxin produced by a virulent isolate of V. dahliae from potato. Detached leaves were injected with aqueous solutions of acetone precipitates of crude toxin and observed for chlorosis and necrosis after 72 h. Toxin dilutions, for which cv Alpha was insensitive and cv Russet Burbank was sensitive, elicited differing responses among the 14 cultivars and clones evaluated. While susceptible cvs, Butte and Norgold, were sensitive, the highly susceptible clone NDA8694-3 was not sensitive. Clones A66107-51 and A68113-4, known to be highly resistant to V. dahliae, were sensitive to the toxin. These results suggest that while the crude toxin might be useful in identifying certain types of resistance/tolerance, it has limitations as a tool for rapid evaluation of potato germplasm for resistance to V. dahliae.

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INFLUENCE OF CELL WALL-RELATED MONOSACCHARIDES ON IN VITRO GROWTH OF GERMINATED SPORES OF MYROTHECIUM RORIDUM PATHOGENIC TO MUSKMELON. J.O. Kuti, T. J Ng, Department of Horticulture, University of Maryland, College Park, MD 20742 and K.C. Gross and H.E. Moline, USDA-ARS, HCQL, Beltsville, MD 20705.

Since the host plant cell wall may serve as a primary carbon source for pathogens after initial cuticle penetration, we have studied the growth of Myrothecium roridum, a muskmelon pathogen, on various monosaccharides. Spores of M.roridum were germinated at five concentrations (0, 0.1, 0.5, 1.0, 5.0 mM) of eight common monosaccharide wall constituents. Arabinose, galactose and glucose supported germ tube growth at all concentrations while galacturonic acid, mannose and rhamnose inhibited growth at all concentrations. Fucose and xylose stimulated growth up to 25% at 0.5mM but inhibited growth at higher concentrations. Noncellulosic neutral sugar analysis of muskmelon fruit cell walls revealed that susceptible genotype 'Iroquois' had consistently higher arabinose, galactose and glucose content than the resistant genotype 'Hales Best'.

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FUSARIUM VARIANTS DEFECTIVE IN KIEVITONE DETOXIFICATION ALSO SHOW IMPAIRED METABOLISM OF PHASEOLLIN AND PHASEOLLINISOFLAVAN. D. A. Smith and C. S. M. Turbek, Department of Plant Pathology, University of Kentucky, Lexington, KY, 40546-0091.

Two cultural variants of Fusarium solani f. sp. phaseoli, both deficient in kievitone hydratase (KHase), were more sensitive to kievitone and less virulent towards Phaseolus vulgaris than the original wild-type (Physiol. Pl. Path. 25:135-147). It is now apparent that these variants are also defective in their abilities to transform phaseollin and phaseollinisoflavan to the known metabolites, la-hydroxyphaseollone (HP) and metabolite-1 (M-1), respectively. Furthermore, growth of the variants is more vulnerable to phaseollin and phaseollinisoflavan than is that of the wild-type. Attempts to procure isolates deficient in KHase, while successful, seem, simultaneously, to have given rise to fungi also lacking in their capacities to generate HP and M-1 despite the apparently different mechanisms involved. There may be a portion of the F. solani f. sp. phaseoli genome devoted to isoflavonoid tolerance.

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GENETICS OF RESISTANCE IN DURUM WHEAT TO SEPTORIA TRITICI. M. van Ginkel and A.L. Scharen, USDA, ARS, Plant Path. Dept., Montana State University, Bozeman, MT 59717

The reaction was studied of 13 durum wheat cultivars to 34 S. tritici isolates, all of Mediterranean origin. Highly significant differences were observed between cultivars and between isolates. The interaction component was insignificant. Generation mean analyses of 65 crosses indicated prime additive gene effects, while dominance effects increasing resistance were often present. Epistasis was negligible. Combining ability analyses of a 10x10 diallel executed for eight S. tritici isolates confirmed the major role of additive gene effects. Different isolates appeared to initiate similar genetic resistance systems in the hosts. Broad-sense heritability estimates averaged 38%. The mean number of effective factors per cultivar was seven. Classical gene-for-gene relationships were not detected. Instead, the existence of host-species specialization was implied. Thus cultivars differed in horizontal resistance and isolates in aggressiveness.

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ALLOZYME RELATIONSHIPS BETWEEN ISOLATES OF FOMES ANNOSUS FROM PINE AND TRUE FIR IN CALIFORNIA. William J. Otrosina, USDA Forest Service, 1960 Addison St., Berkeley, CA 94701

Over 80 isolates of Fomes annosus from pine or true fir were collected throughout California and subjected to isozyme analysis. Genetic analyses of allele frequencies at 9 allozyme loci indicated 70 percent of the loci in isolates from both hosts were polymorphic. The mean number of alleles per locus was 2.3 and 1.9 for the fir and pine isolates, respectively, while heterozygosity in both ioslate groups was generally lower by a factor of 6 and 10, respectively, as compared to calculated Hardy-Weinberg expections. Based upon genetic distance calculations derived from allele frequency data, there is evidence that fir isolates are genetically dissimilar from pine isolates and cluster analysis based upon these data indicate both host tree species and geographic location (lattitude) of isolates are correlated with the observed gene frequency distributions. Studies employing isozyme are being conducted to further define these relationships.

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DETECTION OF RUST RESISTANCE IN LOBLOLLY PINE BY ISOZYME ANALYSIS. H.R. Powers, Jr., D. Lin and M. Hubbes. S.E. For. Exp. Sta., USDA For. Serv., Athens, GA 30602 and Faculty of Forestry, University of Toronto, Ontario, Canada M5S 1A1

Isozyme systems were used to differentiate among pollen samples from loblolly pine selections resistant or susceptible to Cronartium quercuum f. sp. fusiforme. Zymograms of glutamate-oxaloacetate transaminase (GOT) sorted pollen samples into resistant and susceptible groups, except for one intermediate sample that classified as susceptible. In addition, pollen samples collected in

two successive years from specific clones could be recognized by their 6-phosphogluconate dehydrogenase (6-PGD) and glycerate-2- dehydrogenase (G_2 DH) patterns. If, as it appears, isozyme analysis is a simple and rapid bioassay of resistance or susceptibility to fusiform rust, it will provide a major advance in programs to develop rust resistant pines.

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PURIFICATION OF AN EXTRACELLULAR B-1,4-XYLANASE OF PORIA PLACENTA BY GEL FILTRATION AND ULTRAFILTRATION. F. Green III, C.A. Clausen, J.A. Micales and T.L. Highley. U.S. Forest Products Laboratory, Madison, WI 53705.

The brown-rot fungus Poria placenta produces extracellular carbohydrate-degrading enzymes with activities for carboxymethylcellulose, hemicellulose and glycosides. Initial attempts to separate individual enzymes were unsuccessful and suggested the presence of a multienzyme complex. In this study, xylanase was separated from glycosidases by gel filtration (Fractogel 55) and by sequential ultrafiltration through a series of Minitan/Amicon filters. The molecular weight of the xylanase was estimated to be 45 - 50,000 daltons by gel filtration and SDS polyacrylamide gel electrophoresis. The xylanase was extremely stable and could be eluted in an active state from a single band on an SDS polyacrylamide gel; the eluate also retained trace amounts of B-1,4-endoglucanase. Both activities were associated with a single polypeptide. The enzyme is a glycoprotein with a large carbohydrate component (50% w/v).

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EFFECT OF CERATO-ULMIN (CU) ON ELECTROGENIC ION PUMPS OF ELM CELL MEMBRANE AND ION LOSS FROM THE CELLS. Okamoto, H.[1], Otani, H.[2], and Takai, S.[3]. [1] Faculty of Science, Nagoya University, Nagoya, Japan. [2] Faculty of Agriculture, Tottori University, Tottori, Japan. [3] Great Lakes Forestry Centre, Canadian Forestry Service, Sault Ste. Marie, ON P6A 5M7 Canada

CU, a selective toxin to elm, the host of DED displays symptoms (morphological and physiological) similar to those of DED. To elucidate CU action to the membrane of *Ulmus americana*, susceptible to DED, we analyzed 1) activity of electrogenic ion pumps (the source of driving force for active ion transport) at the parenchyma symplast/xylem interface of the young shoot segment and 2) ion loss from leaf cells. Potential change of the xylem parenchyma cell membrane by perfusion of a CU suspension (20 µg/mL) into xylem vessels was a gradual reduction of the passive potential and an increase of the active electrogenic potential to compensate this reduction. Ion loss from leaf cells exposed to CU (25 and 50 µg/mL) was insignificant if the energy-supply to the presumed ion pumps was not interferred with. However, in the presence of N_2 gas or Na_3VO_4 (0.5 mM), both inhibitory to energy-supply, ion loss was evident but not specific (tested for K^+, Na^+, Ca^{2} and Mg^{2+}). In summary, CU causes a non-specific increase in membrane permeability.

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Growth and mortality effects of dwarf mistletoe on uneven-aged ponderosa pine stands in Colorado. H. M. Maffei, F. Hawksworth, and W. R. Jacobi. Dept. Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523

A set of nonlinear models based on 5 cm diameter (dbh) classes were developed for predicting ten year diameter growth and mortality in uneven-aged stands of ponderosa pine infested by Arceuthobium vaginatum subsp. crytopodum. Growth of uninfected and infected trees was reduced (based on severity infection and intertree competition) from a potential growth based on site. The intertree competition effect was best described by: basal area of the dbh class and larger trees (BASL); size class dbh to average stand dbh ratio; and the level of infection of larger trees. Severe mistletoe infection caused over a 50% decrease from potential growth. When trees were heavily infected, their competitive effect was reduced. The best predictors of the percent trees within a dbh class dying over a ten-year period were: percent of severely infected trees in each dbh class, size class dbh, and BASL.

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THE INFLUENCE OF HALF-SIB FAMILY AND TREE SPACING ON INCIDENCE OF PITCH CANKER IN A LOBLOLLY PINE PLANTATION IN EASTERN NORTH CAROLINA. G.B. Runion and R.I. Bruck, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

A loblolly pine plantation was surveyed in the spring of 1984 and 1985 for the number of terminal and lateral shoots exhibiting visual symptoms of the pitch canker disease. Significant differences in the percentage of terminal shoots and average number of lateral shoots exhibiting disease symptoms existed among three half-sib families. Significant differences in these symptom types also occurred among trees planted at nine different spacings; quantity of symptoms was positively correlated with tree spacing. The percentage of symptomatic terminals ranged from 6.2% to 29.9% in 1984 and from 3.6% to 22.9% in 1985; the average number of symptomatic laterals per tree ranged from 1.3 to 4.0 in 1984 and from 1.6 to 3.7 in 1985. Overall percentage of symptomatic terminals was significantly lower in 1985 than 1984. This natural variation in incidence of pitch canker indicates the potential for silvicultural control of pitch canker of loblolly pine in Eastern North Carolina.

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Pathogenicity of seed-borne fungi in Pinus strobus. R.K. Mittal and B.S.P. Wang, Petawawa National Forestry Institute, Chalk River, Ontario, Canada, K0J 1J0

Effect of Alternaria alternata, Cladosporium cladosporoides, Epicoccum purpurascens, Fusarium sporotrichioides, Mucor hiemalis, Penicillium aurantiogriseum, Rhizopus nigricans, Trichoderma viride and Trichothecium roseum on seed germination and seedling development of Pinus strobus was studied in greenhouse. Chilled and non-chilled seeds were inoculated & sown in trays of unsterilized and sterilized nursery soil and in Spencer-Lemaire «Rootrainers» containing a peat-perlite-vermiculite mixture. Total and rate of seed germination; seedling size, biomass and mortality were examined. All fungi except R. nigricans reduced germination. Blight of germinants, damping off, needle tip dieback and top decay of seedlings were common. A. alternata caused significant pre- and post-emergence mortality. F. sporotrichioides, P. aurantiogriseum and T. viride blighted more germinants than other fungi. Seeds inoculated with F. sporotrichioides and T. roseum affected seedling quality. Pathogenicity of other fungi was lower but it varied with species.

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CLOSURE AND COMPARTMENTALIZATION OF SEASONAL WOUNDS IN CLONAL EASTERN COTTONWOOD. L. Shain and J. B. Miller, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Standardized wounds were made during each of the four seasons in 6 clones of eastern cottonwood (Populus deltoides). Trees were wounded at 3-month intervals beginning in Nov. (Set 1) or May (Set 2). Trees were harvested 3 months after their fourth wound; thus, at the time of harvest, each tree had wounds 3, 6, 9 and 12 months old. Older wounds were more poorly compartmentalized than younger wounds in Set 1. In Set 2, however, the older wounds, which were initiated during the growing season, were significantly better compartmentalized than the younger wounds which were initiated during the dormant season. The frequency of decay fungi also was greater in wounds initiated during the dormant season regardless of the wound ages tested. Significant clonal differences in wound closure and compartmentalization were detected. Ethylene production by wounded host xylem is being investigated as an early predictor of these wound responses.

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ETHYLENE PRODUCTION BY RESISTANT AND SUSCEPTIBLE HOSTS NEAR CANKERS CAUSED BY VIRULENT AND HYPOVIRULENT ENDOTHIA PARASITICA. F. V. Hebard and L. Shain, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Bark disks collected from the uncolonized margin of E. parasitica cankers on American chestnut produced from two to ten times more ethylene than control disks. The stimulation declined 1-4 cm from the cankers. Stimulation occurred less frequently at uncolonized margins of cankers induced by hypovirulent E. parasitica or similar margins of cankers on blight-resistant Chinese chestnut and scarlet oak. The lack of stimulation may have occurred after the formation of lignified zones and wound periderm around the cankers. Ethylene was neither evolved nor absorbed by necrotic, cankered tissue. The stimulation of ehtylene production in bark disks may prove useful in the bioassay of metabolites produced by E. parasitica.

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RESISTANCE TO CERCOSPORA SEQUOIAE VAR. JUNIPERI GREATER IN JUNIPERUS VIRGINIANA THAN IN J. SCOPULORUM PROGENIES. Glenn W. Peterson, Rocky Mountain Forest and Range Exp. Stn., Forestry Sciences Laboratory, Univ. Nebr., Lincoln, 68583.

Cercospora sequoiae var. juniperi causes a blight which can kill junipers. Resistance to this fungus was evaluated in progenies of Juniperus virginiana and J. scopulorum established in an eastern Nebraska planting in 1980. Five replications of 4-tree linear plots of 131 J. virginiana and 23 J. scopulorum progenies were arrayed in a randomized block design. Two trees in each plot were inoculated, one in 1982 and another in 1985, by use of infected foliage collected on site. Disease evalua-

tions were made in mid-October 1985 by rating each tree on the basis of percentage of necrotic foliage, according to the following scale: 1 = 0% necrosis; 2 = 1 to 20%; 3 = 21 to 40%; and 4 = >40% necrosis. The average rating for the 131 J. virginiana progenies was 1.6 (range 1.0 - 2.6) and for the 23 J. scopulorum progenies was 2.5 (range 1.8 - 3.2).

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VARIATION IN THE INCIDENCE OF SWISS NEEDLE CAST AMONG DOUGLAS-FIR PROVENANCES.
H. L. Morton and Zhiliang Zhu, University of Michigan, School of Natural Resources, S.T. Dana Building, Ann Arbor, MI 48109.

In 1983, 1864 branch samples were examined from 62 provenances of a 20-yr-old Douglas-fir plantation in southern Michigan. The lowest live foliage was evaluated from 4 cardinal directions for needle retention and discoloration, resulting in a disease rating scale of 0-10 pts. Provenances originated from 32-51°N latitude, 105-121° W longitude, and from 2000-10000 feet elevation. All provenances were infected, but a Hotelling's T^2 indicated those from the lowest elevations had significantly ($P<.05$) less disease. In 1985, a second, 6-yr-old provenance study was examined for percent needle infection from 4444 shoot samples among 24 geographic sources. Origins ranged from 45-48° N latitude, 113-118° W longitude, and 1949-6152 feet elevation. Again, all sources were infected, with an average of 8.3% of the needles infected (range 1.5-34.2%). Only elevation was positively correlated with percent infection, where percent infection=-13.103+0.00525 x elevation ($r^2=0.61$, $P<.05$).

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THE NEW BRANCHING ATMOSPHERIC TRAJECTORY MODEL: IMPLICATIONS FOR MODELING LONG-RANGE SPORE TRANSPORT. J. M. Davis and C. E. Main, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

The Branching Atmospheric Trajectory (BAT) model provides a much-needed refinement in modeling the diurnal changes in the structure of the Atmospheric Boundary Layer (ABL), within which inoculum is both released and transported. During the daytime, the ABL is a well-mixed layer 1 to 2 km thick. Spores released into this layer are soon mixed throughout. At night a surface-based inversion is formed, and the efficient vertical exchange processes characteristic of the daytime ABL are absent. The nocturnal ABL is often marked by dramatic changes in horizontal wind speed and direction with height. Thus spores located within the ground-based inversion are likely to be transported in a different direction and at a different speed than those above the inversion. The model has been applied to the transport of Peronospora tabacina spores.

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AN ASSESSMENT SYSTEM FOR EVALUATION OF CERCOSPORA BLIGHT OF ASPARAGUS. K. E. Conway. Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-0285.

Cercospora blight, caused by Cercospora asparagi, has the potential to become a yield-limiting disease in southern production regions in the USA. An assessment system was developed to study disease progress on the ferns. The system is based on the location of three types of symptoms on the fern (lesions, browning and defoliation). A single number is given for the location of each symptom on the fern, where 0 = absence of symptom, 1 = bottom, 2 = middle and 3 = top third of fern. Numbers are inserted into the formula $X_T = 5.5L_L + 11.0L_B + 16.5L_D$, where X_T = total percent disease, L_L = location of lesions, L_B location of browning and L_D = location of defoliation. Values of X_T were logistically transformed and linear and polynomial regression lines were determined. Compared to an alternative assessment system (rating scale 0-9) using the arc sine transformation, the proposed assessment system better fit the data and could be explained by either a linear or a polynomial regression equation.

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VEGETATIVE COMPATIBILITY GROUPINGS IN FUSARIUM OXYSPORUM F. SP. ASPARAGI. W. H. Elmer and C. T. Stephens. Dept of Botany and Plant Pathology, Mich. State Univ., East Lansing, MI 48823-1312.

Isolates of Fusarium oxysporum f. sp. asparagi (FOA) were collected from 6 states in the U.S. including Michigan and from other countries. Other F. oxysporum (FO) formae speciales such as apii Race 1 and 2, cepae, chrysanthemi, conglutinans Race 1, 2, 3, and 5, gladioli, lycopersici, melonis and niveum were also collected, along with nonpathogenic FO isolates. All isolates were tested for pathogenicity on asparagus seedlings. Over 500 random pairings between mutants unable to use nitrate as a N source were examined on a nitrate medium. Over 13 vegetatively compatible groups (VCG) from isolates pathogenic on asparagus were tentatively identified. The FO formae speciales cepae, gladioli, and apii Race 1 were pathogenic on asparagus seedlings, and fell into some of the VCG. Several VCG consisted of only 2 isolates of FOA, but appeared distinct from the rest. Many nonpathogenic FO isolates were in the same VCG as known FOA isolates. These preliminary findings suggest that FOA is composed of several distinct heterogeneous populations.

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DISEASE PROGRESS OF Alternaria brassicicola ON CABBAGE. D. A. Fontem[1], D. P. Weingartner[2], and J. A. Bartz[1], [1]Plant Pathology Dept. and [2]Agricultural Research Center Hastings, University of Florida, Gainesville, 32611.

Disease progress of dark leafspot (Alternaria brassicicola) was evaluated on cabbage cvs Market Prize, A & C No. 5, and Gourmet, in the winter of 1985-86. Disease severity was scored at various distances from a line source of inoculum using the Horsfall-Barratt rating scale. The curves were fit better to the Weibull function ($3<c<9$) than to the logistic or Gompertz equations. The logistic infection rates ($0.03<r<0.16$) increased with increase in distance from the source of inoculum. No significant differences were detected in the level of resistance of the cvs based on initial disease, apparent infection rates, disease values on the last day of assessment, and AUDPC. The maximum disease severities were 0.65, 0.41, and 0.50, for Market Prize, A & C No. 5, and Gourmet, respectively. Yields for each cv. were negatively correlated with AUDPC or disease on the last day of assessment.

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EFFECT OF WETNESS DURATION AND TEMPERATURE ON INFECTION OF STRAWBERRY FLOWERS AND FRUIT BY BOTRYTIS CINEREA. M. A. Bulger, M. A. Ellis, and L. V. Madden, Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691.

Development of latent infections of immature strawberry fruit from flower infections by Botrytis cinerea, is considered critical for disease development on mature fruit. However, infection of mature fruit by conidia is not believed to contribute significantly to gray mold epidemics. Attached strawberry flowers and fruit were inoculated with a 1 x 10^5 spore/ml suspension and placed under wetness durations of 5-30 hr at temperatures of 5, 10 and 30 C, and 2-24 hr at temperatures of 15, 20 and 25 C. Incidence of petal, sepal and stamen necrosis was recorded after 4 days. The optimum temperature for flower infection was between 15 and 20 C. Flower infection at 5 C was minimal at all wetness durations. No infection resulted from inoculations of immature (green) fruit at any temperature-wetness duration tested. Inoculation of mature (red) fruit resulted in 52 and 100% fruit infection at 20 C for 8 and 24 hr wetness, respectively.

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EFFECT OF WIND AND RELATIVE HUMIDITY ON SPORULATION AND EXTERNAL MYCELIUM FORMATION OF BOTRYTIS ON GRAPE. C.S. Thomas and J.J. Marois, Department of Plant Pathology, University of California, Davis, CA 95616.

Red Emperor grapes were injected with Botrytis cinerea conidia and placed in 2.5 cm diameter wind tunnels. Wind speeds of 0 and 0.3 m/sec were applied to grapes at 35 and 95% relative humidity (RH). Grapes also were exposed to ambient air turbulence at 35% RH. After 8 days the no. of berries with external mycelium and the no. of spores produced by these grapes were evaluated. At 95% RH all grapes developed external mycelium. However, grapes also exposed to wind had a four-fold increase in number of spores produced per grape. At 35% RH 72% of the grapes in still air developed external mycelium and sporulated but berries exposed to wind did not develop mycelium on the surface. Five days after inoculation, fans were turned off for half of the grapes exposed to wind. Three days after wind was stopped values for external mycelium and sporulation were similar to or greater than those of grapes that were never exposed to wind.

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THE SEASONAL OCCURRENCE OF CERATOCYSTIS SPP. ON NITIDULIDS TRAPPED IN CENTRAL TEXAS OAK WILT CENTERS. T. M. Kurdyla, D. N. Appel, Dept. Plant Path. & Micro., Texas Agric. Exp. Sta., College Station, TX 77843, and R. L. Lewis Jr., USDA Forest Service, Hardwood Insect and Disease Research Laboratory, Stoneville, MS 38776.

From 9 March 1984 to 10 May 1985, 4126 free-flying nitidulid beetles were trapped in Texas oak wilt centers and assayed for the presence of Ceratocystis spp. Five percent of the assayed beetles were contaminated with one or more isolates of C. piceae, C. fagacearum, C. perfecta, or C. pluriannulata. Ceratocystis piceae was present on beetles trapped throughout the year, accounting for 80% of the total contamination. The

remaining Ceratocystis spp. were found only on beetles trapped in spring and early summer, having a combined frequency of 1%. The greatest number of contaminated beetles were trapped in April, when beetle activity was at its peak. These additional Ceratocystis spp. may influence the infection of wounds by C. fagacearum on oaks in central Texas.

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EFFECT OF TEMPERATURE ON THE GROWTH OF NORTHERN AND SOUTHERN ISOLATES OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA.
B. L. KEELING, USDA-ARS, STONEVILLE, MS 38776.

The growth of 4 Diaporthe phaseolorum var. caulivora (Dpc) (soybean stem canker pathogen) isolates were compared on potato-carrot agar (PCA) at temperatures between 10C-35C. Two southern (Mississippi) and two northern (Iowa and Indiana) isolates were used. After 4 days at 10C on PCA, the northern isolates grew slightly (4mm) more than the southern isolates. At 15,20,25,30, and 35C the growth of southern isolates exceeded that of northern isolates. Growth of southern isolates exceeded that of northern isolates 3-11mm at 20C, 8-21mm at 25C, and 31-36mm at 30C. Growth of northern and southern isolates was inhibited (to less than 5mm) at 35C. Growth of the northern isolates peaked at 25C and fell sharply to 5-7mm at 30C. Southern isolates also reached maximum growth at 25C, but were not inhibited untill the temperature exceeded 32C. These differences in effect of temperature on growth of northern and southern isolates may influence their geographic distribution.

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ISLAND BIOGEOGRAPHY: A MODEL FOR PHYLLOPLANE COLONIZATION.
L. Kinkel and J. H. Andrews, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

The equilibrium theory of island biogeography provided a model for the immigration and extinction of filamentous fungal species on apple leaf "islands" surface-sterilized in situ. The number of species per leaf reached an equilibrium within about 3 weeks, though species immigrations and extinctions occurred continually. Six of the approximately 12 species present at equilibrium were detected throughout the season, while the identities of the remainder varied. In contrast to many macro-communities, there were no relationships between the number of species or individuals and island area, or between the number of species and individuals, reflecting the contrasting ecology of macro-organisms and microbes. The observed phylloplane colonization pattern of species equilibrium with continual immigrations has implications for the introduction and establishment of biocontrol organisms.

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SURVIVAL OF SCLEROTIA OF SCLEROTINIA MINOR IN THE DIGESTIVE TRACT OF A RUMINANT.
H. A. Melouk, L. L. Singleton, F. N. Owens[1] and C. N. Akem. USDA-ARS, Dept. of Plant Pathology, and Animal Science Dept.[1], Oklahoma State University, Stillwater, OK 74078-0285.

Peanut hay naturally infected with Sclerotinia minor and containing sclerotia was fed for ten days to a cross bred heifer in a holding pen. Fecal and ruminal samples were collected at 6, 7, 8 and 9 days after the feeding began. Sclerotia were recovered from the samples by wet sieving on a series of metal screens. Sclerotia-like bodies retained on the 850 µm screen were picked, surface sterilized in 0.1% NaClO for 3 min and plated on potato dextrose agar containing 100 µg/ml streptomycin suflate. Viable sclerotia were recovered from fecal and ruminal samples regardless of collection time. However, survival of sclerotia was greater in ruminal samples than in fecal samples. Cultures of S. minor generated from fecal and ruminal sclerotia were pathogenic against the peanut cv. Tamnut 74 under greenhouse conditions.

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GROWTH OF Diaporthe phaseolorum var. caulivora ISOLATES ON MEDIA AT VARIOUS pH AND TEMPERATURE LEVELS.
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Isolates of Diaporthe phaseolorum var. caulivora (Dpc) causing soybean stem canker were obtained from Auburn, AL (3), Huntsville, AL (2), Georgia (3), and Mississippi (3) and grown on PDA either acidified with 85% lactic acid to pH 4.5, 5.5, 6.5, or alkalinized with 1 N NaOH to pH 7.5 at temperatures of 15, 20, 25, and 30°C both in light and dark for 54 hr. In culture, the mycelium varied from white to light-brown in color and appressed to flocculent both in light and dark. Maximum mean linear growth of mycelium of 7.7 cm dia in PDA plate in 54 hr. was obtained at pH 4.5 at temperature of 20°C in light. At temperature of 30°C at pH 4.5 in the dark, the mean linear growth of Dpc mycelium was 6.3 cm dia. Significant variations in linear growth among Dpc isolates were noted. Pathogenecity on soybean cultivars is underway to determined possible races of Dpc.

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PROTECTION OF SNAPBEAN SEEDLINGS FROM RHIZOCTONIA ROOT ROT BY BINUCLEATE RHIZOCTONIA-LIKE FUNGI.
Jose E. Cardoso and E. Echandi, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Some nonpathogenic binucleate Rhizoctonia-like fungi (BRLF) protected snapbean (Phaseolus vulgaris) seedlings from root rot caused by Rhizoctonia solani. BRLF extensively colonize the surface but did not penetrate the primary roots and hypocotyls of snapbean seedlings. Exudates from BRLF-inoculated seedlings suppressed germination of sclerotia and hyphal growth of the pathogen. Moreover, the number of infection cushions of R. solani on BRLF inoculated seedlings was 90% less than on seedlings void of BRLF. Surface sterilization of BRLF-inoculated seedlings with 70% alcohol did not reverse the protective ability of BRLF. Seemingly, BRLF induced metabolic changes in snapbean seedlings making them more resistant to root rot.

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RHIZOCTONIA SOLANI AND BINUCLEATE RHIZOCTONIA-LIKE FUNGI IN PEANUT SEED IN A PEANUT-CORN ROTATION.
D. K. Bell and D. R. Sumner, University of Georgia Coastal Plain Station, Tifton, GA 31793

Field microplots (MP) fumigated with metam sodium were infested separately with Rhizoctonia solani AG-4, AG2-T2, AG-2T1, binucleate CAG-2, CAG-3, CAG-4, CAG-5 or noninfested. MP were planted alternately to peanut and corn 3 yr. AG-4 caused 45% peanut stand loss in 1982. There were no differences for stand in 1984 or for pod yield either yr. Isolations in 1982 from seed in pods attached to plants (AP) yielded 2,2,5,1,1 and 6%, respectively, cultures of AG-4, AG-2T2, AG-2T1, CAG-2, CAG-3, and CAG-5. In 1984 AG-4, AG-2T2, AG-2T1, CAG-4 and CAG-5 were isolated, respectively, from 1,1,2, 0.2 and 2% of seed in AP; and AG-4, AG-2T2, AG-2T1, CAG-3, CAG-4 and CAG-5 were isolated, respectively, from 11,2,3,17,1 and 18% of seed in loose pods. Pods left in soil after harvest may contain inoculum of R. solani and related binucleate fungi. All fungi except AG-2T2 and CAG-3 were recovered from soil 3 yr after infestation.

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PERSISTENCE OF BENZIMIDAZOLE FUNGICIDES IN TRUNK INJECTED QUERCUS FUSIFORMIS IN TEXAS.
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Samples of wood from Texas live oaks (Quercus fusiformis) were bioassayed for Arbotect 20-S (2-(4-thiazolyl)benzimidazol) and Lignasan (Methyl-2-benzimidazole carbamate phosphate) at 3, 5, and 7 years after experimental trunk injections for oak wilt (Ceratocystis fagacearum) control. The fungicides persisted in all 12 sampled trees but did not adequately control oak wilt. Growth of C. fagacearum was inhibited by fungicides in some of the samples when spores were sprayed on potato dextrose agar in petri dishes. The percent of each sample type with fungicidal action was 93% at injection points; 87% at 2.54 cm above injection points; 50% at 30 cm above injection points; 33% at 60 cm above injection points; and 18% in callus over injection points. Mean zone diameters of C. fagacearum inhibition by different sample types ranged from 1.8 to 2.6 cm. Benzimidazole fungicides persisted in living and dead wood near injection sites and inhibited C. fagacearum growth in bioassays 7 years after treatment.

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An adjuvant increases survival and efficacy of Colletotrichum coccodes, a mycoherbicide for velvetleaf (Abutilon theophrasti).
L.A. Wymore and A.K. Watson, Dept. of Plant Science, McGill University, Ste.-Anne-de-Bellevue, Quebec H9X 1C0.

Colletotrichum coccodes is a potential mycoherbicide for velvetleaf, a damaging weed in maize and soybean. Inoculum is applied as a spore suspension in water and the pathogen requires a dew period in order to initiate disease. Plants in pots were inoculated, incubated in growth chambers without a dew period, and periodically sampled by plating leaf washings on agar media. There was a 75% reduction in viable spores recovered from inoculated leaves after 24 hr, but some viable spores could still be recovered after 12 days. Viable spores were capable of initiating disease following a subsequent 18-hr dew period. The addition of Sorbo (64% sorbitol, Atkemix Inc., Brantford, Ontario) to the inoculum increased numbers of viable spores recovered from inoculated leaves by up to 20-fold. With Sorbo, three 9-hr dew periods on consecutive nights were as effective for disease

initiation as one 18-hr dew period. Similar results were obtained in the field, but spore survival time was shorter.

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SYSTEMIC ANTIFUNGAL ACTIVITY OF CYMOXANIL AGAINST PSEUDOPERONOSPORA CUBENSIS AND PHYTOPHTHORA INFESTANS. Yigal Cohen and Masha Grinberger, Life Sciences Department, Bar-Ilan University, Ramat-Gan 52100, Israel

Cymoxanil (Curzate ^{R}, 50 WP, 500-1000 a.i./ml) effectively controlled downy mildew in cucumbers and late blight in potatoes incited by either metalaxyl-sensitive or -resistant isolates of P. cubensis and P. infestans, respectively. The fungicide exhibited preventive and curative effects in controlling both pathogens. The compound translocated acropetally from roots and stems to leaves, but not from leaf to leaf. Cymoxanil, however, was phytotoxic to roots of cucumber and tomato. Efficient translaminar translocation of cymoxanil occurred in potato and tomato but not in cucumber. Sandocur-M (a mixture of cymoxanil, mancozeb and oxadixyl) effectively controlled late blight in potatoes in the field where Ridomil MZ58 failed to do so.

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THE EFFECT OF METALAXYL AND FOSETYL-AL ON SPORULATION OF PHYTOPHTHORA CACTORUM IN NATURALLY INFESTED SOIL. M. K. Rahimian and J. E. Mitchell. Department of Plant Pathology, University of Wisconsin-Madison 53706

The effect of metalaxyl and fosetyl-Al on sporulation of Phytophthora cactorum (Pc) in vivo was studied in two phases of manipulation of soil moisture and temperature prior to counting the number of colony forming zoospores (cfz) on a selective medium. In phase 1 (sporangium formation), 2 g airdried naturally infested soil was moistened for 1 day with 1.4 ml of the fungicides at 24C. Metalaxyl at $\geq$25 µg/ml resulted in no cfz. Fosetyl-Al at 100 and 400 µg/ml resulted in 74% and 99% less cfz than the control, respectively. In phase 2 (zoospore release), after soil was moistened for 1 day and flooded for 2 days at 24C, water was replaced with the fungicides and incubated for 2 h at 8C. Metalaxyl at $\leq$100 µg/ml did not result in less cfz than the control. Fosetyl-Al at 400 µg/ml resulted in no cfz and at 100 µg/ml resulted in 44% less cfz than the control. Both fungicides prevented sporulation of Pc in vivo.

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DETECTION OF METALAXYL RESISTANCE IN PERONOSPORA TABACINA ON WILD TOBACCO (NICOTIANA REPANDA) IN TEXAS. M. Reuveni, W. C. Nesmith, M. D. Wiglesworth, and M. R. Siegel, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Isolates of P. tabacina were collected in 1983, 1984 and 1985 from locally and systemically infected wild N. repanda plants near Uvalde, Texas. The sensitivities to metalaxyl of the Texas isolates, as well as of those collected in Kentucky from cultivated tobacco during 1979-1985, were determined either by foliar spraying of whole plants or by using a detached leaf uptake assay (Pestic. Sci. 16:244-250). All of the isolates, except that from Texas in 1985, had the same sensitivity to metalaxyl. The Texas 1985 isolate produced sporulating lesions at two-to-five times the concentration inhibitory to the other isolates. The tolerance to metalaxyl of the Texas 1985 isolate increased (up to 50 times) when the inoculum was obtained from detached leaves treated once with metalaxyl. This increased tolerance did not occur with any of the other isolates.

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METALAXYL RESISTANCE OF PERONOSPORA TABACINA ON TOBACCO IN MEXICO. M. Wiglesworth, W. Nesmith, M. Reuveni, S. Tuzun, M. Siegel, J. Kuč, University of Kentucky, Lexington, KY 40546, and J. Juárez, Tabamex, San Andrès Tuxtla, Veracruz, Mexico.

Isolates of P. tabacina, were collected in January 1986 from metalaxyl-treated and untreated cigar tobacco plantations in the state of Veracruz, Mexico. The sensitivity of each isolate to metalaxyl was determined by a detached leaf assay (Pestic. Sci. 16:244-250). Leaves treated with various levels of metalaxyl (0-100 µg/ml) were challenged with 40,000 sporangiospores/ml obtained directly from freshly sporulating field lesions. These isolates tolerated metalaxyl at 150-225 times the minimum concentration required to prevent lesion development by Kentucky isolates (0.066 µg/ml) using the identical bioassay. Mexican isolates were variable in metalaxyl sensitivity and produced lesions on leaves treated with up to 20.0-66.6 µg/ml while sporulation occured with $\leq$ 15 µg/ml. These data explain why fungicides containing metalaxyl are not controlling tobacco blue mold in Veracruz, Mexico.

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RESISTANCE OF FUNGI TO FUNGICIDES - WHY TO SYSTEMIC FUNGICIDES?
A. Dinoor, Faculty of Agriculture, Rehovot 76100, Israel.

Systemic fungicides having penetrated into the host are in close contact with the pathogen outside and inside the host. Selection pressure is continuous and there is no possible escape by penetration into the plant, unlike the case with non-systemic fungicides. The systemic fungicides are distributed in and on the plants in gradients of concentrations, being more concentrated along leaf margins and less and less towards the centers of the leaves, the petioles and the stems. The relatively more resistant components of the pathogen populations will be selected along such gradients. Therefore they will develop first, with no competition, on petioles and then on main veins.

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EFFECT OF SANITATION, FUNGICIDE APPLICATION AND HAND POLLINATION ON MONILIASIS AND BLACK POD INCIDENCE. V.H.Porras[1], J.J.Galindo[2] and C. Cruz[1]. UCR, San José[1] and CATIE, Turrialba[2]. Costa Rica.

The effect of i)weekly sanitation, ii)fungicide application, iii) total pod removal above 2 m of tree height, and iv) hand pollination, were evaluated on moniliasis (Monilia roreri) and black pod (Phytophthora palmivora) incidence. These four treatments were tested on 18-year-old trees of 6 cacao hybrids. Sanitation reduced moniliasis incidence from 76.5 to 34.1% and black pod from 23.4 to 6.1%. Additional reduction of fungicide application as compared to sanitation was significant in hand pollinated plots but not in naturally pollinated plots. There was no additional reduction of black pod incidence by fungicide application in relation to sanitation. In the hand pollinated plots cacao yield increased from 1496 to 2121 kg/ha with the use of fungicide. In the naturally pollinated plots with sanitation, cacao yield was 1108 Kg/ha. Treatment iii) did not reduce moniliasis and was detrimental to cacao yield (539 kg/ha).

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CHEMICAL CONTROL OF PREMATURE-DEATH OF POTATO SEEDLINGS. H. Torres and C. Martin. International Potato Center, Apartado 5969, Lima 100, Peru.

Premature-death of potato seedlings, caused by a complex of pathogens, mainly Rhizoctonia, Fusarium and Erwinia species is common in the coastal valleys of Peru. Two field trials were carried out during 1984-85 under irrigated desert conditions to study the control with chemicals. Botanical seeds from heat-tolerant clone DTO-33 were sown in trays and 35 days later seedlings were transplanted in Jiffy-sevens to the field. Treatments included the application of fungicides Metalaxyl 5 G, Tolclofos methyl and PCNB + Benomyl (control) in different combinations, with or without a pre-planting application of Dazomet 98, and the use of solarization. There were no significant differences in both trials in the number of plants yielding at harvest time since diseased plants yielded a few small tubers. Significant differences in the number of premature-death plants were obtained in the second trial only. Plots treated with Dazomet + Tolclofos methyl + Solarization yielded up to 150% more than the control.

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THE EFFECTS OF CHLOROTHALONIL APPLIED IN IRRIGATION WATER ON SOIL FUNGI IN PEANUT CULTURE. R. H. Littrell and Donald R. Sumner, Coastal Plain Experiment Station, Tifton, GA 31793.

Applications of chlorothalonil (Bravo 500) in irrigation

water for control of leafspotting diseases of peanut have consistently increased pod yield over yields obtained from applications using conventional ground spray. Greenhouse and field microplots (0.66 m^2) were used to determine if applications of chlorothalonil (1.24 kg a.i./ha) in irrigation water (0.4 cm) had an influence on the soilborne pathogens Rhizoctonia solani, Sclerotium rolfsii, Pythium spp. and other fungi. Populations of R. solani but not S. rolfsii and Pythium spp. were reduced by chlorothalonil. There were no differences among populations of saprophytic soil fungi between ground spray and irrigation water application methods. Lesions on stems, pods, pegs, and hypocotyls were reduced and root growth was stimulated by soil application of chlorothalonil. These results may help explain improvement in pod yield.

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BAY HWG 1608 : A BROAD-SPECTRUM EXPERIMENTAL FUNGICIDE. P. Reinecke, H. Kaspers, H. Scheinpflug, G. Holmwood, Bayer AG, Agrochemicals Division, Research and Development, D-5090 Leverkusen, Bayerwerk, West Germany; and K. Noegel, Mobay Corporation, Agricultural Chemicals Division, 8400 Hawthorn Road, Kansas City, Missouri 64120.

BAY HWG 1608, ethyltrianol (proposed), is highly effective as either a seed treatment (Raxil™) or as a foliar fungicide (Folicur™) on several economically important crops. On cereals, HWG 1608 is effective as a seed treatment for the control of smut (Ustilago spp.) and bunt (Tilletia spp.), and as a foliar spray against leaf and head diseases, including Fusarium, Septoria and Puccinia spp. On peanuts, HWG 1608 provides excellent control of rust (Puccinia arachidis) and leaf spots (Cercospora arachidicola and Cercosporidium personatum) and results in higher yields than the commercial standard. HWG 1608 has also been effective against Mycosphaerella on bananas and against important grape pathogens, including Botrytis cinerea.

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Ro 15-1297: A STEROL INHIBITOR FUNGICIDE WITH PREVENTATIVE AND CURATIVE PROPERTIES. D. F. Myers, Maag Agrochemicals Research and Development, HLR Sciences, Inc., P. O. Box X, Vero Beach, FL 32961.

Ro 15-1297, 1-(2,4 dichlorophenyl)-2-(3-pyridinyl) ethanone O-methyloxime, common name pyrifenox, is an inhibitor of ergosterol biosynthesis. The fungicide possesses low order mammalian toxicity and has preventative and curative properties. Pyrifenox is effective against many foliar and fruit diseases including early and late peanut leaf spots (Cercospora arachidicola, Cercosporidium personatum), apple and pear scabs (Venturia inaequalis, Venturia pirina), powdery mildews of apple and grape (Podosphaera leuchotricha, Uncinula necator), and black sigatoka of banana (Mycosphaerella fijiensis var. difformis). In test evaluations against these diseases, pyrifenox compared favorably to commonly used standards and other sterol inhibitors in development.

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TAXONOMY OF SIX PATHOVARS OF XANTHOMONAS CAMPESTRIS BASED ON SDS-PAGE OF MEMBRANE PROTEINS. **M. L. Derie and N. W. Schaad, Dept. Pl., Soil, & Ent. Sci., Univ. of Idaho, Moscow, ID 83843.**

Six pathovars of Xanthomonas campestris were analyzed by sodium doedecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of membrane proteins, growth on semiselective media, serology, ice nucleation activity, and resistance to antibiotics and triphenyltetrazolium chloride. The following strains were examined: 31 strains of X. c. campestris, 10 each of X. c. carotae, X. c. phaseoli, X. c. malvacearum, and X. c. translucens, and 5 strains of X. c. manihotis. Each group was clearly distinguished from one another from results of SDS-PAGE, growth on semiselective media, serology, and ice nucleation activity. We propose the following five pathovars be elevated to species level: Xanthomonas campestris (Pammel) Dowson nom. rev.; Xanthomonas carotae (Kendrick) Dye nom. rev.; Xanthomonas malvacearum (Smith) Dye nom. rev.; Xanthomonas phaseoli (Smith) Dye nom. rev.; and Xanthomonas translucens (Jones, Johnson, and Reddy) Dye nom. rev. We also suggest the elevation of the pathovar manihotis to species level.

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PHENOTYPIC VARIATION AMONG RACES OF XANTHOMONAS CAMPESTRIS PV. ORYZAE. C. M. Vera Cruz & T. W. Mew, The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Similarity of 131 phenotypic features among 52 isolates belonging to six races of Xanthomonas campestris pv. oryzae was determined. No distinct clusters were detected among the isolates tested. The isolates were homogeneous in cultural, biochemical and physiological characters regardless of the cultivar specificities. However, several features were found to be associated with certain races. More than 90% isolates of all races except race 1 (17%) grew and produced acid on basal medium containing trehalose. More than 80% isolates of races 2 and 5 grew on Na-aconitate medium whereas less than 30% isolates of the other races were able to use Na-aconitate as carbon source. All isolates of races 4 and 6, while 16 to 25% of races 1 and 3 grew in the presence of 0.05% triphenyl tetrazolium chloride. The tetrazolium was inhibitory to all isolates of races 2 and 5.

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Production of ethylene in culture by Agrobacterium tumefaciens and A. rhizogenes. M. L. Canfield and L. W. Moore, Dept. of Botany and Plant Pathology, Oregon State Univ. Corvallis 97331

Ethylene (C_2H_4) is produced by carrot discs inoculated with Agrobacterium tumefaciens or A. rhizogenes (Z. Pflanzenphysiol. 112:471). To determine whether the agrobacteria could produce C_2H_4 independent of the plant, several strains were grown in culture and C_2H_4 accumulation was measured. Agrobacteria grown on a shaker for 48 h in the light in 100 ml of mannitol glutamate medium supplemented with 10 mM L-methionine produced 0.4 mMoles C_2H_4/ml in closed 250 ml flasks. No C_2H_4 was produced in the dark. When 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of C_2H_4 in plants, was substituted for L-methionine, no C_2H_4 was produced. Cell free culture filtrates from bacteria grown in the dark produced C_2H_4 when exposed to light. Production was inhibited by copper sulfate and enhanced in the absence of oxygen. These data support the hypothesis that a flavin-like compound excreted into the culture medium aids in the nonbiological conversion of methionine to C_2H_4.

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A WILT AND CROWN ROT OF PRIMULA CAUSED BY AN ERWINIA SP. S.M. McCarter, M.L. Waindle, and E.H. Moody, Dept. of Plant Pathology, Univ. of Georgia, Athens 30602.

In 1985 Primula plants with crown necrosis and wilt were sent by a commercial grower to the University of Georgia Extension Service Plant Disease Clinic. Isolations yielded mostly pure cultures of buff to tan bacterial colonies on nutrient yeast dextrose agar. Koch's Postulates confirmed pathogenicity. The bacterium was a short, Gram negative, motile rod with peritrichous flagella. It was a facultative anaerobe with an optimum of 25-30 C. It was negative for oxidase, arginine dihydrolase, phosphatase, and reducing substances for sucrose, and positive for catalase, aesculin hydrolysis, nitrate reduction, potato soft rot, and gelatin liquefaction. It produced acid but not gas from glucose, lactose, and sucrose. It caused rapid pitting of sodium polypectate gel at pH 4.5 and 8.0 and liquefaction of Beraha medium. It was slightly susceptible to erythromycin (15 µg). Test results indicate that the bacterium is an Erwinia sp., possibly a strain of E. carotovora.

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INFLUENCE OF TEMPERATURE, VAPOR PRESSURE DEFICIT AND INOCULATION METHOD ON INFECTION OF SQUASH PLANTS BY ERWINIA CAROTOVORA. Jean Carlson Batzer and Andrew C. Schuerger. The Land, EPCOT Center, P. O. Box 40, Lake Buena Vista, FL 32830.

Two techniques were used to inoculate three- to four-week-old squash plants of cultivar "Banana Pink Jumbo" with Erwinia carotovora. At 30°C and a vapor pressure deficit (VPD) of 2.77 mm Hg (90% RH) the highest disease development (100%) occurred when 0.1 ml of a bacterial suspension of 2.0 X 10^8 cfu/ml was injected 0.5 cm into the squash stem. Eighty-percent of the plants developed stem rot when a scalpel dipped into the same inoculum was used to prune off the second true leaf of each plant. When temperature was held at 29°C and VPD was set at constant levels between 18.02 mm Hg (38% RH) and 2.77 mm Hg (90% RH) the percentage of plants developing stem rot increased with decreasing VPD. When VPD was held at 2.77 mm Hg and temperatures were set at constant levels between 10°C and 30°C the percentage of plants developing stem rot increased with increasing temperatures.

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A PROPOSED BASIS FOR VARIETAL DIFFERENCES IN SENSITIVITY OF GRAPES TO CROWN GALL DISEASE. G.L. Cleveland and R.N. Goodman, Dept. of Plant Pathology, University of Missouri, Columbia, Missouri 65211.

A tissue culture system was used to study the basis for varietal differences in sensitivity of grapes to crown gall disease. Stem explants from cultivars with various sensitivities to the disease (as determined by tumor size) were incubated on woody plant medium supplemented with equal concentrations of naphthaleneacetic acid and kinetin (0.5, 1.0 and 2.0 mg/l) at 28 C for 16

days. Increases in fresh weight of callus tissue from the explants were 3.0 to 6.5 times greater for a highly sensitive variety (Chancellor) than for insensitive varieties (Catawba and Concord) at all hormone concentrations. Crown gall tumors are chimeras of transformed and normal plant cells. Our findings suggest that tumor size in these grape cultivars may be a function of the inherent sensitivity of normal cells to stimulation by phytohormones synthesized and excreted by proximate transformed cells. We are in the process of quantitating transformed and normal cells in tumors derived from sensitive and insensitive cultivars.

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OCCURRENCE AND CONTROL OF COPPER TOLERANT STRAINS OF PSEUDOMONAS SYRINGAE ON ALMOND AND CITRUS IN CALIFORNIA. G. L. Andersen and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Forty-five percent of 133 epiphytic isolates of P. syringae isolated from almond and 33% of 127 isolates from navel orange leaves were tolerant of greater than 0.32 mM $CuSO_4$ in a casitone-yeast extract-glycerol (CYE) medium and in aqueous $CuSO_4$ solutions (>4 µg/ml). A bimodal distribution of copper ion sensitivity among P. syringae strains was observed; strains were most frequently tolerant of less than 0.16 mM $CuSO_4$ or more than 0.8 mM $CuSO_4$ in CYE medium. Copper sensitive P. syringae strains were unable to grow on bean leaves treated with $Cu(OH)_2$. However, copper tolerant strains grew at an equivalent rate on copper treated and untreated leaves. Copper resistant strains were not killed but 94% of the cells of sensitive strains were killed by foliar treatment of colonized leaves with $Cu(OH)_2$ unless maganese ethylene bis-dithiocarbamate was added to $Cu(OH)_2$ sprays.

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STUDIES ON BIOLOGICAL CONTROL OF Moniliа roreri BY EPIPHYTIC BACTERIA. J.M. Jiménez[1], J.J. Galindo[1] and C. Ramírez[2], CATIE, Turrialba[1] and UCR, San José[2]. Costa Rica.

Fourty fluorescent Pseudomonas strains were isolated from the phylloplane of 60 to 90-day-old cacao pods. Antagonism against M. roreri was tested in vitro. Antagonistic strains were tested in the field during two seasons at La Lola, Limón (26 C, 3674 mm of rain) on cacao cv UF-29, highly susceptible to moniliasis. The effect of 3, 4, and 6 applications of bacteria (10^7 UCB/ml) on 15 to 90-day-old pods were compared with fungicide application to the pod under natural and artificial inoculation of M. roreri (10^5 conidia/ml). Bacteria reduced moniliasis incidence from 85.1 to 5.6% on artificially, and from 72.9 to 6.25%, on naturally inoculated pods. Similar results were obtained on pods treated with fungicide. Bacteria population of strains applied to cacao pods had a positive correlation with relative humidity and precipitation and negative with solar radiation and temperature. These results show the potential for using biological means of control against M. roreri.

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SYSTEMIC SPREAD OF AGROBACTERIUM TUMEFACIENS BIOVAR 3 IN THE VASCULAR SYSTEM OF GRAPES AND THE DEVELOPMENT OF SECONDARY TUMORS. F.A. Tarbah and R.N. Goodman. University of Missouri, Columbia, MO 65211.

The movement of Agrobacterium tumefaciens biovar 3 was studied in artificially inoculated roots of "Chancellor" and "Catawba" grape cultivars. Subsequent induction of aerial tumors at aseptically wounded stem tissue sites was acertained by using a streptomycin-rifampicin resistant mutant of Ag 63 of biovar 3. Visual evidence of systemic translocation of A. tumefaciens in vascular tissue was obtained from light and scanning electron microscopic observations. Our observations suggest that bacteria per se initiated the secondary tumors that developed at aseptically wounded sites.

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ULTRASTRUCTURAL STUDIES OF THE INFECTION PROCESS BY AGROBACTERIUM TUMEFACIENS BIOVAR 3 TO GRAPEVINE. F.A. Tarbah and R.N. Goodman. University of Missouri, Columbia, MO 65211.

Our study of the infection process of Agrobacterium tumefaciens in grape was facilitated by inoculating small diameter stem discs or delaminated petioles which permitted visualization of the infection process in a limited area and which revealed the primary target cells. Light microscopy revealed that the initial host cell divisions occurred in ray and xylem parenchyma of vascular bundles 48-52 h after inoculation. In inoculated tissue, the number of bacteria became greatly reduced after 36-60 h and those bacteria that persisted displayed distinctive variations in their shape and content. Reduction in bacterial number was characterized by intense lysis which appears to be a prelude to the release of the Ti-plasmid. Some bacterial cells which were attached or in close contact with host cells contained dense inclusions in their cytoplasm. Presumably, bacterial cell inclusion formation and subsequent lysis develop in response to increased osmotic concentration of fluids diffusing from wounded plant cells. Following bacteral cell lysis these dense inclusions, which may be T-DNA containing, reach exposed plant cell membranes there they may be endocytosed.

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PHENOTYPIC VARIABILITY OF PSEUDOMONAS SOLANACEARUM STRAINS ISOLATED FROM A SINGLE POTATO FIELD. R. J. McLaughlin and Luis Sequeira, Univ. of Wisconsin, Madison, WI 53705.

Strains of Pseudomonas solanacearum were isolated on a selective medium from 180 soil samples taken from a 0.3 ha field near Hastings, Florida. Strains were characterized as to antibiotic production and sensitivity, pathogenicity on two potato cultivars, biotype, and hypersensitivity (HR) on tobacco leaves. Eighty-six strains tested for antibiosis against 26 strains from the same area showed 24 different typing patterns. Differences in typing patterns were also evident when 12 strains were tested against 9 exotic strains from potato (4 types). Stem inoculation of potato cv. Ontario (resistant) and cv. Atlantic (susceptible) with 8 strains showed that they differed in aggressiveness. Differences in this test were most evident at relatively low temperatures. All isolates elicited the HR on tobacco cv. Bottom Special. Occasional variability in the oxidation of galactose, arabinose, and lactose was noted in the biotyping tests.

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EFFECTS OF VOLATILES FROM NURSERY AND FOREST SOILS ON THE MICROBIAL PROFILE OF A CONIFER NURSERY SOIL. D.A. Schisler and R.G. Linderman, Dept. of Botany and Plant Pathology, Oregon State University and USDA-ARS Horticultural Crops Research Laboratory, Corvallis, Oregon 97330

Numerous genera of ectomycorrhizal fungi and other soil microbes produce volatile compounds in vitro. The consequence of in vivo volatile production by soil microorganisms is unknown largely due to difficulties in separating volatile effects from other microbially mediated effects. An apparatus which slowly purges volatiles from a "donor" test soil into a "receiver" soil was used to determine differential effects of volatiles from "donor" forest or nursery soils on microbial populations of "receiver" nursery soils. "Donor" soils were either treated or not with methylbromide and aerated steam and were planted or not with Douglas-fir seedlings. "Donor" soil origin and tree presence significantly altered populations of several taxonomic and functional microbial groups in "receiver" nursery soils, demonstrating for the first time that soil volatiles selectively influence soil microbe populations in vivo.

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GEL FORMATION BY THE EXTRACELLULAR POLYSACCHARIDE (EPS) AMYLOVORIN PRODUCED BY ERWINIA AMYLOVORA AND APPLE CELL WALL POLYMERS, A BASIS FOR XYLEM VESSEL OCCLUSION. R.N. Goodman, University of Missouri, Columbia, MO 65211.

Clearly visualized xylem vessel occlusion by gel-like structures, suggested that amylovorin is a participant in the process. Experiments using viscometry and spectropolarimetry (circular dichroism measurements) indicate that the EPS by itself does not form gels. However, EPS at 0.1% and either highly purified apple pectin or tamarind xyloglucan at 0.1% do form gels. These data suggest that vessel occlusion reflects an interaction between EPS and vessel wall polymers. The manner in which this may proceed will be discussed.

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DISEASE DYNAMICS OF THE FLORIDA FORM OF CITRUS CANKER IN SIMULATED EPIDEMICS. Gottwald, T. R., U.S. Department of Agriculture, ARS, 2120 Camden Rd., Orlando, FL 32803, Civerolo, E. L., Garnsey, S. M., Brlansky, R., and Gabriel, D.

Field plots were established at Frederick, MD, to study the disease dynamics of the Florida form of citrus canker. Nursery and replant grove simulations were created by placing an infected plant in the center of nursery and replant plots one of 'Duncan' grapefruit (DG) and 'Valencia' orange (VO). Bacterial spread was monitored by immunofluorescence probe, DNA hybridization probe, and isolation on selective media. Bacterial spread in replant plots to 13.7 m (plot limit) by day 33 in VO and by day 109 in the DG plot. Bacteria spread in nursery plots to 2 m in VO and 3 m in DG nurseries by day 81, and reached 3 m in VO nursery by day 110. Disease incidence was recorded at several locations in the DG nursery by day 81 but not in any of the other three plots. Similar tests have been established in Argentina with the Asiatic form of the bacterium.

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ALTERATION OF LEAF ANGLES AND CIRCADIAN LEAF MOVEMENTS IN SEEDLING SUNFLOWERS INFECTED BY Pseudomonas tagetis. B. W. Kennedy. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Stem inoculation of newly emerged Helianthus annuus L. cv Funk Standard Hybrid G-6625 resulted in severe chlorosis of emerging first leaves on plants grown in controlled environments at 25 C with 12 hr light followed by 12 hr dark. Affected leaf areas were 51% that of controls and the horizontal angle of diseased

leaf laminae were 5-41 degrees greater toward the vertical than controls; greatest magnitude of difference between healthy and disease leaves was at midpoint of the light cycle and was least during the last 4 hr of the dark cycle. Circadian leaf movement rhythms were similar in diseased and control leaves but the magnitude of drop in the angle of diseased leaves in the dark was reduced much less than that of healthy leaves. Mesors representing average angle of diseased leaves was always greater than that of controls; there was no phase shift in movements during light/dark cycles due to disease.

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THE INFLUENCES OF PLANTING DATE, MATURITY GROUP, AND CULTIVAR SUSCEPTIBILITY ON PERITHECIAL PRODUCTION BY THE SOUTHERN STEM CANKER PATHOGEN. T. W. Hobbs, D. V. Phillips, Dept. of Plant Pathology, and P. L. Raymer, Dept. of Agronomy, Univ. of Georgia, Georgia Station, Experiment, GA 30212.

An epidemic of Southern stem canker of soybean, caused by southern biotypes of Diaporthe phaseolorum (Cke. & Ell.) Sacc. var. caulivora Athow & Caldw., occurred during the 1985 crop season in cultivar test plots at the UGA SW Branch Station at Plains, GA. Perithecia of the organism were first detected on 21 Oct. on the crown and root area of the plants. A collection of cultivars representing a wide range of disease susceptibility was made on 10 Dec. Perithecia were most frequent on samples from early-planted, late-maturing (MG 7 & 8) cvs. (54.9%) and least frequent on late-planted (1-mo. delay), early-maturing (MG 5 & 6) cvs.(5.8%). Perithecial production generally increased as susceptibility increased (R^2 = 0.63, p = 0.0001). Perithecia were detected on the highly resistant cv. Tracy-M and all other cultivars except cv. Braxton.

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YIELD REDUCTIONS DUE TO EXSEROHILUM TURCICUM AND DETASSELING OF INBRED FEMALES IN HYBRID SEED CORN PRODUCTION. Kira L. Bowen and W.L. Pedersen, Dept. Plant Path., University of Illinois, Urbana, IL.

Yield reductions due to Exserohilum turcicum (northern corn leaf blight) (NCLB) and/or defoliation due to detasseling were studied on inbred females in hybrid seed corn production. Three female inbreds were grown in a factorial field experiment at two locations. Four rows of inbred females (FRMo17cms, FR632cms, and FRB73cms) were planted alternately with two rows of males (FR634rfc, FR619, and FRMo17rfc, respectively). Six different severities of NCLB were established by fungicide applications to inoculated plants. Mechanically detasseled corn was compared to tasseled inbreds (c-male sterile). Reduction of yield and seed quality (e.g. seed size and seed weight) due to NCLB was significantly (P=0.05). Yield reduction due to detasseling was also significant (P=0.05). Maximum reduction in yield was 44% due to NCLB and 32% due to detasseling on FR632cms. Seed weight was the only parameter measured that showed a significant interaction effect of detasseling and disease.

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SORGHUM RUST: ECOLOGY, LOSSES, AND RESISTANCE. Paul R. Hepperly and A. Sotomayor-Ríos, USDA-ARS, Box 70, Mayaguez, PR 00709.

Lowland tropical oceanic environments favored severe rust on susceptible sorghum lines after flowering and year round in Puerto Rico. Rust losses were calculated based on differences between the yields of oxycarboxin rust fungicide treatments and nontreated checks. During two seasons, moderately (SC 307) and very susceptible (SC 212) sorghum lines showed 20 to 30 and 40 to 50% yield losses, respectively, and losses in 100-seed weights were similar to those for yield in the two lines. Controlling rust resulted in no yield benefit in TAM 428 (moderately resistant) and SC 120 (very resistant). Over 70 low rust source lines were identified but in 3 subsequent plantings only 50 were consistently resistant. High frequency (80%) of dull midrib (dd) was found in low rust source lines. Resistant sources also show increased frequency of tan plant coloration (pp). Resistant sources were crossed with Texas Population 24 which is segregating for genetic male sterility (ms_3ms_3). Recombination and selection of this population is in progress.

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DALE SWEET SORGHUM NOW RESISTANT TO RUST INCITED BY PUCCINIA PURPUREA CKE. Natale Zummo. USDA-ARS, Dept. of Plant Pathology and Weed Science, Miss. State, MS 39762

When Dale sweet sorghum was introduced in 1970 as a mid-season variety adapted for sirup production in Mississippi it was resistant to anthracnose and red rot but was susceptible to rust. Plants with resistance to rust were found in a planting of Dale at Houma, Louisiana in 1978. Seed from these rust resistant plants was increased at Meridian, Mississippi and screened at Houma to produce a rust resistant selection of Dale. Rust resistant Dale has gradually replaced the original Dale throughout the sirup areas of the United States so that this is the only seed of Dale now available.

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Selection for resistance to Septoria musiva in Populus utilizing somaclonal variation. M. E. Ostry, D. D. Skilling, T. L. Ettinger, W. P. Hackett, P. E. Read. North Central Forest Experiment Station, 1992 Folwell Avenue, St. Paul, Minnesota 55108.

Somaclonal variation is variation in any number of traits exhibited by plants regenerated from aseptic cultures. Exploiting this variation has led to increased resistance to several important pathogens of agronomic crops. Our objective is to develop a model system to demonstrate the potential role of somaclonal variation in forestry. Aseptic culture techniques are being used to regenerate plants from cell aggregates, calli, and adventitious shoots from selected hybrid poplar clones. In preliminary results from leaf disc bioassays using Septoria conidia, several regenerated somaclones have shown more resistance to Septoria than did the original clones. Identification and isolation of somaclonal variation for disease resistance in forest trees will greatly augment tree improvement programs and reduce the time it takes to develop superior trees.

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EFFECT OF STEM CANKER ON PERFORMANCE OF SOYBEAN CULTIVARS UNDER DIFFERENT MANAGEMENT PRACTICES. C. S. Rothrock, T. W. Hobbs, and D. V. Phillips, Department of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

The performance of the soybean cultivars Coker 368 and Hutton (moderately resistant and very susceptible to Southern stem canker, respectively) was examined under different tillage and cropping practices. Stem canker was greatest under no tillage. Total disease incidence was 6% and 2% for Coker 368 and 85% and 35% for Hutton under no tillage and conventional tillage, respectively. Disease was also slightly greater under soybean/wheat doublecropping. Yields were similar for Coker 368 for all treatments. Yield of Hutton was 29% lower and 49% lower than Coker 368 for conventional and no tillage, respectively. Yield was 32% lower for Hutton under no tillage than conventional tillage. The data indicate that cultivars with moderate resistance can perform well under management practices which favor disease and that stem canker can result in about 30% greater yield loss under no tillage.

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EFFECT OF TEMPERATURE AND MISTING REGIME ON DEVELOPMENT OF RHIZOCTONIA LEAF SPOT OF TOBACCO. H.D. Shew and C.E. Main. Dept. of Plant Pathology, N.C. State University, Raleigh 27695-7616.

Six-wk-old 'NC 2326' tobacco plants were placed in growth chambers at controlled temperatures and exposed to misting regimes that provided different durations of high relative humidity and leaf wetness. Rice grains infested with Rhizoctonia solani were placed 1cm below the soil surface in each pot 3cm from the plant stem. Pots were observed daily for hymenium formation and basidiospore production. Optimum temperatures for hymenium formation and leaf infection were 20-30 C. Little or no infection occurred above 30 C or below 16 C. Humidities above 95% for at least 12 hr per day were required for optimum pathogen reproduction and leaf infection. Leaf area damage was 1, 15, 24, and 46% with no misting, night

misting once every 20 min, night misting once every 5 min, and 24 hr misting once every 5 min, respectively.

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Effect of Plant Population and Inoculum Density on Disease Progress of Sclerotinia Wilt of Sunflower. B. D. Nelson, D. M. Hertsgaard and R. C. Holley. First and third authors Department of Plant Pathology and second author Department of Statistics, North Dakota State University, Fargo, ND 58105.

Eight field experiments were established at different sites with soils naturally infested with Sclerotinia sclerotiorum to study the effect of plant population and inoculum density (ID) on disease progress (DP) of Sclerotinia wilt of sunflower. Plant populations varied from 37 to 74.1 X 10^3 plants per hectare and ID ranged from .11 to 1.67 sclerotia/800 cm^3 of soil. Analysis of DP data with the Weibull probability density function showed differences in the rate of disease increase between plant populations in six experiments. However, the lowest rates of increase were not consistently associated with low plant populations but rather varied among the populations. Regression analysis indicated a positive correlation between ID and rate of disease increase.

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EFFECTS OF PATHOGEN-INFESTED CROP RESIDUE AND PLANTING SEED ON SEVERITY OF SOYBEAN STEM CANKER. Albert Y. Chambers, University of Tennessee, Jackson, TN 38301.

Observations in 1981-82 indicated that pathogen-infested crop residue and planting seed have distinct and additive roles in development of soybean stem canker, Diaporthe phaseolorum var. caulivora. Soil in microplots (half barrels, ends removed, 1 m apart in pathogen-free soil, rims 15.2 cm above soil line) was unamended and amended with infested crop residue and/or planted with uninfested and infested seed (susceptible cultivar) in 1983-85. Stem canker ratings (0 = no disease symptoms to 10 = 100% of plants affected) ranged from 1.0 in microplots with uninfested seed planted in unamended soil to 9.5 in those with infested seed planted in soil with residue mixed in upper 12.7-15.2 cm. Microplots with residue and uninfested seed rated 7.3; those with infested seed with no residue rated 2.9. Yields averaged 42.8 hl/ha in microplots that received neither residue nor infested seed, 2.9 hl/ha for those that received both, 15.2 hl/ha for those that had residue only, and 34.5 hl/ha for those that had infested seed only.

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LABORATORY AND FIELD EVALUATION OF SOYBEANS FOR REACTION TO WHITE MOLD. J.L. Lockwood and L.B. Kao, Dept. of Botany and Plant Pathology; and T.G. Isleib, Dept. of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824

Further work with a laboratory assay for soybean reaction to white mold (Sclerotinia sclerotiorum)(Chun and Lockwood, Phytopathology 74: 869) has shown that excised stems inoculated at cut stem apices and incubated at 15-20 C developed longer lesions and higher incidences of infection than stems inoculated at leaf axils and incubated at higher temperatures. Stems are now incubated in level trays on moist vermiculite. Of 16 cultivars grown in a naturally infested field, Ozzie, Hodgson 78, Pella, Corsoy 79, Hardin, and Corsoy had the lowest incidence of white mold (0-8%), whereas Gnome, Weber 84, and Hobbit had the highest (40-52%). Disease incidence was inversely correlated with seed yields ($r = -0.94$); for every increase in disease of 10%, yield was reduced by 3.5 bu/A. Laboratory assays of the same 16 cultivars grown in a greenhouse under varied conditions gave a range of correlations with field results ($r = -0.2$ to 0.63).

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LEVELS OF RHIZOCTONIA AND COLLEMBOLA AS AFFECTED BY TILLAGE AND CROPPING SYSTEM. D.H.Rickerl, Agronomy and Soils Department, Auburn University, Auburn, AL 36849.

Field plots on a silt loam and a sandy loam soil were monitored during the 1985 growing season to determine the intensity of soil infestation by Rhizoctonia solani and its natural predators ; Onychiurus encarpatus and Proisotoma minuta. Soil samples were collected every three weeks. A Tullgren extraction system was used to collect the insects, and disease infestation was estimated using a modified stem-trap procedure of Parmeter. Management systems included crop rotations with corn, cotton, soybeans, and peanuts in conventional- and conservation-tillage systems. Insect numbers were 20% greater in the sandy loam soil than in the silt loam soil. Insect numbers were also 20% more under conservation tillage than conventional tillage. Disease levels were more severe in the conservation tillage system. Both insect and disease levels varied significantly with crop and sample date.

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INFLUENCE OF SOYBEAN STEM CANKER ON THE USE OF SUSCEPTIBLE CULTIVARS IN FLORIDA. R.C. Ploetz, R.K. Sprenkel, and F.M. Shokes. NFREC, Route 3, Box 4370, Quincy, FL 32351.

During the severe soybean stem canker epiphytotic of 1983, counties in the Florida panhandle were placed into one of three groups based on the incidence of the disease. A high incidence of stem canker (incited by southern Diaporthe phaseolorum) was detected in certain western and central panhandle counties (stem canker found in 81% to 56% of the fields surveyed in a given county; group 1) while lower incidences were found in neighboring counties (8% to 2%; group 2); stem canker was not found in a third group of counties located in the eastern panhandle (group 3). After 1983, three- to six-fold reductions in the use of susceptible cultivars were noted in group 1 and group 2 counties. Low levels of stem canker found in these counties during the last two seasons may have resulted, in part, from this change in cultivar usage. No significant reduction in the use of susceptible cultivars was detected in the third group of counties in 1984 or 1985.

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THEORY OF SELECTION FOR YIELD IN A DISEASE STRESS ENVIRONMENT. M. L. Carson and Z. W. Wicks III. Plant Science Department, South Dakota State University, Brooking, SD 57007

The effects of selection for plant yield in disease stress environments (Y_{ds}) on yield performance in non-stress environments (Y_{ns}) and disease resistance (X) were examined where disease losses (βX) are a linear function of disease resistance: $Y_{ds} = Y_{ns} - \beta X$. Genetic correlations between Y_{ds} and Y_{ns}, and Y_{ds} and βX were calculated in terms of the genetic correlation between Y_{ns} and βX and the ratio between genetic variances for βX and Y_{ns}. Correlated responses in Y_{ns} and βX to selection for Y_{ds} were compared to those predicted from index selection for low βX and high Y_{ns} with both traits having equal economic weight. Selection for Y_{ds} would result in favorable correlated responses in Y_{ns} and βX except in certain situations where the genetic correlation between βX and Y_{ns} is highly positive. Responses in Y_{ns} and βX from selection for Y_{ds} compared favorably to those predicted from index selection.

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NEW MULTIPLE VIRUS RESISTANT CAPSICUM CULTIVARS, Benigno Villalon, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596

Viral diseases of peppers reduce yield in many pepper production areas throughout the world. The virology program at Weslaco, Texas, has yielded Capsicum cultivars resistant to tobacco etch virus (TEV), pepper mottle virus (PeMV), potato virus Y (PVY), and tobacco mosaic virus (TMV). Other objectives are to develop adapted pungent and non-pungent pepper cultivars possessing multiple disease and insect resistant, with high quality attributes, that can be mechanically harvested. Cultivars released to the pepper industry include: 'TAMBEL-1', 'TAMBEL-2', 'TAM Mild Chile-1', 'TAM Mild Chile-2', 'TAM Mild Jalapeno-1' and 'Hidalgo'-serrano pepper. Further releases include sweet and hot yellow wax, cayenne, pimiento, ancho, cherries and sweet paprika types.

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UNIQUE PARTICLES ASSOCIATED WITH A NEW VIRUS-LIKE DISEASE OF BARLEY. N.L. Robertson and T.W. Carroll, Dept. of Plant Pathology, Montana State University, Bozeman, Montana 59717.

Electron microscopic examination of plants having symptoms of a new virus-like disease of barley in North Central Montana, revealed morphologically unique virus-like particles (VLPs). The VLPs appeared to have diameters between 66-74 nm and variable lengths from 150 to 4,000 nm. Each particle seemed to be enveloped by a single membrane. The VLPs were detected in leaf, awn, sheath, and root tissue only from symptomatic plants. In leaves, the VLPs were seen to accumulate in large masses in the cytoplasm of mesophyll cells. Fewer VLPs were observed in xylem parenchyma and vessels, phloem parenchyma, sieve elements, companion cells, and rarely in epidermal cells. The VLPs appeared to be restricted to the cytoplasm and were never inside chloroplasts, mitochondria, or nuclei. However, maturation of VLPs seemed to be closely associated with the endoplasmic reticulum. Mature VLPs were contained individually or in small groups in cytoplasmic membraneous cisternae.

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Cytological responses of sorghum (Sorghum bicolor Moench) to infection with maize dwarf mosaic virus. C. W. Choi, W. S. Gardner and D. J. Gallenberg. Dept. of Plant Science, South Dakota State University, Brookings, SD 57007

Tissue from sorghum hybrid HOK plants exhibiting the primary acute phase of symptom development 5-6 days after inoculation with maize dwarf mosaic virus (MDMV) strain A was observed with the electron microscope for cytological modifications. Similarly, observations were made on tissue from sorghum hybrid Pioneer 8680 plants exhibiting chronic mosaic (systemic) symptoms 25 days after inoculation with MDMV strain B. Thin sections of tissue infected with either virus revealed numerous cylindrical inclusion bodies typical of the potyvirus group randomly scattered throughout the cells. Laminated aggregates were also observed in Pioneer 8680 tissue infected with MDMV-B but not in HOK tissue infected with MDMV-A. HOK tissue infected with MDMV-A did exhibit cell wall abnormalities, including thickenings and protrusions, associated with extended plasmodesmata, paramural bodies, and extraprotoplasmic sacs.

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DISTRIBUTION AND QUANTIFICATION OF MAIZE CHLOROTIC MOTTLE VIRUS; WITHIN TISSUE IN SINGLE INFECTIONS AND IN MIXED INFECTIONS WITH MDMV-B. C.A. Schmitt, Plant Pathology Dept., University of Nebraska, Lincoln, NE 68583-0722.

The concentration of maize clorotic mottle virus (MCMV) was previously shown to be up to five-fold higher in corn also infected with maize dwarf mosaic virus strain B (MDMV-B), than in singly infected plants. The concentration of MDMV-B was the same in mixed and single infections. Increased MCMV could arise from an increase in the number of infected cells or an increase in number of virions per cell. To distinguish between the possibilities and help explain the synergistic interaction of these two viruses, sections of fixed tissue were labelled with primary rabbit anti-sera IgG, followed by fluorescein tagged goat anti-rabbit IgG. Viral density in infected cells and percentage of infected cells in mixed and single infections was determined, using fluorescent microscopy.

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EVIDENCE SUGGESTING A VIRAL ETIOLOGY FOR LAFRANCE DISEASE OF THE COMMON MUSHROOM. C. P. Romaine, M. P. Wach, K. C. Koons, and B. Schlagnhaufer, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

The presence of double-stranded RNAs(dsRNAs) of MW 2.50, 2.05, 1.95, 1.85, 1.70, 1.10, 0.89, 0.58, and 0.53 x 10^6 in sporophores of Agaricus bisporus was associated with symptoms ascribed to LaFrance disease. Variation in this dsRNA pattern, which involved the deletion of lower molecular weight species and/or the addition of species, occurred between diseased sporophore isolates. The disease-related dsRNAs copurified with spherical virus-like particles in a protocol involving PEG-NaCl precipitation and differential, rate-zonal, and isopycnic centrifugation. The fact that these dsRNAs were labeled in vivo with ^3H uridine indicated that they are actively replicating in diseased mycelium. DsRNA patterns characterized by a predominant species of MW 1.60 x 10^6 prevailed in apparently healthy sporophore isolates. The findings suggest that specific virus(es) incite LaFrance disease and that other viruses are present which are not overtly pathogenic to A. bisporus.

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EVALUATION OF RESISTANCE OF CAPSICUM ANNUUM CULTIVARS TO A TOBAMOVIRUS ISOLATED FROM AEROPONICALLY GROWN PEPPERS IN FLORIDA Kristin G. Pategas and Andrew C. Schuerger, The Land, EPCOT Center, P. O. Box 40, Lake Buena Vista, FL 32830.

A mechanically transmitted virus was isolated from aeroponically grown "Hungarian Wax" pepper plants(Capsicum annuum). Preliminary host range studies, epidermal leaf strips and symptomatology distinguished it as a tobamovirus other than tobacco mosaic virus. Twenty-five pepper cultivars resistant to tobacco mosaic virus were screened under greenhouse conditions for resistance to this tobamovirus. No cultivar was immune. Initial reactions on all cultivars were necrotic local lesions followed by leaf abscission. Systemic symptoms ranged from leaf necrosis (moderate resistance) to plant death (highly susceptible). One cultivar, "Anaheim" (Royal Sluis, Holland), did not develop systemic symptoms. The following cultivars showed moderate resistance: "Bell Boy," "Cal Wonder 300," "Early Thickset," "Emerald Giant," "Resistant Giant #4," "Jupiter," and "Yolo Wonder B." Pepper cultivars "Golden Belle" and "Hungarian Wax" were the most susceptible.

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PROPERTIES OF CHERRY RASP LEAF VIRUS. R.N. Skopp and L.C. Lane. Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Several Nebraska weeds occasionally contained cherry rasp leaf virus (CRLV). Its host range was broad, but symptoms were indistinct and unreliable. Virus was purified from infected hosts grown at cool temperature by differential centrifugation in the presence of SDS. Yields were slightly less than for tobacco ringspot virus (TRSV). The virus cosedimented with TRSV bottom component and contained two RNAs similar in size to TRSV RNAs. It had 3 proteins in equimolar ratio weighing 19-22 Kd. A Scottish isolate was indistinguishable by immunodiffusion but had a distinct virion electrophoretic mobility and capsid proteins with distinct SDS gel mobilities. Despite the atypical protein composition, CRLV appears to be a T=1 icosahedron like other nepoviruses.

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PARTIAL CHARACTERIZATION OF AN ISOMETRIC VIRUS FROM GERANIUM Suzanne S. Hurtt and Ramon Jordan, USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville, MD 20705.

A virus isolated from Pelargonium x hortorum gave local lesions on mechanically inoculated Chenopodium quinoa in 5-7 days without subsequent systemic infection. The virus also infected Nicotiana clevelandii, N. benthamiana, N. debneyi, and cowpea. Extracts of all hosts contained two double-stranded RNA species of Mr = 3.0 and 1.0 X 10^6. Virus was extracted from inoculated leaves of C. quinoa or systemically-infected N. benthamiana in 0.05 M Tris buffer, pH 8, containing 10 mM $MgCl_2$, 1mM DTT, and 0.1% thioglycollic acid and precipitated with 10% PEG (final conc., w/v). Preparations were subjected to differential centrifugation and centrifugation on 10-40% sucrose density gradients. Viral fractions had a 260/280 of 1.7 and a minimum absorbance at 236 nm. Virus was degraded by most electron microscopic stains. Preparations did not react with antisera to pelargonium leaf curl, line pattern, flower break, or ringspot viruses or tobacco, tomato or carnation ringspot viruses.

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INFECTION OF MESOPHYLL PROTOPLASTS OF NICOTIANA CLEVELANDII WITH SWEET CLOVER NECROTIC MOSAIC VIRUS. H.R. Pappu and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Protoplasts, isolated from fully expanded leaves of 35 to 40 day-old Nicotiana clevelandii plants, were successfully infected with purified preparation of sweet clover necrotic mosaic virus (SCNMV) in the presence of poly-L-ornithine. About 80% of the protoplasts survived up to 36 hr in the incubation medium. Indirect immunofluorescence revealed 35 to 40 percent infection 24 hr after inoculation. At various time intervals after inoculation, protoplasts were disrupted in TNE buffer containing 2% SDS. Nucleic acids were extracted with water-saturated phenol/chloroform (1:1) and precipitated with ethanol. Nucleic acid samples from healthy and SCNMV-infected protoplasts were spotted onto nitrocellulose filters and probed with ^{32}P-labelled cDNA prepared to viral RNA by random priming. Synthesis of viral RNA was detected from 12 hr after inoculation and increased with time.

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PURIFICATION AND CHARACTERIZATION OF A GEMINIVIRUS ISOLATED FROM LIMA BEAN (Phaseolus lunatus L.).G. Sánchez; R. Lastra & E. Debrot, I.V.I.C., Caracas,Venezuela;C.A.T.I.E., Turrialba, Costa Rica; F.O.N.A.I.A.P., Maracay, Venezuela.

Inoculated lima bean "Henderson bush" with Lima bean golden mosaic virus were kept at 30ºC and a photoperiod of 16 h. Leaves were harvested 12-15 days after inoculation and macerated in phosphate buffer containing 0.1% Driselase and 0.01M DIECA. The virus particles were precipitated with 4% PEG followed by high speed centrifugation. Pellets were twice extracted with buffer containing.5% Triton X. A linear-log sucrose density gradient was used to purify the virus. The absorption spectrum of the viral band was typical of a nucleoprotein with a 260/280 ratio of 1.4. DNA extracted from the virus particles migrated as two bands (2500 and 2750 bases) in agarose-alkaline gels. The capsid protein migrated as a single band with a MW of 33.000 d. Large inclusions were observed by light microscope in phloem tissue,these inclusions are acumulations of viral particles locacated in the nucleus of phloem cells as revealed by E.M.

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ERADICATION OF PLRV FROM SOLANUM TUBEROSUM AND SOLANUM MURICATUM BY CHEMOTHERAPY OF AXILLARY BUDS. W.B. Jones, R. Locy, and P. Dyer. NPI, 417 Wakara Way, Salt Lake City, UT 84108

Ribavirin (virazole) was effective in eliminating PLRV from several PLRV infested Russet Burbank potato lines and the pepino cultivar Miski prolific. PLRV infested potato plantlets were established in vitro from tubers of PLRV infested plants. Pepino plantlets were established in vitro from infested greenhouse plants. The presence of PLRV was determined by ELISA. Plantlets were maintained on a 300 µM concentration of ribavirin incorporated into a standard agar based medium for shoot-tip culture for 3 (potato) and 5 (pepino) weeks. Plantlets were then placed in soil in a greenhouse and allowed to grow 24 days (potato) and 10 days (pepino). Lower leaves were taken and the virus status of the plantlets was determined. Fifty-three percent (31 out of 58) of the Russet Burbank lines tested free of PLRV and 5 lines were partially free. Twenty-one percent (10 out of 47) of the pepino plantlets tested free of PLRV. It would appear that ribavirin is a useful tool for ridding potato and pepino of PLRV.

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DETECTION OF VIRUS IN BAMBOO PLANTS FROM OKINAWA QUARANTINED IN HAWAII. T. L. German and J. Neill, Dept. of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Edible-bamboo plants (phyllostachys reticulata) imported from Okinawa and placed under quarantine developed chlorotic streaks and were shown to contain flexous rod shaped virus particles. The virus was characterized with respect to dimensions, DEP, TIP, symptom expression on indicator plants, nucleic acid content and coat protein properties. A dot blot hybridization procedure was developed to assay indigenous plants for the presence of this virus. Results of these tests will be presented.

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A POSSIBLE VIRAL DISEASE OF SHIITAKE MUSHROOMS IN THE U.S.A. K.L. Deahl, S.S. Hurtt & M. Miller, USDA, ARS, HSI, VL & FNCL Beltsville, MD 20705 & Lambert Spawn Co., Coatsville, PA 19320

A disease of unknown etiology occurred on Shiitake mushrooms (Lentinus edodes) at three commercial growing houses in Pennsylvania in 1985. The disease, characterized by abnormal growth and morphology of vegetative mycelia and basidiocarps, was found in 85% of the affected crops. Diseased sporophores exhibited a variety of symptoms, including dwarfing, early maturity, hardened gills, and thickened, elongated or barrel-shaped stipes. Detection of double-stranded RNA (dsRNA) in mycelial extracts treated with RNAase and DNAase after CF-11 cellulose chromatography suggested that the disease is of viral origin. After staining with ethidium bromide, two major bands were observed on 5% polyacrylamide gels. Estimated molecular weights for the dsRNA segments were 2.4 and 2.0 x 10^6 daltons. No particles were observed in electron microscope examinations of thin sections and leaf dips, and all attempts to purify the "virus" were unsuccessful.

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MONITORING OF POTATO CLONES WITH RESISTANCE TO POTATO VIRUS X (PVX) AND POTATO VIRUS Y (PVY) EXPOSED UNDER FIELD CONDITIONS IN PERU. C.E. Barrera, and E. N. Fernandez-Northcote. Universidad Nacional Agraria, La Molina, and International Potato Center, Apartado 5969, Lima-Peru, respectively.

Potato clones with known reaction to PVX and PVY were exposed in La Molina and Huancayo fields. Virus free tubers were planted initially. After four field exposures clones which are 'immune' to PVX and PVY were not infected by these viruses. However, clones with high hypersensitivity to PVY, showed 0-6% infection by PVY. A clone with low hypersensitivity to PVY and susceptibility to PVX showed 30 and 90% infection by PVY and PVX respectively. Independently of the level of resistance to PVX and PVY, potato virus S was more prevalent than potato leaf roll, andean potato latent and andean potato mottle viruses. Degeneration in susceptible controls was evident by their symptoms severity index (SI) and yield decrease (20-63%). Clones 'immune' to PVX and PVY showed low SI and yield decrease due to other virus infections. Myzus persicae, Macrosiphum euphorbiae, and Aphis sp., were the most important winged aphids detected.

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RESISTANCE TO WATERMELON MOSAIC VIRUS 1 STRAIN OF PAPAYA RINGSPOT VIRUS ACQUISITION BY MYZUS PERSICAE IN MELON. H. Lecoq*, J.M. Clauzel*, M. Jacquemond*, M. Pitrat** and J.B. Quiot***, INRA, Stations * de Pathologie Végétale and ** d'Amélioration des Plantes, B.P. 94, 84140 Montfavet France, and *** INRA-CIRAD, B.P. 5035, 34032 Montpellier, France.

When infected by a Watermelon Mosaic Virus 1 strain of Papaya Ringspot Virus (PRSV-W) melon line PI 161375 developped milder symptoms than a susceptible cv. and proved to be a significantly less efficient source of virus for Myzus persicae. Virus multiplication (measured by ELISA in leaves used for virus acquisition by aphids) was lower in the resistant than in the susceptible cv. The concentration of Amorphous Inclusion Protein (AIP) (a protein serologically related to Helper Component) was also estimated by ELISA : less AIP were found in the resistant than in the susceptible cv. In a F1 hybrid AIP were in low and virus in intermediate concentrations; however F1 plants were as efficient virus sources for aphids as the susceptible parent. Therefore resistance to virus acquisition by M. persicae seems to be a recessive character which is not simply related to virus or AIP estimated concentrations in the plants.

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A SURVEY OF VARIABILITY AMONG ISOLATES OF BARLEY STRIPE MOSAIC VIRUS. Michael C. Edwards and Roland G. Timian, USDA-ARS, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Approximately fifty isolates of barley stripe mosaic virus were examined to determine the extent of variability among them. RNAs were electrophoresed on 2.5% polyacrylamide/0.5% agarose gels in Tris-acetate buffer. Prior to electrophoresis, purified virus was heated at 75 C for 3 minutes in the presence of SDS and mercaptoethanol. Formaldehyde was then added to a final concentration of 2.2M and the sample heated at 75 C for an additional 10 minutes. Of the isolates examined, 30 had 3 electrophoretically distinguishable RNAs, 20 had 2, and 1 had 4. Coat proteins of the isolates were compared by electrophoresis on 10% SDS gels. Virus preparations were heated to 98 C for 5 minutes in the presence of SDS and mercaptoethanol prior to electrophoresis. While most isolates' coat proteins were of nearly identical moelcular weight, 3 were significantly smaller. Differential host reactions were also compared.

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SOME PROPERTIES OF BEETLE-TRANSMITTED VIRUSES THAT MAY EXPLAIN THEIR TRANSMISSIBILITY. R. C. Gergerich, H. A. Scott and J. P. Fulton, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Ribonuclease in the regurgitant of leaf-feeding beetles has been implicated as the factor responsible for prevention of transmission of most plant viruses. Beetle-transmissible viruses can move through zones of leaf tissue killed by sodium azide or through steam-killed stem sections and cause infection in non-wounded tissues. Non-beetle-transmissible viruses either did not move through killed tissues or did not infect non-wounded tissue. Only beetle-transmissible viruses were detected in the primary leaves and growing point of plants that had been excised at the base and placed in vials of purified virus for two hours. An essential characteristic of beetle transmissible viruses may be their ability to move away from the damaged area of the feeding site and to set up infection at a distant unwounded site where ribonuclease is not present.

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BIOLOGICAL CONTROL OF TAN SPOT OF WHEAT WITH FOLIAR APPLIED BACTERIA CULTURES. B.L. Norman and W.W. Bockus, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Over 100 bacteria isolates collected from 20 sources including plant roots, soil, and plant refuse were tested in a greenhouse bioassay for suppression of tan spot Pyrenophora tritici-repentis (Died.) Drechs. The isolates were grown in stationary broth cultures for 3-5 days at 21±3 C. Broth containing live cells was subsequently sprayed onto 4-5 leaf stage TAM 105 wheat. After drying, the leaves were inoculated with a spore suspension of the tan spot fungus and placed in a

mist chamber for 48 hr. Lesion severity was rated about 6 days after the mist treatment. Twenty-seven bacteria isolates reduced disease severity scores by 30% to 87%. In similar tests Mancozeb fungicide suspensions (1000 ppm) inhibited severity by about 35%. Suppressive bacteria isolates were also strongly inhibitory to growth of the tan spot fungus in vitro. Field trials and other tests using culture filtrate and/or live cells are currently under way.

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A DEGENERATIVE DISEASE OF LEUCOSTOMA CINCTA, THE CAUSAL AGENT OF CYTOSPORA CANKER OF PEACH. S.A. Hammar, D.W. Fulbright, and G.C. Adams. Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824.

A nonsporulating fungus was isolated from a North Carolina peach tree canker and tentatively identified as Leucostoma cincta on the basis of cultural morphology. This isolate appears to have a degenerative disease characterized by abnormal growth rate, swollen hyphae, lysis of hyphal tips, reduced or absent conidiomata, and reduced virulence. The degenerative disease is associated with double-stranded RNA (dsRNA) as determined by CF-11 chromatography and analyzed by polyacrylamide gel electrophoresis. Isolates containing dsRNA induce significantly smaller cankers in apple fruit and peach stem sections as compared to non-dsRNA infected field isolates. Less degenerated strains derived from hyphal transfer of the infected isolate have fewer dsRNA bands in gel electrophoresis and decreased dsRNA concentrations. These strains also show increased virulence.

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COLONIZATION OF CUCUMBER ROOTS BY BACTERIAL ANTAGONISTS IN CONTAINER MEDIA AMENDED WITH COMPOSTED HARDWOOD TREE BARK. O. C. H. Kwok, P. C. Fahy and H. A. J. Hoitink. Dept. of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691 and BCRI, NSW Dept. of Agric. PMB10, Rydalmere, Australia.

Movement of spontaneous rifampicin-resistant mutants of Flavobacterium balustinum, Pseudomonas putida and Xanthomonas maltophilia, that suppress Rhizoctonia and Pythium damping-off, was followed on cucumber roots in CHB media from seeds coated with the antagonists (10^7 CFU/seed). All three mutants colonized entire root systems (10^7 and 10^4 CFU/cm root near the seed and root tip, respectively) in autoclaved CHB media that were not irrigated after tests were set up. In media prepared with high temperature conducive compost, colonization was less extensive (10^{3-5} CFU/cm root). In suppressive CHB media mutants colonized only the first 4 cm of roots below the inoculated seed. Mutants were detected in container media around seeds but not in other areas of pots. Irrigation distributed mutants thoughout the container medium. Mutants persisted at least 45 days after seeding.

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OBSERVATIONS ON MORPHOLOGICAL AND ULTRASTRUCTURAL CHANGES IN MICROSCLEROTIA OF CALONECTRIA CROTALARIAE DUE TO FREEZING. E.P. White and G.T. Berggren, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, La., 70803.

Calonectria crotalariae (Loos) Bell & Sobers, which causes Red Crown Rot in soybeans, overwinters in Louisiana fields. Microsclerotia(ms), which overwinter in soil and plant debris are reported to be the primary propagules for survival and dispersal. While freezing lowers ms germination levels, incubation at 26C for one month following freezing promotes an increase in the previously depressed germination levels. Observations using scanning and thin section electron microscopy will be presented showing changes in the morphology of the ms which occur during the freezing and incubation process and changes in the ultrastructure of the ms cells.

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CYANINE POTENTIOMETRIC PROBES FOR MITOCHONDRIAL ACTIVITY IN HAUSTORIA OF ERYSIPHE GRAMINIS. Wm. R. Bushnell[1], Z. Liu[1], and Kurt Mendgen[2]. 1) USDA-ARS, Cereal Rust Laboratory, Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108; 2) Faculty of Biology, Univ. of Konstanz, D-7750 Konstanz, W. Germany.

The fluorescent cyanine dyes, $DiOC_4(3)$ and $DiOC_7(3)$ at 1×10^{-6} M were taken up by haustoria of Erysiphe graminis f. sp. hordei in living epidermal cells of barley coleoptiles. Mitochondria were visible within 15-30 min by epifluorescence microscopy as fine, filamentous threads in the central body and fingers of haustoria. $DiOC_7(3)$ was specific for mitochondria; $DiOC_4(3)$ sometimes also stained unidentified globular organelles. Mitochondrial fluorescence could be monitored in individual haustoria with a microscope photometer. Fluorescence was lost in 5-10 min after host tissues were treated with DNP (1×10^{-4} M) or CCCP (1×10^{-6} M) indicating that mitochondrial fluorescence was dependent on the potential across the mitochondrial membrane. The probes offer a new method to monitor respiratory activity of haustoria in situ.

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BIOLOGY, SPORE DISPERSAL AND CONTROL OF RHYNCHOSPORIUM SECALIS (OUD.) DAVIS, CAUSAL AGENT OF BARLEY LEAF BLOTCH. Carvajal, M. Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México. Apartado Postal 70-233. Delegación Coyoacán, 04510 México, D.F.

A study has been made of some factors which influence infection and epiphytic development of Rhynchosporium secalis, the fungal pathogen of barley that causes leaf blotch. Infective barley stubble and seed from previous crop as sources of primary infection has been demostrated. The rain criteria for splash distribution of inoculum was precipitation of at least 2 mm per hour. The infection ocurred from 1°C with 8 hours of leaf surface wetness and 70% relative humidity follow spore deposition. The life cycle, effects of weeding and sown density, the mexican race of R. secalis and chemical control were studied as well.

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ANTAGONISM OF COCHLIOBOLUS SATIVUS TOWARDS PYRENOPHORA TRITICI-REPENTIS ON WHEAT LEAVES. W. C. da Luz and G. C. Bergstrom. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Following mixed inoculation with conidia of Cochliobolus sativus (incitant of spot blotch) and Pyrenophora tritici-repentis (incitant of tan spot), wheat leaves developed necrosis attributable mostly to C. sativus. Spot blotch also predominated over tan spot following sequential inoculations where P. tritici-repentis preceded C. sativus by up to 6 hr. Antagonism occurred even when mixed inocula contained only 20% C. sativus. C. sativus inoculation resulted in reduced conidium germination, slowed germ tube development, and reduced appressorium formation by P. tritici-repentis. C. sativus was also inhibitory to the mycelial growth of P. tritici-repentis in culture. Tan spot development may be suppressed in the field where spot blotch is favored by a conducive environment. Perhaps C. sativus or its metabolites could be manipulated to produce an effective biological control for tan spot of wheat.

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THE BIOLOGY AND EPIDEMIOLOGY OF PUCCINIA ALLII (DC) RUD. ON ALLIUM TUBEROSUM ROTTLER (CHINESE CHIVE). *M.L. So, Biology Department, Hong Kong Baptist College, 224 Waterloo Road, Hong Kong.*

Puccinia allii (porri) (DC) Rud, infects a number of Allium species in most fields in Hong Kong. These species include A.ascalonicum L. (Shallot), A.fistulosum L. (Chinese Onion), A.sativum L.(Garlic) and A.tuberosum Rottler (Chinese Chive). However, no spermagonial or pycnial stages had ever been observed throughout the year (10-35°C). Uredial and telial stages could be found at the same time and telial stage arose not because of low temperatures or ageing of the leaf. All varieties of Chinese Chive are susceptible to the parasite. Infection depends very much on the wind pattern and availability of landing surfaces on the leaves. Infection on leaves was not related to leaf age. Other factors such as soil fertility weather, wind pattern and rainfall all affect disease development.

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FACTORS INFLUENCING DEVELOPMENT OF SCLEROTINIA TRIFOLIORUM APOTHECIA IN VIRGINIA. K. L. Reed, E. L. Stromberg and S. W. Van Scoyoc, Dept. of Plant Pathol., Physiol. & Weed Sci. and Dept. of Agronomy, VPI & SU, Blacksburg, VA 24061.

Ascospores of Sclerotinia trifoliorum constitute the primary inoculum for Sclerotinia crown and stem rot of alfalfa. Microenvironmental factors influencing the development of apothecia from sclerotia were investigated in field microplots. The microplots were infested with sclerotia at two-week intervals from August through December 1984 and from October through December 1985. Sclerotia were observed for apothecium development three times weekly. Microenvironmental factors measured on-site and recorded hourly included: air and soil temperatures, relative humidity, rainfall, duration of leaf wetness, solar radiation, and wind speed and direction. Sclerotia produced apothecia from November 1984 through March 1985, with the greatest number appearing in December, and from November 1985 through January 1986, with greatest number appearing in November.

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EFFECT OF WATER STRESS ON THE SEVERITY OF PHYTOPHTHORA ROOT ROT IN TOMATO. Jean Beagle-Ristaino and J. M. Duniway, Dept. of Plant Pathology, University of California, Davis, CA 95616.

The effect of water stress on Phytophthora root rot in processing tomato cv 6203 was evaluated in growth chamber experiments. Three-wk-old tomato seedlings were either inoculated with zoospores of Phytophthora parasitica or left uninoculated. Plants were water stressed by witholding water until leaf water potential reached -9 bars prior to inoculation, after inoculation, or were watered regularly to maintain leaf water potentials between -3 and -4 bars. Incidence of damping-off and root rot severity were rated 7 days after inoculation. Plants subjected to water stress prior to or after inoculation had a 30% and 33% incidence of damping-off and root rot severity ratings of 3.3 and 3.0, respectively. Inoculated but nonstressed plants had root rot severity ratings of 2.2 to 2.6 and less than 2.5% damping-off. Nonstressed or stressed and noninoculated plants had no damping-off and no root rot symptoms. In conclusion, water stress either before or after inoculation increases the severity of Phytophthora root rot.

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VARIATION IN MACROPHOMINA PHASEOLINA (TASSI) GOID. IN DIFFERENT HOSTS AND CONDITIONS OF WATER STRESS. M. Diourte, J. P. Stack, and M. J. Jeger. Dept. Plant Path. & Microbiol., Texas Agri. Exp. Sta., College Station, TX 77843

Variation in pycnidia was found among isolates of M. phaseolina from cotton (Mp9), pecan (Mp6), and peanut (Mp14, Mp4A). Length:width ratios ranged from 1.48:1.58 and 1.89:2.27 for pycnidia and pycnidiospores, respectively. Cotton, peanut, and sorghum plants were grown in nonsterile soil infested with microsclerotia of Mp9, Mp14, or a sorghum isolate Mp16. There was variation in assessed plant stress due to the Mp isolates; symptoms were more severe on plants in Mp16-infested soil than on plants in Mp9 or Mp14 soil or in noninfested soil. There was no difference between plants in infested or noninfested soil watered daily. Plants in infested soil watered every 3-days showed more stress. Number of root lesions did not vary with Mp isolate, but more lesions occurred on plant roots watered at 3-day intervals. Frequency of isolation of Mp from root lesions, although low, was greater in plants watered daily.

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DISEASE GRADIENTS OF SUGARCANE SMUT. M. T. Momol, R. A. Schmidt and L. H. Purdy. Dept. of Plant Pathology, University of Florida, Gainesville, FL.

The sugarcane cultivar, CP 75-1091, that is susceptible to smut (caused by Ustilago scitaminea Syd.) was planted in the field in Southern Florida. To characterize disease gradients, a 122-m long line source was established and disease incidence (percentage of infected plants) was monitored for two years (plant cane and first ratoon). Incidence of smut which developed 1.5, 3.0, 7.6, 13.7, 22.9 and 29.0 m from the line source was fitted to seven transformation equations. Gregory's model [$\log_{10}(y)$ vs $\log_{10}$(distance)] provided the best fit to the data. Using Gregory's transformation, slopes became flatter over time with slope coeficients of -0.75, -0.45, -0.16, -0.16 and -0.09 for October/1984, April, June, July and October/1985, respectively. The Logit(y) vs $\log_{10}$(distance) transformation also gave a good fit with flatter gradients over time. Based on the primary gradient, disease spread occured up to a distance 25-30 m from a line source of inoculum.

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INOCULUM POTENTIAL OF PHYTOPHTHORA CINNAMOMI AND P. PARASITICA IN PINEAPPLE ROOT ROT. **J. K. Tomita, K. G. Rohrbach, and M. Aragaki. University of Hawaii Honolulu, Hawaii 96822.**

A technique was developed for laboratory inoculations of individual pineapple roots. In one modification, direct root inoculations with single zoospores of P. cinnamomi or P. parasitica resulted in infection frequencies of 10% and 20% respectively. In a second modification, individual roots were covered with 5 grams of soil in longitudinally divided PVC pipes and inoculated with concentrations of $2x10^2$ zoospores/gram of soil, 10% infection of roots were obtained with each. At $2x10^4$ zoospores/gram of soil for P. parasitica, 10% of the roots were infected in 12 hours and by 48 hours 80% were infected. At $2x10^4$ zoospores/gram of soil, 90% and 70% of roots from untreated crowns were infected by P. cinnamomi or P. parasitica respectively, and 5% infection by either species was obtained with crowns treated with Aliette. This method will provide a rapid assay for pineapple root rot development which should prove useful in studies of epidemiology, disease resistance or chemical control.

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MELAMPSORA STEM CANKERS AND FUNGICIDE CONTROL ON DOUGLAS-FIR. Gary A Chastagner, John M. Staley, and Ralph S. Byther. Washington State University, Puyallup, WA 98371.

During June 1984, stem cankers, caused by Melampsora occidentalis, severely damaged developing shoots on Douglas-fir Christmas trees. Lesions frequently developed at the bases of heavily rusted needles, which later dropped off. Mycelia with conspicuous orange colored oil drops were seen in sections through stems with lesions. Pycnia and aecia were produced on a few lesions. Development of lesions into cankers distorted or killed entire shoots making trees unmarketable. Highest levels of needle infection and stem cankers developed on trees closest to overwintered cottonwood leaves with telia. Rust resistant trees among severely cankered trees did not develop cankers. An application to new shoots (3 cm in length) of triadimefon, triforine, ziram, ferbam, bitertanol or propiconazol reduced the incidence of diseased needles by at least 88%. Mancozeb, anilazine, zineb, fenarimol, oxycarboxin, and chlorothalonil reduced disease incidence by 63-76%. Controlling needle infection prevented the formation of stem cankers.

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BROWN PANEL CANKER OF RUBBER IN JAMBI PROVINCE, INDONESIA. K.J. Jones and Nezryetti, Universitas Jambi, Telanaipura, Jambi, Indonesia.

A large proportion of rubber trees (Hevea brasiliensis) in Jambi province, Indonesia, are affected by a Brown Panel Canker. The renewing bark on the tapping panel becomes dry and lumpy, with disorganization of lactiferous tissues and phloem. This results in the inability to retap the panel which reduces the yield and shortens the productive life of the affected trees considerably. A Fusarium sp. was consistently isolated from affected trees at three locations. The Fusarium was shown to produce cankers and localized growth abnormalities when inoculated onto 1 year old rubber trees in pots in the greenhouse.

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HISTOPATHOLOGY OF COLLETOTRICHUM TRIFOLII ON ALFALFA. A.C.L. Churchill[1], C.J. Baker[2], N.R. O'Neill[3], and J.H. Elgin, Jr.[3], [1]Dept. of Botany, Univ. of Maryland, College Park, MD 20742, [2]MPPL and [3]GQEL, USDA-ARS, Beltsville, MD 20705.

The symptomatology and development of individual races of Colletotrichum trifolii were observed on alfalfa clones derived from the cultivar 'Arc'. Plants were spray-inoculated with a single race 1 or race 2 isolate in compatible and incompatible combinations. By 5 days post-inoculation, lesions were visible along stems of susceptible clones. Resistant plants showed only occasional pinpoint yellow discoloration on the leaves. Immature appressoria were most evident by 7 to 12hr post-inoculation, and by 16hr, 86% of the spores examined had formed pigmented appressoria. Penetration pores were visible in appressoria as early as 7hr but were most evident by 16hr. There was no significant difference among resistant and susceptible treatments in immature and mature appressorium formation or in percentage of appressoria that formed penetration pores. This suggests that expression of alfalfa resistance to C. trifolii occurs after fungal penetration of the epidermis.

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THECAPHORA CUNEATA (SCHOF.) CLINT. ON GRINDELIA CAMPORUM GREENE. IN ARIZONA. P. T. Rotkis, S. M. Alcorn, S. McLaughlin, R. L. Gilbertson, Dept. of Plant Pathology, University of Arizona, Tucson, AZ. 85721

Grindelia camporum (a potential comercial source of terpenes and resins) has been grown in experimental plots near Tucson since 1980. In October 1983, Thecaphora cuneata was observed in distorted inflorescences of over half the tetraploid and diploid plants examined. Infected and non-infected plants were similar in size and appearance; only a small number of flower heads on each infected plant were affected. The smut disease occurred again in both diploid and tetraploid plants in August of 1984. In a 1985 field planting, T. cuneata was found in plants grown in a new area from seeds collected from infected 1984 lines but not in plants grown from seeds collected from a non-infected 1984 line. T. cuneata also was found in plants grown from the non-infected line if seeds were sown in infested soil. This is the first report of this disease on G. camporum in Arizona.

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EFFECTS OF COTTON-LEAF DERIVED VOLATILES ON THE GROWTH OF ASPERGILLUS FLAVUS. H. J. Zeringue, Jr., and Susan P. McCormick, USDA/ARS, Southern Regional Research Center, P. O. Box 19687, New Orleans, LA 70179.

Microbial-free compressed air was passed continuously for 2- or 7-day test periods through an enclosed system containing wounded or non-wounded leaves of glanded or glandless cotton; the resultant emitted volatiles were bubbled through liquid cultures of A. flavus. After 2 days incubation, wounded glanded and wounded glandless cotton leaves volatiles retarded the growth of A. flavus when compared to controls. After 7-day incubation, there was a stimulation of fungal growth in cultures which received volatiles from wounded or non-wounded glanded cotton leaves when compared to controls. Volatile profiles of the leaves were obtained by GC/MS on the leaves at 2- and 7-day time periods. Purified compounds of the major identified volatiles were assayed with A. flavus to determine which of the volatiles might be responsible for the bioactivity described. Results of the assays will be presented and discussed.

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STRESS METABOLITES FROM A FUNGAL-COTTON OVULE INTERACTION. J. E. Mellon and S. McCormick, USDA, ARS, Southern Regional Research Center, P. O. Box 19687, New Orleans, LA 70179

A study involving the inoculation of culture-derived cotton ovule tissue with Aspergillus flavus revealed a pronounced hypersensitive reaction. A study was initiated to determine if the metabolic response of developing ovule tissue to fungal-induced stress included the production of antimicrobial metabolites. Cotton ovule cultures derived from glanded and glandless cotton ('Stoneville 208 7A' cultivars) were initiated and maintained using standard techniques, inoculated with A. flavus, and incubated at 30°C for 6 days. The stress metabolites were extracted from the inoculated ovule tissue with 85% aqueous acetone. Preliminary analysis by thin layer chromatography indicated the presence of antimicrobial lacinilene-type and gossypol-type compounds, in addition to scopoletin. Non-inoculated control tissue contained very low levels of these stress metabolites. Structure verification and quantitation of the isolated stress metabolites will be presented.

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THE EFFECT OF GLYPHOSATE ON RESISTANCE OF TOMATO TO FUSARIUM CROWN AND ROOT ROT DISEASE. R.A. Brammall and V.J. Higgins. Dept. of Botany, Univ. of Toronto, Toronto, Ontario M5S 1A1.

Sub-lethal exposure to the herbicide glyphosate (1.0 mM for 24h prior to inoculation) induced susceptibility to Fusarium oxysporum f.sp. radicis-lycopersici in the resistant cultivars CR6 and Larma. Ultrastructural examination of glyphosate-treated, inoculated root tissues revealed a marked change in the cell wall modifications normally induced in the resistant host. Papillae which formed following glyphosate exposure were composed primarily of callose and lacked the inclusion of conspicuous electron-opaque material normally associated with these structures. Similarily, electron-opaque material was no longer deposited on cortical cell walls at sites adjacent to intercellular hyphae. A decrease in phenolic substances at these sites was confirmed by red rather than blue staining with Toluidine blue O and by the loss of autofluoresence. Depression of aromatic amino acid synthesis, the presumed primary mode of action of glyphosate, may explain the loss of host resistance.

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INDENTIFICATION OF THE FESCUE ENDOPHYTE (ACREMONIUM COLNOPHIALUM) WITH PROTEIN A SANDWICH ELISA. B.B. Reddick and M.H. Collins. Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN. 37901

The direct double sandwich enzyme-linked immunosorbant assay (DS-ELISA) was compared to indirect protein A sandwich ELISA (PAS-ELISA) with antiserum made against protein fractions of the fescue endophyte (Acremonium coenophlialum). Higher absorbance readings at higher antigen dilutions, and lower background absorbance readings at lower antigen dilutions were obtained with PAS-ELISA than with the DS-ELISA method. Optimun conditions for PAS-ELISA will be discussed.

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INVOLVEMENT OF A TOXIN IN THE SYMPTOMATOLOGY OF TAN SPOT OF WHEAT. A. Tomas, W.W. Bockus and J.E. Leach, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Stationary 14-day-old culture filtrates of Pyrenophora tritici-repentis (Pt) were infiltrated into leaves using a Hagborg device. Filtrates were concentrated 5-fold in a rotary evaporator and diluted 0, 1:2, 1:4, and 1:8 before use. Three to 5 days after treatment, infiltration sites were rated as 0, no symptoms; 1, faint chlorosis; 2, marked chlorosis, slight necrosis; 3, marked necrosis with or without yellow halos; 4, extensive chlorosis and necrosis; 5, collapse of tissue ahead of inoculation site. When infiltrated, leaves of TAM 105 (susceptible) and Red Chief (resistant) wheat and Larker barley (highly resistant) produced values of 2.7, 0.5, 0.1 respectively at the 0 dilution rate; 2.3, 0.6, 0.0 at 1:2; 1.5, 0.3, 0.0 at 1:4; and 1.0, 0.0, 0.0 at 1:8. Pt appears to produce a toxic metabolite in vitro which mimics the disease symptoms and susceptible/resistant reactions obtained with the fungus itself.

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USE OF ANTIBODIES AGAINST STEMPHYLIUM BOTRYOSUM PHYTOTOXIN TO COMPARE PHYTOTOXINS FROM OTHER FUNGAL SPECIES. D. K. Heiny and D. G. Gilchrist, Department of Plant Pathology, University of California, Davis, California 95616.

A host-nonspecific phytotoxin (PT) causing local necrosis is produced by the cool-temperature biotype of Stemphylium botryosum Wallr. pathogenic on alfalfa. Purification of culture filtrates by gel filtration, chromatofocusing, HPLC ion exchange, non-denaturing electrophoresis, or isoelectric focusing fails to separate three major polypeptide bands from each other in toxic fractions visualized by SDS-PAGE. Immunogammaglobulins produced in response to individual polypeptides or a PT preparation containing all polypeptides detect all three bands present in PT on Western blots. Antibodies purified against the 27Kd polypeptide by affinity blot techniques detect all three bands, indicating common antigenic determinants among the polypeptides. A few other fungi, including saprophytic isolates and species pathogenic on other hosts, produce PT with polypeptides that react with antibodies against S. botryosum PT.

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THE OCCURRENCE OF RED CROWN ROT IN LOUISIANA. D. K. Berner, G. T. Berggren, J. P. Snow and M. E. Pace, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803.

Red crown rot (Calonectria crotalariae (Loos) Bell and Sobers) was first discovered on soybeans in Louisiana in 1976 in St. John the Baptist parish. Since then the disease has been reported in an ever increasing range and is now reported in 16 of Louisiana's soybean producing parishes. Overwintering microsclerotia densities as high as 98/g soil are found in severely infested fields. In these fields yield loss estimates range as high as 50%. Fungicides have proven ineffective and control has been limited to non-host crop rotations. Recent investigations have indicated that delayed planting and/or the use of cultivars of maturity groups VII and VIII provide a means of greatly reducing disease pressure.

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GROWTH AND DAMPING-OFF RESPONSE IN DOUGLAS-FIR AND MICROBIAL SHIFTS RESULTING FROM HUMIC AMENDMENTS TO NURSERY SOILS. D. A. Schisler and R. G. Linderman, Dept. of Botany and Plant Pathology, Oregon State University, and USDA-Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, Oregon 97330

Fusarium spp. are conspicuously absent from coniferous forest soils, yet frequent conifer nursery soils. To test the hypothesis that loss of humus from nursery soils may affect Fusarium spp. survival, 3 nursery soils were amended with 4 levels of 3 organic materials high in humic content. Humic-induced increases and occasional decreases in tree growth varied with soil origin. A humic amendment that stimulated tree growth in all soils also increased numbers of several soil microbial groups (total bacteria, actinomycetes, chitinase producers, and facultative anaerobes). Fusarium- and Pythium-induced damping-off declined in 1 of 3 soils for all amendments. Ectomycorrhizae were increased by only 1 amendment and then in only 1 soil. The complex nature of soil-humic interactions and the physiological action of these substances on roots and microbial cells complicates predicting the efficacy of humic amendments to nursery soils.

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ASSOCIATION OF A VIRUS-LIKE PARTICLE WITH A DISEASED ISOLATE OF LEUCOSTOMA CINCTA, THE CAUSAL AGENT OF CYTOSPORA CANKER OF PEACH. B.A. Snyder, G.C. Adams, and D.W. Fulbright, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824.

Hyphae of a fungal isolate tentatively identified as Leucostoma cincta taken from a peach tree canker in North Carolina, were compared to an isolate of Leucostoma taken from a canker in a Michigan orchard using transmission electron microscopy. Cultures of the North Carolina isolate differ from the Michigan isolate in pycnidial formation and in the production of enlarged swollen hyphae and hyphae with bulbous tips. An ultrastructural examination of this isolate revealed the presence of a virus-like particle (VLP) in high concentration within the fungal cytoplasm. The VLPs are 32.0 $\pm$ 3.0 nm in diameter, frequently exhibit an electron dense core and are surrounded by a capsid. Nuclei, mitochondria and lipid droplets occur in relative abundance in the infected hyphae although there is a paucity of other organelles.

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A NEW VIRUS DISEASE OF EUSTOMA (LISIANTHUS). D. E. Mayhew and M. E. Sorrell. Analysis and Identification Unit, California Department of Food and Agriculture, 1220 N Street, Sacramento, CA 95814.

Eustoma Russellianum plants showing symptoms of stunting, vein clearing, yellow mosaic, and flower color break, were found to be infected with a mechanically transmissible isometric virus. The virus, found in high concentration in leaf tissue, measures approximately 28 nm. and has a limited host range. Virions aggregate in the cytoplasm and nuclei of infected leaf parenchyma cells, and are closely associated with the outer membranes of mitochondria and chloroplasts. This virus is not related to cucumber mosaic, the only other known virus infecting members of the Gentianaceae.

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TOBACCO ETCH VIRUS-INDUCED FLOWER COLOR BREAKING IN FLOWERING TOBACCO. P. R. Desjardins, P. J. Sasaki, R. J. Drake and S. A. Swiecki, University of California, Riverside, California 92521.

Tobacco etch virus was shown to be the sole causal agent of flower color breaking in naturally infected Flowering Tobacco. The virus was identified by cytological studies in the light and electron microscopes, by serological studies, by symptomatology in certain hosts and by electron microscopy of purified virus. In the cytological studies in the electron microscope nuclear and cytoplasmic inclusion bodies typical of etch virus were observed. When reinoculated into the host, the isolated, purified virus caused the complete disease. In Southern California, Flowering Tobacco will bloom two successive years if cut back after flowering the first year. In two different locations the disease did not appear until the second year suggesting that insect vectors were playing a role in the introduction and spread of the virus.

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REACTION OF BAHAMIAN HOT PEPPER TO SINGLE AND DOUBLE INFECTION WITH TOBACCO MOSAIC VIRUS (TMV) AND POTATO VIRUS Y (PVY). J. L. Sherwood[1], B. B. Reddick[2], and K. E. Conway[1], Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078[1,3], and Entomology and Plant Pathology Department, University of Tennessee, Knoxville, TN 37901[2].

Field grown Bahamian hot pepper (Capsicum annum L.) from Bixby, OK showed mild stunting and leaves with mild mosaic, or severe stunting and leaves severely chlorotic to necrotic. TMV or PVY was found in plants with the milder symptoms. TMV and PVY were found in plants with severe symptoms. TMV was identified by indicator host, leaf dip electron microscopy, and agar double diffusion tests (ADDT). PVY was identified by indicator host, serologically specific electron microscopy and ADDT. Bahamian hot pepper inoculated with TMV and/or PVY showed symptoms similar to those found in the field. TMV or PVY resulted in stunting and mosaic. TMV and PVY resulted in severe stunting and systemic necroses. A synergistic interaction between TMV and PVY in Bahamian hot pepper is indicated.

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MULTIPLE GENES IN COWPEA CONTROL NONNECROTIC RESISTANCE TO SOUTHERN BEAN MOSAIC VIRUS. H. A. Hobbs, C. W. Kuhn, and K. E. Papa, Dept. of Plant Pathology, Univ. of Georgia, Athens 30602.

Individual plants of F_1, F_2, and F_3 progeny of crosses between three cowpea genotypes resistant to southern bean mosaic virus and a susceptible genotype were evaluated for virus accumulation and symptoms. Virus accumulation was determined by absorbance readings at 260 nm after ultracentrifugation. The moderate resistance of Early Pinkeye (100-150 μg of virus/g of leaf tissue) was conferred by a single gene with partial dominance for resistance. Resistance in Iron (10-50 μg/g) appeared to be controlled by at least three genes with incomplete dominance. The resistance of extremely resistant PI 186465 (0.001-0.1 μg/g) was apparently controlled by one gene with partial dominance for resistance; however, minor genes also appeared to be operating and affecting virus accumulation and symptoms. We speculate that genes in Early Pinkeye and Iron are controlling virus replication and the major gene in PI 186465 is controlling virus movement.

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OCCURRENCE OF VIRUS-INDUCED FRUIT NECROSIS OF TOMATOES IN JORDAN. Mani Skaria, Abeer A. Hyassat, and Mohammed M. Khudair. JVASP (USAID-Washington State University), Ministry of Agriculture, P.O.B. 2099, Amman, Jordan.

Severe necrosis on tomato fruits was noticed in certain cultivars grown under plastic houses in the Jordan Valley. So far, tomato fruit necrosis has not been reported from Jordan. Some infected plants showed mild mosaics on leaves. Host range study showed necrotic local lesions on Chenopodium quinoa, Nicotiana glutinosa, N. rustica, and N. tabcum hybrid 4-5 days after inoculation, whereas beans and tomatoes showed no leaf symptoms even after 4 weeks. The dilution end point in water was between 10^3-10^4. N. rustica plants inoculated with higher virus concentration (10^0 and 10^1) developed systemic infection and the plants died after three weeks. The thermal inactivation point was between 85-90C when the sap was heated for 10 minutes.

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VARIABILITY AMONG STRAINS OF SWEET POTATO FEATHERY MOTTLE VIRUS. J. W. Moyer, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

This investigation was conducted to evaluate the reliability of accepted indexing procedures for sweet potato feathery mottle virus (SPFMV). Nine isolates of SPFMV from Africa, Asia, Central and South America and the United States were evaluated for their host ranges, symptoms on indexing hosts and their serological relationships. One isolate was limited to the Convolvulaceae, all other isolates induced local lesions on one or more Chenopodium spp. and two isolates also caused a mosaic on Nicotiana benthamiana. Symptom expression by Ipomoea setosa and sweet potato clone W, which are standard indexing hosts, was highly variable; two isolates could only be detected symptomatically under optimal environmental conditions. All isolates were serologically related, with eight of the nine isolates determined to be closely related by ELISA. This study indicates that variability in results of standard indexing procedures may be due to differences in virus strains.

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Response of selected sorghum (Sorghum bicolor Moench) hybrids to inoculation with maize dwarf mosaic virus strains A and B. C. W. Choi, D. J. Gallenberg and W. S. Gardner. Department of Plant Science, South Dakota State University, Brookings, SD 57007

Plants of twenty sorghum hybrids were mechanically inoculated in the greenhouse separately with maize dwarf mosaic virus (MDMV) strains A and B and observed for symptom development and type. MDMV-A generally induced systemic mosaic symptoms,

although a few hybrids also exhibited local symptoms. MDMV-B generally induced local necrotic symptoms, although two of the 20 hybrids exhibited reactions simular to those of MDMV-A, and five hybrids produced no reactions following inoculation with MDMV-B. Thirty eight additional hybrids were inoculated and the severity of symptom development rated on a scale of 0-3 for each virus strain. As expected, the general severity of symptoms increased with time. Final proportions of plants exhibiting symptoms ranged from 30-100% for MDMV-A and 0-95% for MDMV-B among the 58 hybrids.

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CITRUS VIRUS AND VIRUS-LIKE DISEASES IN THE JORDAN VALLEY. Mani Skaria and Sami F. Batarseh, JVASP (USAID-Washington State University), Ministry of Agriculture, P.O.Box 2099, Amman, Jordan.

A survey of virus and virus-like diseases of citrus was initiated as part of a program towards the production of disease-free planting materials. Indexing based on symptomatology, use of indicator plants, and/or culture shows the presence of citrus stubborn caused by Spiroplasma citri on sweet oranges, grape fruit, and pummelos. Exocortis was found on citrons and lemons, and infectious variegation on lemons. Psorosis was found on sweet oranges and grapefruit, and xyloporosis on sweet orange. The status of tristeza in Jordan is not yet confirmed. Sour orange is the root stock used in 95% of the total 5200 hectare citrus plantings, therefore, the presence of aphid vectors warrants routine indexing for Tristeza. Current surveys have failed to find greening disease or its vectors.

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COWPEA SOUTHERN BEAN MOSAIC VIRUS IN BEAN LEAF BEETLE HEMOLYMPH. M. D. Robison and H. A. Scott, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Hemolymph from bean leaf beetles, Cerotoma trifurcata, fed as briefly as 1 min on cowpea southern bean mosaic virus-infected 'Crimson' cowpea, contained virus when assayed on 'Georgia 21' cowpea, a local lesion host. Variations in recoverable virus occurred with individual beetles and could not be attributed to differences in age of beetles, sex, length of acquisition feeding, or quantities of tissue ingested. Comparisons of beetles acquisition-fed on two cultivars of cowpea which support high ('Crimson' cowpea) and low ('Monarch' cowpea) virus titers, however, showed that beetles fed on 'Crimson' cowpea had more virus in the hemolymph and transmitted more frequently than beetles fed on 'Monarch' cowpea.

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EFFECT OF WHEAT SOILBORNE MOSAIC VIRUS ON YIELD OF WINTER WHEAT CULTIVARS. R. M. Hunger and J. L. Sherwood, Plant Pathology Dept., Oklahoma State Univ., Stillwater, OK 74078-0285.

Field plots of 12 winter wheat cultivars (cv.) were assessed visually three times in spring, 1985 for wheat soilborne mosaic virus (WSBMV). A disease severity index (DSI) was calculated (Wis. Agr. Exp. Sta. Bull. 531, 1958) based on ten plots/cv. with 7-10 plants/plot. Foliar samples collected from each plot at each assessment were evaluated by ELISA, and yield of each plot was determined at the end of the season. Negative and significant (P=0.01) correlation was obtained between yield and DSI at all assessments for susceptible cv., and negative and significant (P=.05) correlation was obtained at the first assessment for resistant cv. Negative and significant (P=0.05) correlation was obtained between yield and ELISA at the first assessment for susceptible cv., and was not significant for resistant cv. at any assessment. Thus, visual assessment is the best indicator of the effect of WSBMV on yield, and ELISA is useful only if used early in the season on susceptible cv.

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WHEAT SPINDLE STREAK MOSAIC DEVELOPMENT AND ITS REDUCTION OF GRAIN YIELD AND QUALITY. Barry M. Cunfer, James W. Demski, and David C. Bays. Dept. of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

The development of wheat spindle streak mosaic virus (WSSMV) on wheat was studied from planting through harvest. WSSMV was detected by ELISA in the roots of three susceptible cultivars 30 days after planting. After 60 days chlorosis and stunting were evident. Virus titer in roots doubled compared to the 30 day value and the titer in shoots was similar to that in roots. WSSMV was detected in the sap of triturated roots of two resistant cultivars, but not in shoots; the plants exhibited no symptoms. Number of tillers of infected susceptible Florida 301 and Coker 797 declined 43 and 78%, respectively following a severe freeze in late January compared to controls planted in fumigated soil. Grain yield was reduced 56 and 87%. Test weight and 1,000 kernel weight were significantly reduced so that flour made from the grain did not meet minimum milling and baking quality standards.

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CUCUMBER MOSAIC VIRUS CAUSES WHEAT DISEASE. M B von Wechmar, Department of Microbiology, University of Cape Town, Rondebosch 7700, South Africa.

In the 1984 season Cucumber mosaic virus (CMV) caused a severe disease in wheat in the eastern Transvaal, leading to an estimated yield loss of 40-50 percent. Symptoms, first noticed at ear emergence, were uneven elongation of shoots, yellowing of leaves, the appearance of sterile yellow-white ears. Severely infected plants died early. Diagnosis was by ELISA, immuno-electroblots and ISSEM with antiserum against three different isolates of CMV. A protein MW of 24.5 was determined on PAGE and aphid transmission was with Myzus persicae and Rhopalosiphum padi. Distribution of CMV amongst shoots differed and was not always related to symptoms. The infection could be traced to Sakurajima radishes. It is probable that primary infection was started by aphids feeding in CMV-infected vegetable crops in the Lowveld and carried South by air currents. Seedborne CMV was detected in local and other wheat seedsources.

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AIMS AND ACTIVITIES OF INDUSTRY'S FUNGICIDE RESISTANCE ACTION COMMITTEE (FRAC). M. Wade, Shell Research, Ltd., Sittingbourne, Kent, ME9 8AG, UK; C. M. Smith, E. I. du Pont de Nemours & Co., Inc., Stine-Haskell, P. O. Box 30, Newark, DE 19714.

FRAC is an inter-company committee of GIFAP (International Group of National Associations of Agrochemical Manufacturers) dedicated to extending the effectiveness of fungicides with risk of resistance and to limiting crop damage due to resistance. Through educational and research programs, FRAC communicates information on resistance and fungicide-use strategies to agricultural, academic, industrial, and regulatory sectors worldwide and promotes cooperative action to solve resistance problems. FRAC coordinates 4 Working Groups for fungicides at risk (acylalanines, benzimidazoles, dicarboximides, demethylation-inhibitors). Through resistance monitoring and research programs, Working Groups develop and recommend technical resistance-management strategies. FRAC believes that cooperation by all those involved in fungicides is vital for effective resistance management.

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LABORATORY RESISTANCE IN RHIZOCTONIA SOLANI TO SEVERAL FUNGICIDES. M.L.Gullino, A.Gualco and A.Garibaldi, Istituto di Patologia vegetale, Via Giuria 15, 10126 Torino, Italy.

Strains of Rhizoctonia solani Kühn respectively resistant to iprodione (RI), tolchlofos-methyl (RT) and pencycuron (RP) were selected under laboratory conditions. RI and RT strains showed cross resistance to other dicarboximides and PCNB, while were sensitive to pencycuron and flutoluanil. RP strains were inhibited, in vitro, by dicarboximides,tolchlofos-methyl, pencycuron and flutoluanil. RI, RT and RP strains showed a decreased virulence as compared with the sensitive parent isolates. Under greenhouse conditions RI strains showed a reduced ability to grow as saprophyte in soil. All these characteristics seem to make resistant strains of R.solani not very fit.

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EVALUATION OF A MODIFIED PEANUT LEAFSPOT ADVISORY. C. A. Matyac and J. E. Bailey, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616

Two modifications of the Jensen and Boyle advisory model were evaluated on six peanut genotypes. The new models allowed progressively longer periods of high relative humidity and higher temperatures before spray recommendations were issued. The new models resulted in 16 and 33% reduction in fungicide application as compared to the current advisory. There were no statistically significant differences in areas under the disease progress curves, apparent infection rates nor area under the defoliation curves (AUDEFC) among epidemics under the modified or non-modified advisory for all genotypes tested. However, yield was reduced for two of the six genotypes using the modified advisory. The apparent infection rate was the most

sensitive epidemiological parameter in differentiating genotypes or fungicide spray schedules while AUDEFC values were the least sensitive. Genotype x advisory interactions indicate modified advisories will be useful for resistant genotypes.

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GENETICS OF USTILAGO HORDEI: MUTAGENESIS AND MEIOTIC RECOMBINATION. Caroll E. Henry, Bethsheba Bullock, Valerie Smith, Evelene Steward-Clark. Department of Biological Sciences, Chicago State University, Chicago, Illinois 60628.

Seventeen auxotrophic and two morphological mutants obtained by UV and chemical mutagenesis were employed in the detection of linkage in Ustilago hordei (Pers.)Lagerh. For mutagenesis a 10^8 sporidial suspension of I_4A or E_3a standard mating type was subjected to a fluorescent lamp output of $7x10^3$ ergs/m^2/sec. for 4 min. or incubated with reciprocal shaking at 21°C with 5% EMS (ethyl methane sulfonate) for one hr. or 1mg/ml MNNG (N-methyl-N-nitro-N-nitrosoguanidine) for 10 min. After appropriate dilutions, sporidia were plated on VCM (Vogel's complete medium) for 5-7 days. Morphologicals were detected visually and auxotrophs by selective plating. Barley (Hordeum vulgare L.) seeds were inoculated with aliquots of compatible auxotrophs in various mutant x mutant combinations. Heterozygous diploid teliospores resulting from smutted inflorescences were plated on VCM. Random analysis of haploid sporidia gave linkage values.

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APPLE POWDERY MILDEW: EVALUATION OF PROTECTIVE SPRAY PROGRAMS IN WESTERN COLORADO. H. J. Larsen, C.S.U.-Orchard Mesa Res. Center, 3168 B 1/2 Rd., Grand Junction, CO 81503.

Spray programs with triadimephon at 28 and 56 mg ai/l provided better protection against apple powder mildew for Jonathan apples that did programs with sulfur/dinocap (at 1.47 g and 337 mg ai/l, respectively) or sulfur only (at 1.47 & .74 ai/l). Application of triadimephon (at 56 mg ai/l) before and after bloom, followed by a sulfur/dinocap program, protected fruit best (22% better than the control). Application of two sulfur sprays before and after bloom, followed by five sprays of triadimephon at the lower rate, protected foliage and terminal buds best (38 & 28% better than the control, respectively). The standard sulfur/dinocap program provided intermediate protection for foilage, fruit, and terminal buds. A sulfur only program provided least protection in all categories. Average fruit size (diam.) was not significantly affected by treatment although fruit from traidimephon-treated trees generally tended to be slightly smaller.

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ULTRASTRUCTURAL OBSERVATIONS OF ENDOGONE PISIFORMIS & CYTOCHEMISTRY USING ENZYME-GOLD & LECTIN-GOLD COMPLEXES . Jabaji-Hare, S. H., P. M. Charest & J. A. Fortin. Depts. of Forestry & Phytology, Laval University, Quebec, Canada. G1K 7P4 .

The ultrastructural observations of the mycelia of Endogone pisiformis (Endogonaceae) revealed that the hyphae were coenocytic and formed intercalary & terminal swellings. In young cells, the cytoplasm was granular & rich in nuclei, ribosomes, mitochondria and membranes. While in older cells the cytoplasm was replaced by lipid bodies and glycogen particles. The enzyme-gold & lectin-gold labeling proved to be useful specific markers for the localization of appropriate molecules in E. pisiformis. α-amylase-gold complex localized glycogen particles in the cytoplasm, while phospholipase-gold complex labeled phospholipids in the cell wall & lipid bodies. Chitin was localized in the cell wall by the use of wheat germ agglutinin. Other lectin-gold complexes such as concanavalin A (Con A) and a lectin from Ulex europaeus localized mannose and fucose respectively. Mannose was found in electron translucent areas of the cytoplasm and fucose was localized in lipid bodies, membranes and nuclei. No siginificant labeling was observed in control experiments.

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Relationship of bound calcium and inoculum concentration to the effect of postharvest calcium treatment on decay of apples by Penicillium expansum. W. S. Conway and K. C. Gross, USDA, ARS, Hort. Crops Quality Lab, Beltsville, MD 20705 and C. E. Sams, Univ. of Tennessee, Knoxville, TN 37996

Golden Delicious apples were pressure infiltrated (68.95 kPa) at harvest with 0,1,2, or 4% solutions of $CaCl_2$ and stored at 0 C. After 6 months, the fruits were removed from storage and wound inoculated with a conidial suspension of Penicillium expansum, the concentration of which was either 10^4, 10^5, or 10^6 spores per milliliter. After 7 days at 20 C, the fruits were rated for decay severity, and the cell walls of similarly treated but noninoculated fruits were extracted and analyzed for calcium concentration. As the calcium concentration of the solutions with which the fruits were infiltrated increased, the amount of cell wall bound calcium also increased. As the cell wall calcium content increased and the inoculum concentration decreased, the resulting percent reduction in decay increased. These results indicate that as the inoculum concentration decreases, the relative effectiveness of increased cell wall bound calcium in reducing decay increases.

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W.A. Powell, E. Gobbi, N.K. Van Alfen, 2-D Gel Analysis of Polypeptides from Virulent and Hypovirulent Strains of Endothia parasitica, Utah State Univ., Logan, Ut.

Transmission of hypovirulence in Endothia parasitica has been shown to be associated with the transfer of dsRNA during anastomosis. Analysis of whole cell lysates on 2-D gels show significant differences in protein patterns between a virulent strain (EP155) and it's converted hypovirulent form (EP713). We have reconstructed these two strains in a way that ensures nuclear homogeneity. These strains were found to contain the same mitochondrial genomes as determined by restriction endonuclease mapping. The only detectable genetic difference between these virulent and hypovirulent strains is the dsRNA contained in the hypovirulent form. Preliminary results using these two strains show differences in their peptide pattern as detected by 2-D gels. These changes represent less than 10% of the total detectable proteins. These results imply that the cause of hypovirulence is not general debilitation but rather an effect on specific polypeptides.

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CYCLIC NUCLEOTIDES AND BEAN RUST UREDOSPORE DEVELOPMENT. L. Epstein, R.C. Staples, and H.C. Hoch*. Boyce Thompson Institute for Plant Research, Ithaca, NY 14853; New York State Agr. Exp. Sta., Cornell University, Geneva, NY 14456.*

Ten millimolar cyclic AMP (cAMP) or cyclic GMP (cGMP) induced bean rust uredospore germlings to undergo one round of mitosis, a process normally associated with appressorium formation. In addition to mitosis, cAMP induced development of disoriented septa, but not appressoria. In order to eventually assess the possibility of cyclic nucleotide regulation of mitosis, we used a cAM[^{32}P] photoaffinity probe to identify three cyclic nucleotide binding proteins which bound both cAMP and cGMP. AT[^{32}P] phosphorylation of one peptide in uredospore germling extracts was stimulated by cGMP but not cAMP. Uredospores contain ca. 23 and 1500 pmoles cGMP and cAMP/g dry weight, respectively, as determined by radioimmune assays.

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TEM OBSERVATIONS ON DEGRADATION OF WESTERN HEMLOCK AND SWEETGUM BY THE WHITE-ROT FUNGUS CORIOLUS VERSICOLOR. T. L. Highley and L. Murmanis, Forest Products Laboratory, Madison, WI 53705.

Cell walls of hemlock and sweetgum, degraded by the white-rot fungus, Coriolus versicolor, and observed by TEM show a different mode of attack in the two species. Sweetgum was attacked by hyphae both from the lumen and cell corners. Hemlock was attacked only from the lumen. The compound middle lamella of hemlock was not attacked until removal of the secondary wall was completed; the cell corners were particularly resistant to degradation. In sweetgum, the middle lamella and cell corners were severely degraded without appreciable degradation to adjacent cell wall material. Delignification occurred throughout the cell wall in sweetgum without substantial structural alteration of the remaining cell wall. However in hemlock, lignin was degraded simultaneously with destruction of the cell wall. These results support earlier findings that, due to slower degradation of softwood lignin, C. versicolor degrades softwoods slower than hardwoods.

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POSSIBLE RELATIONSHIP OF FRUIT MATURITY AND CALCIUM TO QUIESCENT INFECTION IN MUSKMELON. B.D. Bruton* and W.S. Conway**, USDA-ARS, *PH&SPRU, P.O. Box 267, Weslaco, TX 78596, **HCQL, Beltsville, MD 20705.

The major postharvest decay of Texas grown muskmelon fruit is due to quiescent infections caused by Diaporthe melonis. Fungal growth on tissue extracts of 10 through 50 day postanthesis fruit increased significantly with age. Maintenance of the quiescent condition may be related to nonavailability of proper nutrients in young fruit which become available as the fruit matures. A gradient exists in muskemlon fruit in which bound Ca as well as total Ca concentration is greatest in the rind and lowest near the seed cavity. As the fruit matures, there is a corresponding decrease in bound Ca throughout the fruit. Bound Ca has been shown to maintain structural integrity and thus retard decay in other fruits. As the muskmelon matures, cell wall Ca becomes more soluble which may reduce the resistance of the cell wall to maceration by fungal enzymes.

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INCIDENCE OF PATHOGENIC MUCOR SPP. IN ANJOU PEAR ORCHARD SOILS IN THE OKANAGAN VALLEY OF BRITISH COLUMBIA. P.L. Sholberg and G.R. Owen, Agriculture Canada, Research Station, Summerland, British Columbia V0H 1Z0

Stem end rot of stored Anjou pears caused by Mucor piriformis is a yearly recurring problem. Studies were initiated in 1985 to determine the source and distribution of this fungus. Fifty-one orchards were surveyed for Mucor spp. by sampling the top two inches of soil beneath the pear tree canopies within the orchards. Mucor spp. were found in 49 of the 51 orchards sampled with 15 orchards having extremely high levels of Mucor spp. Single spore isolates which grew at 10°C were made from the cultures isolated from the soil of the 49 orchards. These isolates were tested for pathogenicity on Anjou pear fruit. Results showed that 36 of the 49 orchards had pathogenic Mucor spp. The pathogenic species were divided into two groups according to the height of the sporangium. The species with tall sporangia have been identified as Mucor piriformis but those species with short sporangia remain to be identified.

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IN VITRO FUNGITOXICITY OF AN ETHANOLIC EXTRACT OF NEEM SEED ON RHIZOCTONIA SOLANI AND FUSARIUM OXYSPORUM. J. C. Locke, USDA, ARS, Florist & Nursery Crops Laboratory, Beltsville, MD 20705.

The Indian neem tree, Azadirachta indica Juss. (Syn. Melia azadirachta L.), contains a number of biologically active compounds that are associated with various plant parts, especially the seed. The possible fungitoxic activity of these compounds from the seed was studied utilizing two soilborne fungal pathogens, Rhizoctonia solani Kuehn and Fusarium oxysporum Schlecht. An ethanolic extract of neem seed (1:1) was diluted with water, filter sterilized, and added to sterile Czapek Dox broth in 250 ml flasks. The test fungi, grown on Czapek Dox agar, were introduced as 5 mm plugs into the individual flasks each containing 40 ml of growth medium. After 14 days of incubation in the dark at 22C, mycelial mats were harvested and dry weights determined. Rhizoctonia was more sensitive than Fusarium to the neem seed extract. Vegetative growth (dry weight) was reduced by as much as 70 and 30% respectively in a 1% solution of the seed extract.

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EFFECT OF SLOW RELEASE METALAXYL ON CONTROL OF PYTHIUM ROOT ROT OF POINSETTIA. Robert D. Raabe, Department of Plant Pathology, University of California, Berkeley, CA 94720

To determine the effectiveness of slow release metalaxyl, rooted poinsettia cuttings, cv. Improved Rochford, were planted in 10.16 cm pots in a soil-less mix which had been infested with Pythium irregulare and P. debaryanum. The inoculum was grown on sterilized millet seeds and was added to the mix one week before planting. At that time, spikes of slow release fertilizer containing metalaxyl were added to the mix. Three fertilizer levels and two levels of metalaxyl were used. Controls consisted of fertilizer spikes alone and fertilizer spikes plus monthly metalaxyl drenches. After 2 months, plants were rated as to height, bract width, and disease. Composite samples were used to determine the number of Pythium propagules in the treatments. Both levels of metalaxyl gave good control of root rot and this correlated with low levels of propagules in the mix at all 3 fertilizer levels. In the absence of metalaxyl, the amount of disease and the numbers of propagules increased greatly with an increase in the fertilizer level.

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SPATIAL PATTERN DYNAMICS OF PROPAGULES OF MACROPHOMINA PHASEOLINA. C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Propagule density of Macrophomina phaseolina in a sandy loam soil has been monitored since spring 1983. A 10-g portion from a single, centric soil core in each of 180 6x6 m contiguous quadrats was assayed each spring and fall prior to and after cultural operations. Crops in the field were corn, cotton, and corn in 1983, 1984, and 1985, respectively. Inoculum density (ID) decreased when corn was present but increased when cotton was present. Aggregation of propagules, as indicated by Morisita's index, increased after planting during each year. ID increased from 43 to 66 propagules/10 g soil and aggregation decreased between 15 Feb and 22 May 1984 with cultural operations after the 1983 corn crop; ID remained stable and aggregation decreased with tillage and planting in spring 1985 following cotton in 1984. Consideration of the dynamics of spatial pattern in addition to inoculum density provides additional insight into the effects of crops and cultural practices on population dynamics of M. phaseolina.

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EFFECTS OF ROOT GROWTH OF CORN ON INOCULUM DENSITY AND SPATIAL PATTERN OF MACROPHOMINA PHASEOLINA. O. M. Olanya and C. L. Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

The effect of root growth on inoculum density (ID) and spatial pattern of Macrophomina phaseolina was investigated in plots with and without the presence of corn plants. Soil cores were obtained at monthly intervals from contiguous quadrats (18x18 or 15x15 cm) at each of two locations, respectively. ID was measured from a 10 g subsample of air dry soil using a selective assay. Soil characteristics from each plot were determined. Frequency count ID data were best described by the Poisson distribution and values of Morisita's index were very close to one which indicated a random or near random pattern of propagules. A split plot analysis of variance in time indicated nonsignificant ($p=0.05$) differences in ID and spatial pattern in plots with and without corn. Growth of corn roots through soil thus had no detectable effect on ID or spatial pattern of M. phaseolina during a growing season.

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GENETIC DIVERSITY WITHIN FUSARIUM OXYSPORUM F. SP. LYCOPERSICI AS DETERMINED BY VEGETATIVE COMPATIBILITY (HETEROKARYOSIS) TESTS. K. S. Elias and R. W. Schneider, Dept. of Pl. Path. and Crop Physio., La. Agric. Expt. Sta., LSU Agric. Ctr., Baton Rouge, LA 70803.

Fusarium oxysporum is a common soilborne fungus in agricultural soils. Although most formae speciales are morphologically indistinguishable, a technique that involves selection of nitrate reductase deficient (Nit) mutants makes it possible to identify vegetative compatibility groups (VCG) that delimit formae speciales and races. More than 100 isolates of F. oxysporum f. sp. lycopersici, including Race 3, have been collected from within Louisiana, major tomato growing regions within the U.S., Taiwan, Australia, Europe, and North Africa. Many nonpathogenic isolates of F. oxysporum from symptomless tomato roots also were collected and tested. Some conclusions concerning the genetic diversity of this pathogen with respect to origin of races within f. sp. lycopersici, other formae speciales, and nonpathogenic Fusaria will be discussed.

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GERMINATION OF SPORANGIA OF PYTHIUM SPECIES IN RESPONSE TO VOLATILES FROM GERMINATING COTTON SEEDS. E. B. Nelson, Univ. of Arkansas, Dept. of Plant Pathology, Fayetteville, AR 72701.

Sporangia of Pythium ultimum, P. sylvaticum, and P. irregulare grown under fungistatic conditions germinated readily when exposed to volatiles from germinating cotton (Acala SJ-2) seeds. Direct germination of P. ultimum sporangia was evident 4, 2, and 6 hr after the start of seed imbibition at 15, 25 and 35 C, respectively. Maximum sporangium germination occurred within 6-8 hours. Volatiles collected during the initial 6 hr of germination were separated by gas chromatography, identified and quantified. Ethanol, acetaldehyde, acetone, methanol and ethane were detected as principle components of volatiles accumulating during the first 6 hr of germination. Ethane production was detectable only from seeds germinating at 15 C. Levels of all other volatile compounds increased as imbibition temperature increased. Maximum volatile production occurred 24 and 36 hours after imbibition at 35 and 25 C, respectively.

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ASSESSMENT OF RHIZOSPHERE POPULATION DENSITIES OF FUSARIUM SPP. ASSOCIATED WITH CORN STALK ROT AND CONSERVATION TILLAGE. L. G. Skoglund, W. M. Brown, Jr., and E. R. Ruppel, Dept. of Plant Pathology & Weed Science and USDA-ARS, Colorado State University, Fort Collins, CO 80523.

The second year of a study investigated the effects of three tillage regimes (reduced till, chisel till, and conventional till) and three herbicides (alachlor, triazine, and EPTC) on stalk rot of corn caused by Fusarium spp. Rhizosphere population densities were determined by the soil dilution plate technique on Komada's Fusarium-selective medium. The number of colonies of total Fusarium spp. (TOTF), F. moniliforme (FM), and F. subglutinans (FS) from seven day old cultures were recorded. Differences among tillage treatments were significant for TOTF, FM, and FM + FS but not for FS. Population densities of Fusarium spp. were significantly higher for reduced and chisel till plots than for conventional till plots. Reduced till plots had population densities significantly greater than chisel till plots for FM only. As in 1984, herbicides had no effect on fungal population densities.

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CYLINDROCLADIUM ROOT ROT OF SPATHIPHYLLUM. J.Y. Uchida and M. Aragaki. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

A severe root rot, characterized by wilting and chlorotic foliage, resulted in large losses of mature, blooming spathiphyllum plants at a commercial nursery in Hawaii. The disease was caused by Cylindrocladium spathiphylli Schoulties et al., although variances from the published description of the species were observed. Conidiophore terminal vesicles of C. spathiphylli are described as globose; those of the Hawaiian isolates are predominantly globose, but vesicle shapes are also sub-globose, ellipsoid, spatulate, or ovoid. Conidia of C. spathiphylli are described as single septate; those of Hawaiian isolates are predominantly single septate, although 2-septate conidia are common, and occasional 3-septate conidia also occur. Percentage of 2-septate conidia varied from 2 to 40% among isolates. In preliminary inoculation studies, Spathiphyllum cv. 'Tasson' was severely infected, while S. floribundum cv. 'Silver Streak' appeared to be resistant.

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CHARACTERISTICS AND PATHOGENICITY OF TWO FUNGI ISOLATED FROM BERMUDAGRASS AFFECTED WITH SPRING DEAD SPOT. N. Tisserat, A. Nus, and J. Pair, Depts. of Plant Pathology and Horticulture, Kansas State University, Manhattan, KS 66502.

Two fungi were isolated consistently from dead patches of bermudagrass (Cynodon dactylon) afflicted with spring dead spot disease in two locations in Kansas. One fungus was similar in culture morphology and color, in growth rates at various temperatures on PDA, and in sensitivity to fungicides on amended PDA, to that of Phialophora graminicola, while the second fungus was similar in most characteristics except color (buff) to that of Leptosphaeria korrae. Neither the anamorph or teleomorph states of these fungi have been observed. Common bermudagrass inoculated with the L. korrae-like isolate developed extensive root and crown discoloration but no foliar symptoms after incubation for 3 months in the greenhouse. The effects of cold temperatures on the development of foliar symptoms will be discussed. Inoculations with the P. graminicola-like isolate failed to cause root or foliar symptoms under the conditions tested.

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ANALYSIS OF TOXIN GENE PRODUCTS FROM USTILAGO MAYDIS VIRUS P4. G.K. Podila, R.F. Bozarth, and W.H. Flurkey. Dept. of Life Sci. Indiana State University, Terre Haute, IN 47809

The Ustilago maydis virus(UmV) is a mycovirus that replicates latently in the corn smut fungus Ustilago maydis. An unusual feature of UmV P4 is that it codes for a host-specific killer toxin, which kills sensitive strains of U.maydis. The virus has a segmented double stranded RNA(dsRNA) genome, consisting of three major sizes, namely, Heavy(H), Medium(M), and Light(L). Translation studies performed in vitro show that the M2 dsRNA segment of UmV P4 codes for the toxin. The in vitro translation product immunoprecipitated with toxin antibodies and had a molecular weight of 33 kd, whereas the purified toxin from UmV P4 had a molecular weight of 12 kd. Pulse chase experiments conducted in vivo with 35-S Methionine also yielded a cellular protein of 33 kd and a secreted protein of 12 kd which immunoprecipitated with toxin antibodies. These results show that the UmV P4 toxin is synthesized as a high molecular weight precursor which undergoes processing to yield a 12 kd secreted form of killer toxin.

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A SIMPLE SYSTEM FOR ELECTROPHORESING DENATURED RNA. R.N. Skopp and L.C. Lane. Dept. of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

Gel electrophoresis conveniently estimates sizes of denatured RNAs. Methyl mercuric hydroxide, though convenient, is an expensive and toxic denaturant. Irreversibly denatured (glyoxal or formaldehyde) RNAs have usually been detected with the insensitive reagent, acridine orange. Irreversibly denatured RNAs resolve well by electrophresis on plastic-backed 1% agarose. Prior to electrophoresis, the gel is equilibrated with running buffer (75 mM N-ethylmorpholine, 50 mM H_3PO_4) containing 2% formaldehyde. For glyoxal treatment, the reagent need not be purified but should be neutralized with base. RNAs stain rapidly with highly sensitive silver methods. Brome mosaic virus and E. coli rRNAs give consistent sizes.

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REGENERATION OF RUSSET BURBANK PLANTS FROM TISSUE INFECTED WITH POTATO LEAF ROLL VIRUS (PLRV). H.M. Griffiths and R.D. Locy, 417 Wakara Way, Salt Lake City, Utah 84108

The distribution of PLRV within tissue of potato plants infected by the virus varies. Within a population of plants regenerated from infected tissue some could be virus-free. Whether these virus-free plants would have increased resistance to PLRV infection is unclear. Plants of Russet Burbank were regenerated from petiole sections obtained from plants which had high virus titers for PLRV (determined by ELISA). Sixty-one percent of the petioles initiated led to plant regeneration. A high proportion of regenerates (63%) were virus-free or had only very low titers for PLRV. These plants were clonally propagated and established in the greenhouse. Shoot-tip cultures of Russet Burbank, which were shown to be virus-free were transferred from tissue culture conditions to the greenhouse. All the plants were susceptible to PLRV. Even though virus-free plants can be regenerated from tissue heavily infected with PLRV they are apparently as susceptible to the virus as the parent plants.

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IMMUNO-GOLD DECORATION AS A TECHNIQUE FOR MEASURING SEROLOGICAL RELATIONSHIPS AMONG STRAINS OF MAIZE DWARF MOSAIC VIRUS AND SUGARCANE MOSAIC VIRUS. J. D. Alexander and R. W. Toler. Dept. of Plant Path. and Micro., Tex. Agr. Exp. Sta., College Station, TX 77843.

Immuno-electron microscopy coupled with immuno-gold decoration was used to compare serological relatedness of sugarcane mosaic virus (SCMV) strains A and E, and maize dwarf mosaic virus (MDMV) strain A to MDMV strain B. Particles of each of these four strains trapped on carbon-coated parlodian grids were treated with identical suspensions of anti-MDMV-B IgG-coated colloidal gold (15nm) in two replications each. Mean numbers of labels per particle were 1.1 for MDMV-A, 8.8 for SCMV-A, 14.0 for SCMV-E, and 14.4 for MDMV-B. The Tukey-Kramer multiple comparison test indicated no significant difference between means for MDMV-B and SCMV-E; however, in all other strain comparisons means were significantly different. Since antisera to different strains 'perceive' the serological relationships from different 'points of view', it will be necessary to decorate SCMV and MDMV strains using IgG produced to each of the strains involved if they are to be serologically mapped using this technique.

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COMPARISON OF A DOT IMMUNOBINDING ASSAY WITH ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF TWO CEREAL VIRUSES. Michael C. Edwards and Yuzhi Zheng, USDA-ARS, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

An indirect dot immunobinding assay (DIBA) was compared with direct ELISA for the detection of barley stripe mosaic (BSM) and oat blue dwarf (OBD) viruses. First, antigen was dotted onto a nitrocellulose membrane and dried. After blocking, the membrane was incubated with primary IgG (rabbit), washed, incubated with alkaline phosphatase conjugated anti-rabbit IgG, washed again, and placed in a substrate solution (NBT and BCIP). The use of very dilute concentrations of cross-absorbed primary antibody was essential for the elimination of nonspecific reactions. Rate of color development was greatly affected by the substrate buffer. Less than 100pg of purified BSMV and a 1:16000 dilution of BSMV infested sap were detectable by DIBA, as compared to 2.5pg/µl of purified BSMV and a 1:32000 dilution of BSMV infested sap detectable by ELISA. In both DIBA and ELISA tests, sensitivity of OBDV detection was greatly affected by the choice of sample buffer.

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INTEREST OF SDS-IMMUNODIFFUSION FOR CUCUMBER MOSAIC VIRUS SEROTYPING IN FIELD SAMPLES. H. Lot and H. Lecoq, INRA, Station de Pathologie Végétale, BP 94, 84140 Montfavet France

Two CMV serotypes are observed in vegetables grown in France. Strain serological characterization is erratic in field samples using standard Outcherlony immunodiffusion tests, and is only possible after multiplication on a susceptible host before testing crude extracts or concentrated virus. SDS immunodiffusion (SDS-ID) is useful for CMV detection (Purcifull et al., 1981, Phytopathology, 71, 1221). It is now found efficient for differentiating CMV-DTL and CMV-ToRS serotypes in field samples. When high titered antisera are used spur formation are clearly observed between homologous and heterologous antigens. When using appropriate dilutions only homologous reactions are detected. Reliability of the method was established using different CMV isolates, in various hosts and testing at different times after infection. SDS-ID is widely used for Potyvirus detection in field samples. It may now be jointly used for CMV serotyping. When serotyping is not required CMV is better detected by using a composite antiserum containing a mixture of sera against each serotype.

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cDNA CLONES FROM BARLEY YELLOW DWARF VIRUS. D.J. Barbara, P.P. Ueng, E.E. Kawata, B.A. Larkins & R.M. Lister. Botany & Plant Pathology, Purdue University, W. Lafayette, IN 47907.

A library of cDNA clones derived from the RNA of the MAV isolate (sensu Rochow) of barley yellow dwarf virus was constructed in the bacterophage lambda vector gt11. 15 of 600 clones screened serologically were found capable of expressing coat protein. Restriction enzyme mapping showed that at least 90% of the genome is represented in the library and that the coat protein coding region is located toward the 3' end. When subcloned into the plasmid pUC18 and used in Northern hybridization, clones from the coat protein region of the genome hybridized to MAV-RNA; those from all other parts of the genome hybridized to MAV-RNA and to RNA of the immunologically related isolate PAV. None hybridized strongly to the RNA of RPV, an immunologically distinct isolate. Similar libraries are being constructed for the PAV and RPV isolates.

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SEROLOGICAL RELATIONSHIPS BETWEEN INCLUSION PROTEINS OF SEVERAL STRAINS OF MDMV AND SCMV. S. G. Jensen, N. J. Van Pelt, and E. M. Ball, Agricultural Research Service, U.S. Department of Agriculture and Department of Plant Pathology, University of Nebraska, Lincoln Nebraska 68583-0722.

Antiserum was produced in rabbits to the 65.8 kDa inclusion protein induced in sorghum [Sorghum bicolor (L.) Moench] by a strain B-like isolate of maize dwarf mosaic virus (MDMV). Serological endpoints were determined on dot blots employing alkaline phosphatase coupled antibody. The antiserum was tested against similar inclusion proteins induced by other strains of MDMV and sugarcane mosaic virus (SCMV). Several B-like isolates and some strains of SCMV reacted strongly with the antiserum. Other B-like isolates and some SCMV strains reacted weakly. MDMV-A strains all reacted weakly. Antigenicity did not correlate with protein size which ranged from 64.2 to 67.5 kDa.

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VIROID BINDING PROTEINS FROM INFECTED AND UNINFECTED TOMATO LEAVES. A. Hadidi, USDA, Agricultural Research Service, Microbiology and Plant Pathology Lab., Beltsville, MD 20705

Analysis of a 70,000Xg pellet "post-nuclear" fraction from infected tomato leaves (Lycopersicon esculentum Mill, cv. Rutgers) revealed an association of potato spindle tuber viroid (PSTV) with cellular constituent complexes. The proteins of the complexes as well as those of the 70,000Xg supernatants from infected and uninfected cells were separated by SDS-gel electrophoresis, renatured, blotted on nitrocellulose membranes, and then incubated with PSTV. The PSTV-protein complexes were cross-linked and the PSTV bound protein bands were detected by hybridization with labeled cloned ssPSTVcDNA. Proteins of 17-20 Kd, and of about 28Kd, 30Kd, and 52Kd were found in the complexes. These proteins, with the exception of the 28Kd protein, were also found in the soluble fractions from infected and uninfected cells. A 35Kd protein, that also bound PSTV, was found in the soluble fractions from infected but not uninfected cells.

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NUCLEOTIDE SEQUENCE OF A NATURALLY OCCURRING DELETION MUTANT OF FIGWORT MOSAIC VIRUS. H. B. Scholthof, R. D. Richins, M. K. Handley and R. J. Shepherd, Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546

A frequently occurring deletion mutant of figwort mosaic virus (FMV), a member of the caulimovirus group, has been identified in Datura stramonium plants systemically infected with the severe strain of FMV. Viral DNA from this mutant has been isolated and cloned in pBR322 to give a recombinant plasmid (pFMVm5). The viral portion of pFMVm5, when excised from its cloning vector, failed to induce symptoms when inoculated to D. stramonium. The position and size of the deletion was mapped by restriction endonuclease analysis and by DNA sequencing. Comparison of the nucleotide sequence of the deletion mutant with the sequence of a wild type strain of similar origin (pFMV-DxS) revealed that approximately 1250 base pairs of open reading frames IV and V have been deleted in pFMVm5. The deletion appears to occur as a result of RNA splicing since consensus donor and acceptor sequences (AAGGTA and TACAGG, respectively) were found bordering the deleted region. This observation supports the current model for caulimovirus replication via reverse transcription of an RNA intermediate.

599

EFFECT OF BARLEY STRIPE MOSAIC VIRUS ON THE ISOZYME PATTERNS OF ACID PHOSPHATASE, RIBONUCLEASE, AND ALCOHOL DEHYDROGENASE IN FOUR BARLEY CULTIVARS. F. C. Wu and R. G. Timian, Department of Plant Pathology and USDA-ARS, North Dakota State University, Fargo, ND 58105.

Electrophoretic isozyme patterns of acid phosphatase, ribonuclease, and alcohol dehydrogenase extracted from 14 day old seedlings of four barley cultivars, either uninoculated or 7 days after inoculation with the CV52 strain of barley stripe mosaic virus (BSMV), were examined. Increased relative activities of all three enzymes were found in the two susceptible barley cultivars Black Hulless and Manchuria after inoculation with the virus. However, in the resistant cultivars C.I. 4885 and Silver King, plants inoculated with the virus had decreased activities of these enzymes with one exception. The increase of the enzymatic activities in the susceptible cultivars suggest an involvement of these enzymes in the defense mechanism of the plant.

600

IMMUNOLOGICAL AND BIOLOGICAL STUDIES OF PRUNUS NECROTIC RINGSPOT ILARVIRUS USING A MOUSE MONOCLONAL ANTIBODY. J. A. Aebig[1], R. L. Jordan[2], and H. T. Hsu[2].
[1]American Type Culture Collection, Rockville, MD 20852 and [2]U. S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705.

A monoclonal antibody which reacts with Prunus necrotic ringspot ilarvirus (PNRSV) was used in immunochemical studies and neutralization of infectivity assays. The antibody was able to detect the 27000 relative molecular weight coat protein of PNRSV in Western blot analysis. The monoclonal antibody also detected all polypeptide fragments that were generated after incubation of whole virus with proteolytic enzymes. In neutralization of infectivity studies, the antibody blocked virus infectivity although it did not precipitate the antigen in Ouchterlony double diffusion tests.

601

MONOCLONAL ANTIBODIES TO CLOVER YELLOW VEIN AND BEAN YELLOW MOSAIC VIRUSES. M. R. McLaughlin, USDA-ARS, Crop Sci.Res.Lab., Forage Res. Unit; S. W. Scott, Dept. of Plant Pathol. and Weed Science; and A. J. Ainsworth, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Hybridomas were produced by PEG-fusion of FOX-NY myeloma cells (Taggart Hybridoma Technology™, HyClone Laboratories, Logan, Utah) with spleen cells from BALB-C mice immunized with clover yellow vein virus (CYVV-Pratt), bean yellow mosaic virus (BYMV-KY204-1) or BYMV-Scott. Cells were cultured in RPMI 1640 medium containing 2.38 X 10^{-2} M sodium bicarbonate, 1.0 X 10^{-3} M sodium pyruvate, 15% fetal bovine serum, 2.0 X 10^{-3} M L-glutamine, 100 U penicillin/ml, 0.1 mg streptomycin/ml, and 250 ng amphotericin B/ml. Hybridomas were selected by addition of aminopterin to 4.0 X 10^{-7} M, adenine to 7.5 X 10^{-5} M, and thymidine to 1.6 X 10^{-5} M. Hybridomas were screened for antibody production by ELISA. Purified virus was bound to ELISA plates directly or by virus-specific rabbit polyclonal IgG, then cell culture fluids were added, followed by alkaline phosphatase conjugates of goat anti-mouse IgG and IgM. Virus-specific antibody was produced by 306 of 2,304 hybridomas, and 38 of these were cloned by limiting dilution. Monoclonal antibodies were produced in culture and in mouse ascites fluid. When selected monoclonal antibodies were tested against all three viruses, common and virus-specific epitopes were detected.

602

MOUSE MONOCLONAL ANTIBODY TO RICE GRASSY STUNT VIRUS AND ITS USE FOR DISEASE DETECTION. H. T. Hsu, P. Q. Cabauatan*, and H. Hibino*. USDA-ARS, Beltsville, Maryland 20705 and *IRRI, P. O. Box 933, Manila, Philippines.

A hybridoma cell line secreting IgG2b antibodies reactive to two strains of rice grassy stunt virus (RGSV) was established by the limiting dilution cloning technique. Infected plant sap diluted to 10^{-4} gave a positive reaction using monoclonal antibodies (MA) in double antibody sandwich ELISA. In dot-blot immunoassays (DBI) peroxidase labelled-MA gave similar positive reactions to both RGSV-infected and controlled healthy plant extracts. When alkaline phosphatase labelled-MA and a mixture of nitro blue tetrazolium and 5-bromo-4-chloro-3 indolyl phosphate p-toluidine salt were used in DBI no detectable background was observed with controlled healthy plant extracts. The RGSV antigen in 2.5 ul of a 10^{-3} dilution of infected sap could be detected by DBI. In latex tests, RGSV antigens could be detected with MA in a 10^{-3} dilution of infected sap.

603

SOME PROPERTIES OF MOUSE MONOCLONAL ANTIBODIES PRODUCED TO TULIP BREAKING VIRUS. H. T. Hsu, J. M. Franssen*, J. Hammond, A. F. L. N. Derks*, and R. H. Lawson. USDA-ARS, Beltsville, MD 20705 and *Bulb Research Center, Lisse.

Mouse monoclonal antibodies (Mab) produced to tulip breaking virus (TBV) were selected by indirect ELISA on antigen coated plates and were compared with polyclonal rabbit antiserum (Pab) for detection of TBV. In double antibody sandwich ELISA, lower A_{405} readings were obtained on Mab-coated plates than on Pab-coated plates. Low absorbancy was observed with enzyme-labelled Mab (Mab-E) on Mab-coated plates. Combination of Mab-E and Pab-coating gave better sensitivity than enzyme-labelled Pab (Pab-E) with Mab-coating. The best result was obtained when Pab-E was used on Pab-coated plates. In indirect ELISA on antigen-coated plates, detection of TBV was better with Mab-E than with Pab-E. TBV monoclonals could be used to differentiate strains of TBV and to distinguish at least 10 other potyviruses.

604

AN ANALYSIS OF MAIZE DWARF MOSAIC VIRUS STRAINS BY DOT IMMUNOBINDING ASSAY. M. A. C. Langham and R. W. Toler, Dept. of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

Maize dwarf mosaic virus (MDMV) strains A, B, D, E, F, and O were compared by indirect dot immunobinding assay utilizing goat antirabbit IgG labelled with horseradish peroxidase and 4-chlor-1-napthol color substrate. Sap dilutions from each strain were analyzed against antisera produced to MDMV-A representing early, mid, late, and very-late immunization and to each strain from early-mid immunization. Bleedings from two different rabbits were utilized for each antiserum represented. All results were analyzed by regression analysis. Strains A, B, E, and O of MDMV demonstrated distinct serological differences. MDMV-O was the most distantly related strain with a weak relationship to MDMV-A and MDMV-B and no relationship to MDMV-E. Strains D and F of MDMV closely paralleled the reactions of MDMV-A and could not be clearly separated from MDMV-A.

605

RELATIONSHIPS AMONG ISOLATES OF PEANUT MOTTLE VIRUS AND OTHER POTYVIRUSES: NUCLEIC ACID HYBRIDIZATION ANALYSIS. Mandhana Bijaisoradat and C. W. Kuhn, Dept. Plant Pathology, University of Georgia, Athens 30602.

Relationships of eight isolates of peanut mottle virus (PMV), peanut stripe virus (PStV), peanut mild mottle virus (PMMV), and tobacco etch virus (TEV) were established using nucleic acid hybridization analysis. Complementary DNAs to viral RNAs were synthesized by the random primer method with minor modifications. The nucleic acids were hybridized in solution under high stringency to R_0t=1.35. After treatment with S1 nuclease, assay of the hybrids showed that there was strong sequence homology among the nucleic acids of different isolates of PMV, ranging from 80-100%. TEV was more closely related to isolates of PMV than it was to PStV and PMMV, which were closely related to each other and have less than 50% homology with PMV. Nucleic acid hybridization results correlate with serological data, which showed no difference among PMV isolates. PMV was serologically related to TEV but not to PStV and PMMV.

606

DETECTION AND IMMUNOBLOT ELECTROPHORESIS OF CASSAVA dsRNAs. C.J. Gabriel, USDA-Agricultural Research Service, US Plant Introduction Station, Glenn Dale, MD, 20769 and B.L. Nolt, Centro Internacional de Agricultura Tropical (CIAT), Apartado Aereo 6713, Cali, Colombia.

Several species of double-stranded RNA (dsRNA) were found to occur in the cassava (Manihot esculenta) clone Secundina, which was thought to be virus free. Major dsRNA species reacted with dsRNA antibodies in immunoblot electrophoresis experiments. A large dsRNA molecule (6.6 X 10^6 M_r) was shown to be graft transmitted and concentrated in a nucleus rich fraction from infected plants. Virus-like particles were not detected in plants containing the large dsRNA using a variety of purification procedures. Smaller dsRNAs, ranging mainly between 0.61 to 0.41 X 10^6 M_r, were also detected in some plants which also contained the large dsRNA. These smaller dsRNAs were not transmitted by grafting. DsRNAs could be eliminated by meristem tip culture.

607

DETECTION OF WHEAT SPINDLE STREAK MOSAIC VIRUS BY ELISA AND A DOT-IMMUNOBINDING ASSAY. K. Zagula Haufler[1], D.J. Barbara[2], D.W. Fulbright[1] and R.M. Lister[2], Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824[1] and Dept. of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907[2].

Direct double antibody sandwich (DAS) ELISA in microtiter plates and dot-ELISA on nitrocellulose membranes were evaluated as alternatives to immunosorbent electron microscopy (ISEM) for detecting wheat spindle streak mosaic virus (WSSMV) in crude sap and in partially-purified preparations from wheat leaves. Preliminary results indicate that the dilution end-point for detecting WSSMV in both crude and chloroform-clarified sap by dot-ELISA was at least fourfold greater than that obtained by DAS-ELISA. Similarly, dot-ELISA resulted in at least a fifty-fold increase in sensitivity in partially-purified preparations. Both forms of ELISA are sensitive and potentially useful techniques for rapidly screening large numbers of wheat varieties for WSSMV. Comparisons with ISEM will be described.

608

SEROLOGICAL DETECTION OF SWEET POTATO FEATHERY MOTTLE VIRUS IN SWEET POTATO. J. W. Moyer, Dept. of Plant Pathology, North Carolina State University, Raleigh, 27695-7616.

A dot-blot immunobinding assay was developed for detection of sweet potato feathery mottle virus (SPFMV) in sweet potato. The relatively low titer and irregular virus distribution and with the high concentration of interferring substances (e.g. phenolics and latex) in sweet potato tissue have prevented the adoption of the double antibody sandwich variant of ELISA. Highly purified SPFMV was used as the immunogen for antiserum production. The antiserum was purified by cross absorption with extracts from healthy sweet potato followed by concentration with sodium sulfate precipitation and fractionation by protein A sepharose column chromatography. Visualization was by the indirect method using alkaline phosphatase - goat anti-rabbit conjugate. The method was suitable for detection of SPFMV in sweet potato plants and in plantlets in in vitro culture.

609

PRODUCTION OF MONOCLONAL ANTIBODIES TO WHEAT SOILBORNE MOSAIC VIRUS (WSBMV). Z. Bahrani[1], J. L. Sherwood[1], and M. R. Sanborn[2]. Plant Pathology Department[1] and Botany and Microbiology Department[2], Oklahoma State University, Stillwater, OK 74078-0285.

Stable hybridoma cell lines secreting monoclonal antibodies (MCA) to WSBMV were produced by fusing spleen cells from BALB/c mice immunized with an isolate of WSBMV from OK to mouse myeloma cell line P3x63Ag8.653. Hybridoma clones produced antibodies of the IgG_{2a}, IgG_{2b}, and IgG_3 subclasses. The MCA also reacted with an isolate of WSBMV provided by M. K. Brakke (University of NE), but not with wheat streak mosaic virus, tobacco mosaic virus, brome mosaic virus, tomato spotted wilt virus or a number of other potyviruses tested. The immunoreactivity of the MCA was compared to polyclonal rabbit serum for detection of WSBMV in ELISA and a dot-immunobinding assay. The two antibody sources reacted similarly in ELISA, but the MCA was superior to polyclonal serum in the dot-immunobinding assay.

610

DOSE-DEPENDENT INDUCTION BY LACCARIA LACCATA OF CONDENSED TANNINS AND RESISTANCE TO FUSARIUM OXYSPORUM IN PRIMARY ROOTS OF DOUGLAS-FIR. N. E. Strobel and W. A. Sinclair, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

When primary roots of Douglas-fir (DF) were grown in a 1% agar-nutrient salts medium amended with living mycelium of the ectomycorrhizal fungus Laccaria laccata (LL) at 0, 0.5, 1, 2, or 3 g (moist weight)/100 ml, LL suppressed root elongation, induced the accumulation of condensed tannins, and enhanced the resistance of roots to Fusarium oxysporum (FO) in a dose-dependent manner. This corroborates previous reports of root protection and induction by LL of condensed tannins in roots. Exposure to LL did not significantly affect the amount of ethanol-extractable (noncondensed) phenolic compounds in the roots. The most abundant of these compounds, (+)-catechin, had little effect on growth of FO when administered (in vitro) at concentrations up to 0.01 M. Noncondensed phenolic compounds may be unimportant in root protection.

611

GREENHOUSE SCREENING AS A METHOD FOR PREDICTING FUSIFORM RUST RESISTANCE IN THE FIELD. R. L. Anderson, Rt. 3 Box 1249-A, Asheville, NC 28806; and C. H. Walkinshaw, P. O. Box 2008 GMF, Gulfport, MS 39505; USDA Forest Service.

Predictions of fusiform rust in plantings of loblolly and slash pines have improved significantly. Progeny tests and greenhouse inoculations provide a data base for selection indices and prediction equations. Results at the Resistance Screening Center show that the best predictive variables (r^2=70%) for slash pine field infection are proportion (p) galled seedlings, p bark symptoms but no swelling, p rough bark, and p galls less than 25 mm. These are read 6 months after inoculation. Best predictor variables for loblolly pine (7-month readings) are p galled seedlings, p smooth bark, and p fat galls. Predicting disease severity and rust mortality is being approached by using greenhouse and field symptom types. Proportion with bark symptoms but no swelling appears the best predictor of rust mortality of slash pines at age 6. This variable is better than p galled seedlings for incidence and severity of rust.

612

COMPARTMENTALIZATION RESPONSE TO OAK WILT BY TWO EUROPEAN WHITE OAK SPECIES. F.H. Tainter, L.M. Haugen and J. Pinon, Department of Forestry, Clemson University, Clemson, SC 29634-1003; and INRA, Laboratoire de Pathologie Forestiere, Champenoux 54280, Seichamps, France.

Twelve seedlings of each of 3 Leucobalanus species (Quercus sessiliflora, Q. pedunculata, and Q. alba) were inoculated with Ceratocystis fagacearum and grown at 24, 28, and 32°C. Twig tips, upper and lower stems, and roots were sacrifice sampled at 3, 28, 53 and 76 days, cultured for recovery of C. fagacearum, and examined with light and electron microscopy. Seedlings of all 3 species had some wilt by day 76 but remained alive. C. fagacearum was recovered from 9/29 twigs, 3/29 upper stems, 11/29 lower stems, and 6/29 roots. Densely stained vertical strands of xylem with tyloses were present in all four sample locations, with strongest histological reaction at 28 days and after. Hyphae of C. fagacearum were sparse, but were observed in fibers and in vessels. Hyphae often appeared moribund but were sometimes ensheathed in a distinct structure of unknown origin.

613

BIOCONTROL OF TWO POSTHARVEST DISEASES OF APPLES WITH A YEAST. W. J. Janisiewicz, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Mixtures of spores of two apple pathogens, Penicillium expansum (blue-mold) and Botrytis cinerea (grey-mold), were used for inoculation of wounded Golden Delicious apples protected with different concentrations (10^5-10^7 CFU/ml) of an antagonistic yeast (LT-4-12) isolated from apple leaves. Spore mixtures consisted of 10^4 spores/ml of B. cinerea for all sample units and P. expansum 10 to 10^7 spores/ml in ten fold increases. On unprotected checks inoculated with spore mixtures, grey-mold prevailed at the lower concentrations of P. expansum (up to 10^2 spores/ml) both diseases appeared at 10^3-10^4 spores/ml and only blue-mold developed at higher spore concentrations. On apples protected with the antagonist prior to challenge with the fungal spore mixtures significant protection occurred.

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INFECTION COURT ON BLUEBERRY FOR BOTRYOSPHAERIA DOTHIDEA. T. I. Abdelgawad and F. F. Hendrix, Department of Plant Pathology, University of Georgia, Athens 30602.

Botrytis cinerea annually causes flower and twig blight in blueberry fields in Georgia, with incidence reaching 38% in 1984. In addition to direct loss caused by the fungus, the relationship to development of Botryosphaeria dothidea canker was studied. The latter often colonizes dead twigs before penetrating the xylem of living stems. It can produce fruiting bodies only until the bark is exfoliated. Botrytis was isolated from twigs which it had killed from March until mid June. However, primarily B. dothidea was isolated from similar twigs after mid June, indicating that B. dothidea succeeds Botrytis. Blueberry plants at full-bloom were inoculated with Botrytis and when twig blight was visible, the plants were inoculated with B. dothidea. The initial blighted area was increased and fruiting bodies of B. dothidea were observed 4 weeks after inoculation.

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PATHOGENIC VARIATION AMONG ISOLATES OF THREE COLLETOTRICHUM SPP. FROM STRAWBERRY. Barbara J. Smith, USDA-ARS, Small Fruit Res. Sta., Poplarville, MS 39470; and L. L. Black, Dept. Plant Path. & Crop Physiol., La. Agric. Expt. Sta., LSU Agric. Ctr., Baton Rouge, LA 70803.

Colletotrichum fragariae Brooks (Cf), C. acutatum Simmonds (Ca), and C. Gloeosporioides (Penz.) Penz. et Sacc. (Cg) (=Glomerella cingulata) all cause anthracnose diseases of strawberry. Thirteen Cf, five Ca, and two Cg isolates obtained from strawberry fruit and plants were tested for their virulence to 14 strawberry cultivars and one breeding clone. Pathogenic variation was evident among the three Colletotrichum species and among isolates within each species. All isolates caused a rot of ripe fruit. Following a plant spray inoculation, Cf isolates generally caused more severe petiole and crown symptoms than Ca isolates, which caused more severe symptoms than Cg isolates. However, some cultivars were more susceptible to certain Ca or Cg isolates than to some Cf isolates. All of the Cf, four of the five Ca, and one of the two Cg isolates tested caused a crown rot of certain cultivars.

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PATHOGENIC VARIATION AMONG COLLETOTRICHUM FRAGARIAE ISOLATES. Barbara J. Smith, USDA-ARS, Small Fruit Res. Sta., Poplarville, MS 39470; and L. L. Black, Dept. Plant Path. & Crop Physiol., La. Agric. Expt. Sta., LSU Agric. Ctr., Baton Rouge, LA 70803.

Colletotrichum fragariae Brooks causes anthracnose-crown rot of strawberry and may be devastating in both nurseries and fruit production fields of the southeastern U.S. Thirteen isolates of C. fragariae collected from various locations were tested for their virulence to 14 strawberry cultivars and one breeding clone. Following a plant spray inoculation, all isolates caused varying degrees of petiole lesions and leaf spots on most cultivars as well as a crown rot of the more susceptible cultivars. The cultivar Surecrop was the most suceptible of the cultivars tested while the breeding clone MSUS 70 was more resistant than any cultivar. None of the cultivars was resistant to all Colletotrichum isolates. The cultivars, Surecrop, Sunrise, Florida 90, Rosanne, Apollo and Tennessee Beauty, and the breeding clone, MSUS 70, are hosts that separate the isolates into ten races based on differential disease severity ratings.

617

RESPONSE OF SOUTHERNPEA (VIGNA UNGUICULATA) TO INFECTION BY THE SOUTHERN BLIGHT PATHOGEN (SCLEROTIUM ROLFSII) P. D. Dukes and R. L. Fery, U. S. Vegetable Laboratory, ARS, USDA, 2875 Savannah Highway, Charleston, SC 29407

Field studies were conducted in 1982 through 1985 to evaluate epidemiological parameters of southern blight (SB) on southernpea, to characterize the relationships between inocula levels, plant age and disease development, to determine the impact of the disease on yield, and to access the range of genetic variability. Disease symptoms varied greatly between genotypes and environments. Greatest disease development occurred when younger plants were inoculated; inoculum level had less influence on disease. Results of replicated studies using both inoculated and uninoculated plots showed that SB can cause great losses in yield in some genotypes but not in others. Seed yields were reduced up to 53% in some genotypes, and losses occurred in some cases where SB symptoms were not obvious. Reduced pod numbers accounted for most of the reduced seed yield.

618

EFFECT OF VIRUS INFECTION ON ROOT INITIATION AND SURVIVAL OF ASPARAGUS TISSUE CULTURE CLONES. T.A. Evans and C.T. Stephens. Dept. of Botany and Plant Pathology, Mich. State Univ., East Lansing, MI 48823-1312.

Commercial asparagus (Asparagus officinalis) is currently planted from seed but there is a growing interest in the use of tissue culture clones. The worldwide occurrence of several viruses in asparagus has led to an investigation the effect of virus infection on the propagation of asparagus via tissue culture. Axillary buds excised from spears of field-grown asparagus were grown on several modified Murashige-Skoog (MMS) media. Tissue explants from asparagus plants infected with asparagus virus 2 (AV-2) or asparagus virus 1 (AV-1) singly had average reductions in root initiation of 32% and 70% of controls, respectively, after 4 weeks. Explants from asparagus plants infected with both AV-1 and AV-2 had average reduction in root initiation of 82%. After 10 weeks, 85% of explants derived from virus-free plants, 70% of explants from AV-2-infected plants, 38% of explants from AV-1-infected plants and only 10% of explants from AV-1 and AV-2-infected plants still survived.

619

DEVELOPMENT OF CONTROL PROCEDURES FOR TOMATO SPOTTED WILT VIRUS (TSWV) DISEASE. J. J. Cho, W. C. Mitchell and R. Mau, University of Hawaii, Kula, 96790 and Honolulu, HI 96822.

Twenty-six insecticides were evaluated in the laboratory and eight selected for field tests. Methomyl, azinophos-methyl, acephate, mevinphos, cypermethrin and fluvalinate were effective in reducing thrips populations when applied once or twice a week at a rate of 200 gallons per acre. However, insecticides were not effective in controlling the incidence of TSWV in lettuce. Silver and clear polyvinyl alcohol screens were used as physical barriers and placed over lettuce beds or raised two feet over the tops of plants. Disease incidence was significantly reduced by these barriers where incidence ranged from 4.2% to 10.0% as compared to 90.8% for the uncovered control. Intermittent overhead mist irrigation applied daily from 7 am to 2 pm at 15 and 30 minute intervals for a duration of 2 and 4 minutes respectively were compared with drip irrigation. In four experiments TSWV disease incidence was reduced by both overhead misting treatments as compared to drip irrigation. Significant differences were observed in two of these experiments.

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TECHNIQUES FOR EVALUATING INDIVIDUAL PISUM SATIVUM PLANTS FOR REACTION TO THREE DISEASES. J. B. Mullen and D. J. Hagedorn, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

A procedure, using new techniques, has been developed for evaluating individual pea (Pisum sativum) plants for reaction to wilt (Fusarium oxysporum f. sp. pisi Race 1), powdery mildew (Erysiphe pisi) and the pea enation mosaic virus (PEMV). Pea seedlings were grown in vermiculite, root-dipped in a conidial suspension of the wilt pathogen when 10-12 days old, and tranplanted into a steamed sand-soil mixture contained in 12.5 cm clay pots. At the 6-7 node stage two leaves were removed, and each detached leaf placed over parafilm with the petiole protruding through into sterile water in a petri dish. One leaf was inoculated by dusting with E. pisi conidia; the other leaf was rubbed with PEMV inoculum. Inoculated leaves were incubated at 20°C under artificial light for 14 days. Expected disease reactions to all three pathogens were obtained with both resistant and susceptible pea cultivars.

621

THE EFFECT OF FROST ON BLACK CHAFF DEFVELOPMENT IN WHEAT. H. Azad & N.W. Schaad. Dept. of Plant, Soil & Ent. Sci., Univ. of Idaho, Moscow, ID 83843

The relationship of Xanthomonas campestris pv. translucens (X.c.t). to frost injury and subsequent colonization and infection of wheat plants was investigated. Nine strains of X.c.t. were ice nucleation positive in the Lindow droplet test at -8C. Wheat, barley, bean, and corn plants sprayed with suspensions containing 10^9 CFU/ml of X.c.t. (3 strains) resulted in frost injury ratings of 3 to 5 (0 = no injury, 5 = dead plant) after 20 min at -4C, whereas control plants rated 1 or <1. In addition, disease severity and frost injury increased when needle inoculated plants were exposed to -3C for 15 min at 0, 1, 2, or 3 days after inoculation. Populations of this bacterium as low as 5-10 CFU/cm^2 of leaf resulted in measurable frost injury at -3C. It appears that frost damage in wheat can result from epiphytic populations of X.c.t. and that such frost damage can in turn increase black chaff severity.

622

EFFECT OF RHIZOBIUM SPP. ON MACROPHOMINA PHASEOLINA. M. J. Zaki and A. Ghaffar. Department of Botany, University of Karachi, Karachi-32, Pakistan.

In dual culture plate assays indigenous Rhizobium strains isolated from nodules obtained from fields of Karachi inhibited radial growth of Macrophomina phaseolina. Rhizobium leguminosarum, R.meliloti and R.japonicum causing growth inhibition in vitro also caused a significant reduction in severity of Macrophomina root rot of Mung bean, okra and sunflower in green house experiments. These data suggest that the potential exists for reducing Macrophomina root rot by employing nodulating Rhizobium strains which are highly antagonistic to M.phaseolina.

623 Withdrawn

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BIOLOGICAL CONTROL OF GERANIUM RUST BY A BACTERIAL ANTAGONIST. J. R. Kytter and F. L. Lukezic. USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701, and Dept. Plant Path., The Pennsylvania State Univ., University Park, PA 16802

Twelve cultures of Bacillus spp. were isolated from leaf surfaces of Pelargonium X hortorum, cv. Snowmass, and tested in vitro for antagonism to Puccinia pelargonii-zonalis, causal agent of geranium rust. An isolate of Bacillus subtilis inhibited urediniospore germination and was selected for a replicated greenhouse trial. A filtrate from a nutrient broth culture (CF) of this isolate incubated for 48 hr was atomized onto plant leaves 1 to 2 hr prior to rust inoculation. The number of rust pustules was reduced 75% by the CF treatment compared with controls. A live bacterial suspension applied to leaves decreased infection but was not as effective as the CF. In most areas of the U. S., geranium rust is minimized primarily by inspection and quarantine procedures. These results suggest that geranium rust may be amenable to biological control.

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Antagonism of fluorescent pseudomonads to Agrobacterium tumefaciens strains isolated from apple. M. L. Canfield and L. W. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Agrobacterium radiobacter K84 has not prevented crown gall of apple seedlings as it has in other woody plants. Forty A. tumefaciens strains isolated from apple tumors were insensitive to K84 in vitro. Therefore, other bacteria that utilize opines were evaluated for their antagonism to A. tumefaciens. Fluorescent pseudomonads were isolated from roots and tumors of apple seedlings and tested for their ability to utilize octopine and nopaline, then screened for antagonism to three K84 insensitive A. tumefaciens strains from apple. Seventy-three of 103 opine utilizing strains produced a zone of inhibition on mannitol glutamate medium against the three A. tumefaciens strains. Both the size and number of tumors were reduced on tomato plants when two of these antagonists were coinoculated with each of 12 A. tumefaciens strains in a ratio of 10:1 cfu/ml. Higher ratios may be needed to give complete control.

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SELECTION OF BACTERIA ANTAGONISTIC TO PSEUDOMONAS CICHORII ON CHRYSANTEHMUM LEAVES. P. S. Randhawa and C. R. Semer IV, Technical Business Group, Yoder Brothers, Inc., Alva, FL 33920.

A simple technique that allows direct observations on disease development and minimized biological variation has been developed to select antagonistic bacteria. Young leaves of chrysanthemum cv. Mountain Peak are detached, surface sterilized with 0.5% sodium hypochlorite, blotted dry and laid (abaxial side up) on 0.5% water agar in petri plates. On one side of mid-rib two drops (10 ul each of suspension of a test bacterium in water (absorbance = 0.1 at 535 nm) are placed at selected sites on leaf surface and on the other side of mid-rib only water drops are placed. A sterile dissecting needle dipped in inoculum (1×10^7 cfu/ml of water) of Pseudomonas cichorii is used to scratch (0.1 mm superficial wound) the leaf surface through drops of the test bacterium (treatment) and water (control). After four days at 26C, a greyish black, round lesion (10 mm diameter) develops in controls. Twenty nine of 79 strains tested inhibited lesion development by 81-100%.

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A BACTERIAL DNA SEGMENT CAPABLE OF INHIBITING THE EXPRESSION OF ANTIBIOSIS TO THE TAKE-ALL PATHOGEN. A. R. Poplawsky and A. H. Ellingboe, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Two soil-inhabiting, mitomycin-resistant bacterial strains (Pseudomonas fluorescens B10 and gram-negative strain I21) are not effective in controlling take-all of wheat (causal agent: Gaeumannomyces graminis var. tritici, Ggt) in growth chamber bioassays, and are antibiosis-minus (do not inhibit the growth of Ggt in vitro). Clone banks of these strains were constructed in the cosmid, pLAFR1 and the mitomycin-sensitive E. coli strain HB101. Testing on mitomycin medium indicated that the clone banks were complete. Each clone bank member was mated with the antibiosis-plus Pseudomonas fluorescens biocontrol strain NRRL B-15135. None of the B10 transconjugants differed from NRRL B-15135 in antibiosis, but one I21 transconjugant showed a reduction in antibiosis. This isolate contained pLAFR1 with a 23 kb insert, and plasmid-less derivatives showed antibiosis similar to NRRL B-15135.

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A METHODOLOGY FOR MULTIPLE DISEASE RESISTANCE TESTING IN COMMON BEANS. Josias C. de Faria, CNPAF, EMBRAPA, Caixa Postal 179, 74000 Goiânia,Goiás - Brasil.

Several bean diseases including rust, angular leaf spot (ALS), bacterial common blight(CB) and anthracnose seriously limit bean production in Brazil and Latin America. We have developed a methodology to sequentially test common beans in greenhouse and nursery beds for the reaction to the causal agents of the above diseases. Bean cultivars, segregating population, or breeding lines are first inoculate with the agents of rust and CB on primary leaves of 8-9-day old plants. 17-19-day old plants are inoculated with Isariopsis griseola. Disease readings are taken at days 16-17 for CB, 20-21 for rust, and 32-35 for ALS. Inoculation with Colletotrichum lindemuthianum is done at 27-30 days after planting and disease reading is taken 10 days later. No significant visual interaction has been oberved among the diseases which would hamper germplasm evaluation. In a MDR breeding program the disease testing procedure of 4 years could be done in a single generation when the test is done between families at homozygose.

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THE SURVIVAL OF XANTHOMONAS CAMPESTRIS PV. CITRI ON CITRUS FRUIT. Harold E. Moline, USDA-ARS, Horticultural Crops Quality Laboratory, Beltsville, MD 20705

Valencia oranges were dipped in a suspension of the Florida strain of Xanthomonas campestris pv. citri (Xcc) and its survival was determined at 0° and 20°C under low (60-75%) and high (90-95%) relative humidity (RH) conditions, which were selected to simulate those found in packinghouses and storage. The bacteria were not able to survive on fruit surfaces at 20°C for more than 24-32 hours at low RH. At high RH the bacteria survived for only 6-8 hours. If infested fruit were stored at 0°C at either low or high RH, active bacteria could be isolated from the fruit for 7-8 days. The activity of 2 antibacterial agents was also tested against the Florida, Japanese, Brazilian and Mexican strains of Xcc. No bacteria survived after incubation for 2 minutes in 3600 ppm or higher concentrations of sodium orthophenophenate but all 4 strains survived in concentrations as high as 1800 ppm, the recommended treatment rate is 1000-1500 ppm. No bacteria survived after a 2 minute immersion in 50, 100 or 250 ppm chlorine; its recommended treatment rate is 250 ppm.

630

IN VITRO SENSITIVITY TESTING OF REPRESENTATIVE GROUPS OF PHYTOPATHOGENIC BACTERIA TO VARIOUS CHEMICAL COMPOUNDS. L.V. Coziahr and A.K. Vidaver, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

There has been little in vitro standardized testing of various chemicals (particularly copper compounds) for their effectiveness as bacterial disease control agents. An in vitro test was developed in which members of the phytopathogenic bacterial groups Erwinia, Pseudomonas, Corynebacterium and Xanthomonas were established as bacterial lawns on nutrient broth (NBY) agar plates. Kocide 101, Copper 53 Fungicide, Bordeaux mixture, Liquid Copper Fungicide, Tri-basic, Zineb 75 WP, Maneb, Dithane M-45, streptomycin, cupric sulfate (analytical) and zinc sulfate (analytical) were applied to three-hour-old bacterial lawns at recommended rates in 5 µl droplets. Sterile distilled water was used as a control. Measurements of the diameter of the zone of inhibition were taken at 24, 48 and 72 hours. Differences were found in sensitivity to the chemical treatments among the four groups of phytopathogenic bacteria tested and between pathovars and strains. This test shows potential for being a rapid means of screening chemicals for their effectiveness in disease control.

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ANTIMICROBIAL ACTIVITY OF NAPHTHOQUINONES PRODUCED BY FUSARIA. R. A. Baker and J. H. Tatum, USDA, ARS, SAA, Winter Haven, FL 33883-1909; and S. Nemec, USDA, ARS, SAA, Orlando, FL 32803.

Fusarium solani and F. oxysporum isolates cultured from citrus roots and grown on liquid mineral salts-glucose media produced naphthoquinones, naphthofurans, and anthracenediones. From 22 identified compounds, 10 were impregnated in filter paper discs and tested for antimicrobial activity against certain plant pathogenic bacteria and nonpathogenic fluorescent pseudomonads. These compounds were also screened for activity against eight citrus fungal pathogens. All ten compounds had antibacterial activity, but several strains of bacteria were not sensitive to any of the compounds. Pseudomonas viridiflava was the most sensitive of the plant pathogens, reacting to eight. Only cis-dihydrofusarubin exhibited antifungal activity, and only against Phytophthora parasitica. All 22 compounds were tested for activity against eight bacterial pathogens of humans. None were active against six gram-negative bacteria, but several of the compounds exhibited moderate to strong activity against Staphlococcus aureus and Streptococcus pyogenes.

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EFFECT OF NITROGEN AND DISEASE DEVELOPMENT ON EXPRESSION OF RING ROT IN FIVE POTATO VARIETIES. G.D. Easton and M.E. Nagle, Department of Plant Pathology, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350

Ring rot caused by Corynebacterium michiganinse pv. sepedonicum is a threat to all potato growers. Near Mt. Vernon, WA in a potato seed growing area, freshly-cut seed pieces of five potato varieties were dipped into a ring rot tuber slurry and hand planted. Symptoms on foliage of Norgold Russet, Red Pontiac, White Rose, Nooksack and Russet Burbank appeared first 77, 92, 105, 105 and 115 days, respectively, after planting. High N, 268 Kg N/ha compared to 45 Kg N/ha, had no effect on symptoms. These results indicate that ring rot inspection of fields for seed certification should be timed according to the variety.

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SPECIFICITY OF EPIPHYTIC INTERACTIONS OF PSEUDOMONAS SYRINGAE STRAINS ON LEAVES. S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Twenty-one Ice^+ and Ice^- strains of P. syringae were spray inoculated onto bean, corn, potato, and tomato leaves, incubated in moist conditions to permit large populations to develop and evaluated for their ability to act as "antagonists" to prevent the subsequent growth of isogenic rifampicin resistant or Ice^+ strains or heterologous strains applied as challenge inoculum on leaves. While no significant differences in total bacterial population size of antagonistic strains were measured on a given plant species, antagonists differed in their ability to inhibit the growth of challenge strains. Significant interactions between antagonistic and challenge strains in reducing the population size or numbers of ice nuclei of challenge strains occurred on all plants. No significant enhancement of the competitive exclusion of P. syringae by isogenic strains compared with non-isogenic strains occurred in all pair-wise comparisons.

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ENHANCED ROTTING OF CHRYSANTHEMUM CUTTINGS INFECTED WITH ERWINIA CHRYSANTHEMI UNDER ANAEROBIC CONDITIONS. P. S. Randhawa and C. R. Semer IV. Yoder Brothers, Inc., Alva, FL 33920.

Stem cuttings of Chrysanthemum x morifolium Ramat. were surface sterilized and inoculated by a cut given at the basal end with a scalpel previously dipped in suspension of Erwinia chrysanthemi. By this inoculation technique 7 µl bacterial suspension was deposited at the cut surface. Cuttings were placed in petri plates and incubated for 72 h at 25C in polyethylene bag (aerobic conditions) or in an anaerobic jar containing H_2 and CO_2 (anaerobic conditions). Semi-anaerobic conditions were provided by wrapping the cuttings in three layers of polyethylene wrap. Amount of inoculum required to rot the cuttings by 50% was 500, 13 and 5 colony forming units, under aerobic, semi-anaerobic and anaerobic conditions, respectively. Color of the rotted tissue was greyish black under aerobic and green under anaerobic conditions.

635 Withdrawn

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LOGNORMAL DISTRIBUTION OF EPIPHYTIC XANTHOMONAS CAMPESTRIS PV. PHASEOLI. C. Ishimaru and A.K. Vidaver, Dept. of Plant Path., D.P. Coyne, Dept. of Hort., Univ. of NE, Lincoln, NE 68583-0722.

Epiphytic populations of Xanthomonas campestris pv. phaseoli (Xcp) were determined by plating the washings from various dry bean cultivars onto MXP, a semi-selective medium. Individual leaves and pods, as well as bulk leaf samples (10 leaves per sample), were assayed. The estimated mean number of Xcp per leaf from bulk leaf samples was always greater than that from single leaves. This difference resulted from the fact that Xcp was lognormally distributed on three susceptible cultivars. The distribution was not lognormal for resistant cv. GN 'Tara'. Two colony types of Xcp were recovered, small and large. The former were always pathogenic on cv. 'Dark Red Kidney' and produced a diffusible brown pigment; the latter were variable for those characteristics. Thus, epiphytic populations of Xcp on certain dry bean cultivars were lognormally distributed and heterogeneous with respect to pathogenicity.

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PATHOGENICITY OF ERWINIA ANANAS TO MUSKMELONS IN TEXAS. B. D. Bruton, USDA/ARS, Subtropical Res. Lab., Weslaco, TX, 78596; J. M. Wells, USDA/ARS, Rutgers U., New Brunswick, NJ 08903; and G. E. Lester, USDA/ARS, Weslaco, TX.

Muskmelons grown in the Rio Grande Valley, Texas in 1985, and stored for 14 days at 4 C and 85% relative humidity developed conspicuous watersoaked epidermal lesions. Lesions were firm, slightly yellow-brown, up to 10 mm in diameter and extended 1-2 mm below the epidermis. Isolations on TSA agar yielded colonies of yellow bacteria which caused similar lesions in 60% of cucumber, 42% of cantaloupe and 19% of Honeydew melon wound sites. The bacterium was rod-shaped, facultatively anaerobic, motile, and was identified as Erwinia ananas. Strains reacted serologically with antisera against pineapple and Honeydew melon strains of E. ananas. Analysis of total cellular fatty acids of the muskmelon strains was similar to that for E. ananas: saturated/unsaturated acids ratio of 1.38, and percentage of hydroxy-substituted, branched chain and cyclopropane acids of 5.8, 0.5 and 4.5%, respectively.

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IN VITRO EXPRESSION OF THE RESISTANCE OF LETTUCE TO THE CORKY ROOT ROT TOXIN. J. Kao, D. H. Mitten, and D.E.H. Dunning. PLANT GENETICS, INC., 1930 Fifth Street, Davis, CA 95616

The bacterial pathogen of lettuce corky root rot produces a host-specific toxin in axenic culture. The toxin can discriminate resistant and susceptible lettuce cultivars at seedling and at callus levels. The seedling fresh weight of the resistant cultivars treated with the toxin were 122 to 228% of the non-treated control. The seedlings of the toxin-treated susceptible cultivars weighed 60 to 88% of the non-treated control. Calli of the resistant cultivars grown in a toxin-containing medium remained friable and light green in color and weighed 52 to 93% of the calli grown in a toxin-free medium. In contrast, calli of the susceptible cultivars treated with the toxin became necrotic and weighed 2 to 32% of the control calli. A small portion of the susceptible callus tissues survived the toxin treatment and continued to develop into shoots. However, plants derived from these shoots were susceptible to the bacterial infection in a greenhouse inoculation test.

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IDENTIFICATION OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI MUTANTS WITH ALTERED PATHOGENICITY USING A DETACHED BEAN LEAFLET BIOASSAY. A. Arunakumari and A.K. Vidaver, Dept. of Plant Pathology, Univ. of NE, Lincoln, NE 68583-0722.

A rapid and reproducible detached leaflet bioassay was developed to isolate pathogenicity and virulence mutants of the common blight pathogen, Xanthomonas campestris pv. phaseoli. Leaflets of Phaseolus vulgaris cv. 'Red Kidney' were inoculated with a pipetman tip (1-200 ul) touched to a bacterial colony. The tip was pressed gently onto 3 or 4 points on either side of the midrib. Leaflets were incubated under continuous light and 100% relative humidity. Wild-type strains produced typical symptoms of common blight in 8 to 10 days. X. c. pv. translucens, X. c. pv. vesicatoria and a nonpathogenic mutant of X. c. pv. DRL827, developed only a brown ring at the site of inoculation after 1-2 days. Of 1,800 mutants of DRL827 obtained by chemical mutagenesis, we identified 3 prototrophic nonpathogenic and 6 prototropic low virulence mutants. Results from detached leaflet bioassays correlated with those from greenhouse tests.

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INTERACTION BETWEEN PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA AND NONHOST PLANT SUSPENSION CULTURE CELLS. Ann. G. Matthysse and Mary C. Deasey. Department of Biology, University of North Carolina, Chapel Hill, NC 27514.

Pseudomonas syringae pv. phaseolicola induces a reaction similar to a hypersensitive response (HR) when incubated with carrot suspension culture cells. This reaction is prevented by the presence of a 0.2 µ filter between the bacteria and the plant cells and thus requires physical contact between the bacteria and plant cells. Mutants of the bacteria which fail to elicit an HR in vivo also fail to show the reaction with suspension culture cells. Both wild type and mutant bacteria grow in the plant tissue culture medium. During incubation with plant cells new bacterial proteins are made. Wild type bacteria show a transient binding to the plant cells. The mutants may be altered in this binding reaction.

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PATTERNS OF COLONIZATION OF VASCULAR TISSUES BY CLAVIBACTER XYLI SUBSP. XYLI IN STALKS OF SUGARCANE CULTIVARS DIFFERING IN SUSCEPTIBILITY TO RATOON STUNTING DISEASE. N. A. Harrison and M. J. Davis, Univ. of Florida, IFAS, Research and Education Center, 3205 College Avenue, Ft. Lauderdale, FL 33314.

A modified immune-blot assay was used to detect and enumerate vascular bundles containing C. x. subsp. xyli in internode tissues from diseased sugarcane cultivars CP 72-1210, CP 44-101 and CP 70-1133 which are susceptible, intermediate and resistant, respectively, to ratoon stunting disease. The proportion of infected bundles in stalks differed significantly between cultivars. Less infected vascular tissue was detected in CP 70-1133, as compared to CP 44-101 and CP 72-1210. In all cultivars, the proportion of infected bundles declined in internodes with increasing sampling distance from the base of each stalk. A highly positive correlation was found between the number of infected bundles and populations of C. x. subsp. xyli in sap extracts from corresponding internode tissues. The ratio of bacterial populations to infected bundles was similar for each cultivar at all sampling locations on stalks.

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DIFFERENTIAL RESPONSES OF POTATO TISSUE CULTURES TO INOCULATION WITH STRAINS OF PSEUDOMONAS SOLANACEARUM. Y. Huang, J. P. Helgeson and L. Sequeira, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

Callus tissue culture derived from five individual clones of the cultivated potato diploid, Solanum phureja (selected from progeny of a resistant x susceptible cross) exhibited either compatible or incompatible responses when inoculated with six different strains of P. solanacearum. There was a perfect correlation between these reactions and the susceptibility or resistance of the parent plants. In tissue cultures, incompatibility was characterized by rapid (60 hr) browning and collapse of the tissues as is typical of a hypersensitive response (HR). In the compatible response, the tissue remained light green in color during the same period. Diffusible factors, produced by P. solanacearum when in contact with incompatible callus tissues or cell suspension cultures, appeared to be responsible for the HR. This system has a great deal of potential for studying the biochemistry of HR induction in potato.

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MULTIPLICATION OF PSEUDOMONAS SYRINGAE PV. TABACI RACE 0 AND 1 IN LEAVES OF RESISTANT AND SUSCEPTIBLE TOBACCO. K. K. Knoche and R. W. Fulton, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The multiplication of Pseudomonas syringae pv. tabaci race 0 and 1 in leaves of two cultivars and a breeding line of tobacco was compared. Bacterial suspensions of race 0 and 1 were infiltrated into tobacco leaves with a hypodermic syringe and needle. The bacterial populations in the leaves were determined daily by dilution plating of tissue samples. Multiplication was highest in compatible combinations, Havana 142-race 0, Havana 142-race 1 and Havana 503-race 1, and was lower in incompatible combinations, Havana 503-race 0, 8A2S4-9-race 0 and 8A2S4-9-race 1. Typical wildfire symptoms developed in compatible combinations as the population reached approximately 10^7 cfu/ml, which corresponded to the beginning of stationary phase. However, no symptoms developed in incompatible combinations.

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GENOMIC FINGERPRINTS OF XANTHOMONAS CAMPESTRIS PV. CITRI STRAINS FROM ASIA, SOUTH AMERICA, AND FLORIDA. John S. Hartung and Edwin L. Civerolo, USDA, Agricultural Research Service, HSI, Fruit Laboratory, Beltsville, MD 20705.

Genomic DNA was prepared from strains of Xanthomonas campestris pv. citri isolated in seven nations and in Florida. After the DNA was digested with restriction endonuclease Eco R1, the fragments were separated by polyacrylamide gel electrophoresis and the resulting genomic DNA fingerprints were compared. The eleven strains which belonged to the Asiatic or A group were indistinguishable by this technique. Likewise, the 5 strains which belonged to the cancrosis B group were also indistinguishable from each other. The A and B groups were clearly differentiated by this technique. However, the strains isolated from diseased citrus in Florida showed a wide variety of genomic fingerprints. These results are inconsistent with the idea that a new strain of X. campestris pv. citri has been recently introduced into Florida but support the idea that the X. campestris strains isolated from Florida citrus are samples of an endemic flora.

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AN AGAR PLATING ASSAY FOR THE DETECTION OF CLAVIBACTER MICHIGANENSE SUBSP. MICHIGANENSE IN TOMATO SEEDS. M. Fatmi & N.W. Schaad, Dept. Plant, Soil & Ent. Sci., Univ. of Idaho, Moscow, ID 83843

Clavibacter michiganense subsp. michiganense (C.m.m.), the causal agent of bacterial canker of tomato, is a very destructive seedborne pathogen. A seed assay based upon SCM agar was developed for detecting seed lots contaminated with C.m.m. To extract the pathogen, 10,000 seeds were placed in a plastic bag containing 150 ml of 2-3C sterile 0.05 M PO_4, buffer pH 7.4, 0.02% Tween 20 and agitated at room temperature in a 'Stomaker' (Tekmar Co.) for 5 and 15 min. Samples of 0.1 ml were pipeted in triplicate onto SCM plates. The plates were incubated at 26C for five-six days and the speckled gray mucoid colonies of C.m.m. counted. C.m.m. was detected at a range of 1.0 x 10^3 to 7.5 x 10^5 CFU per sample in three of eight commercial seed lots. Furthermore, C.m.m. was detected at a range of 3.5 x 10^3 to 1.0 x 10^5 CFU in 4 samples of a mixture consisting of 100 seeds from a naturally contaminated (1% level) seed lot and 9,900 seeds from a noncontaminated lot.

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PURIFICATION AND CHARACTERIZATION OF CALMODULIN FROM THE BEAN RUST FUNGUS. L. Laccetti, R.C. Staples, and H.C. Hoch*. Boyce Thompson Institute for Plant Research, Ithaca, NY 14853; NY State Agr. Exp. Sta., Cornell University, Geneva, NY 14456.*

We have purified to apparent homogeneity, the calcium-binding regulatory protein, calmodulin (CaM), from Uromyces appendiculatus uredospores. Typical yields were 20 to 40 µg CaM/g spores. The purification procedures included the removal of heat labile proteins by boiling, precipitation of CaM at an acidic pH, and chromatography on DEAE cellulose and phenyl-sepharose. Final purity was obtained by electroelution from SDS PAGE gels. The purified CaM displayed the typical molecular weight shift from 15.9 to 16.7 kDa after electrophoresis in the presence of Ca^{+2} or EGTA, respectively, and stimulated cAMP phosphodiesterase in a Ca^{+2}-dependent manner. Several CaM inhibitors tested inhibited the in vitro stimulation of cAMP phosphodiesterase by bean rust CaM and inhibited spore germination when present in micromolar amounts. We compare such characteristics as molecular weight, pI, amino acid composition and antigenicity with other calmodulins.

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DELAY OF BACTERIALLY INDUCED HYPERSENSITIVE CELL DEATH IN SUSPENSION-CULTURED TOBACCO CELLS BY ALKALINE TREATMENT. J. L. Salzwedel, M. E. Daub, and J.-S. Huang. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Inoculation of suspension-cultured tobacco cells with Pseudomonas syringae pv. pisi elicits the hypersensitive reaction (HR). Hypersensitive cell death was followed over time by decreased fluorescence of fluorescein diacetate-stained suspension cells. Addition of 10 mM 1,4-diazabicyclo(2.2.2)octane (DABCO) to unbuffered,, inoculated tobacco cell suspensions resulted in an increase in pH from 5.8 to 9.0 and a delay in HR onset of over six hours. Addition of KOH to similar suspensions also increased pH and delayed HR cell death with length of delay depending on the alkalinity. Multiplication of the bacteria was not inhibited by the higher pH. With morpholinoethanesulfonic acid (MES) buffer (pH 6.0) maintaining pH in inoculated suspensions, DABCO and KOH did not delay HR cell death. The pH of the medium may affect the reported K^+/H^+ exchange mechanism of HR.

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HR INDUCED RESISTANCE IN CUCUMBER. J. A. Smith, R. Hammerschmidt and D. W. Fulbright. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Pseudomonas syringae p.v. syringae, pathogenic on wheat, elicits a hypersensitive response (HR) on cucumber and will induce systemic resistance when infiltrated into the first true leaf. HR induced resistance causes a systemic increase in peroxidase activity and is similar in every respect to pathogen induced resistance. The transposon Tn5 has been used successfully to generate mutants in P. syringae p.v. syringae. One particular mutant which fails to produce an HR is also unable to induce peroxidase or systemic resistance. The mutant has no reaction on the host plant, wheat. Currently, work is underway to characterize the mutation and obtain other mutants altered in their ability to induce resistance.

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SCREENING THE IR-1 COLLECTION OF SOLANUM SPECIES FOR RESISTANCE TO BACTERIAL WILT. E. Barlow[1], L. Sequeira[1], and R. Hanneman[2], [1]Dept. of Plant Pathology, and [2]USDA-ARS, Dept. of Horticulture, University of Wisconsin-Madison, Madison, WI 53706.

True seed of 1573 Solanum accessions, representing 102 species, were obtained from the Inter-Regional (IR-1) Potato Collection, Sturgeon Bay, WI. Approximately 50 seeds of each of two accessions were planted in a grid pattern in flats containing Jiffy mix. Seedlings were grown at 22°C in the greenhouse for 21 days. Flats were not watered for 3 days and then seedlings were inoculated by drenching each flat with 5 l of a mixture of P. solanacearum strains 8 (Race 1) and 81 (Race 3), at final concentrations of 6.0x10^5 and 1.2x10^6 CFU/ml, respectively. A knife was thrust between rows to injure the roots. Inoculated seedlings were placed at 28°C and monitored for wilt symptoms for 21 days. Survival rates among lines ranged from 0-100%. Several species such as S. demissum and S. phureja contained many lines with resistance to P. solanacearum and may be good sources of germplasm for breeding for bacterial wilt resistance.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR PECTIC LYASES. R. S. Livingston, D. A. Galuska, M. J. Klopmeyer and A. Kelman, Department of Plant Pathology, University of Wisconsin-Madison, 53706.

Monoclonal antibodies (MAbs) were produced against two major extracellular endopectate lyases (PL) of Erwinia carotovora subsp. carotovora (Ecc) to measure PL in potato tuber tissue during the infection process. The enzymes, PL I (MW = 42K) and PL II (MW = 40K), were purified by ammonium sulfate precipitation, ion exchange, gel filtration and agarose affinity chromatography from culture supernatants of Ecc (AK241). Spleen cells, from Balb/c mice immunized with PL I or PL II, were fused with NS1 myeloma cells and supernatants from the resultant hybridomas screened by an indirect ELISA. Of 30 monoclonal antibody-producing hybridomas that screened positive, 4 MAbs were specific for the PL I, 9 were specific for the PL II, and 17 recognized both the PL I and PL II enzyme preparations. The majority of the MAbs were of the IgG_1 isotype.

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PRODUCTION OF MONOCLONAL ANTIBODIES TO ERWINIA CAROTOVORA SUBSP. ATROSEPTICA LIPOPOLYSACCHARIDE. DeBoer, S.H. and Mc Naughton, M.E. Agriculture Canada, Research Station, 6660 N.W. Marine Drive, Vancouver, B.C., Canada, V6T 1X2

Monoclonal antibodies to E. carotovora subsp. atroseptica (Eca) strain 31 (SR8), serogroup I, lipopolysaccharide (LPS) reacted with purified LPS in ELISA and with whole cells in immunofluorescence (IF). In immunoblots of LPS separated on polyacrylamide gels, the monoclonals reacted with O-side chain containing LPS units but not the LPS R-core. In IF, they reacted with all serogroup I and XXII strains but not with ECA serogroup XVIII and XX strains, E. carotovora subsp. carotovora, or E. chrysanthemi. Blackleg-infected potato stems and tubers gave strong positive reactions in contrast to healthy potato tissue in both ELISA and IF. However, Eca could not be consistently detected in symptomless, contaminated tubers.

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ENHANCED PRODUCTION OF ROOT SURFACE PEROXIDASES BY PSEUDOMONAS PUTIDA COLONIZATION. Frederick G. Albert and Anne J. Anderson, Department of Biology, Utah State University, Logan, UT 84322

Plants exhibit a variety of biochemical changes in response to microbial challenge. Pseudomonas putida, a soil bacterium, may enhance plant growth. The bacterium aggressively colonizes root surfaces of Phaseolus vulgaris at levels of 10^6 cells /g tissue. Colonization causes changes in enzyme activities associated with root surfaces. A 15-fold increase in peroxidase, and a 1000-fold increase in IAA oxidase were observed in root washes of plants inoculated with P. putida compared to noninoculated plants, both grown under sterile conditions. Consequently, the ratio of activities for peroxidase, as measured by phenolic oxidation, to IAA oxidase was much lower in the P. putida inoculated system. Native PAGE revealed several peroxidase isozymes, found only in small quantities in root washes of sterile plants, to be enhanced in preparations of P. putida inoculated roots. The isozymes were purified by chromatography, and characterized by electrophoresis.

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A METHOD FOR DETECTING SOILS SUPPRESSIVE TO PSEUDOMONAS SOLANACEARUM. W. C. Ho[1], L. L. Chern[2] and W. H. Ko[2]. [1]Taiwan Seed Service, Shinshieh, Taichung, Taiwan and [2]Department of Plant Pathology, University of Hawaii, Hilo 96720

The streptomycin-resistant mutant of Pseudomonas solanacearum produced distinct colonies on a soil extract agar supplemented with streptomycin (10% soil extract, 500 ppm glucose, 500 ppm peptone, 500 ppm K_2HPO_4, 50 ppm PCNB and 1000 ppm streptomycin) in 3 days at 28 C. More than 90% of cells of the streptomycin-resistant mutant added to the conducive soil were recovered with this medium. The population of the mutant remained more or less constant in the conducive soil for 1 month. Population change of the mutant in 3 days was used to assess suppressiveness of the 76 soil samples collected from different areas in Taiwan. About 31% of the soil samples tested were suppressive to P. solanacearum, with recovery rates of less than 30%. The population of P. solanacearum decreased to less than 1% of the original level in 3 days in 5 of the soil samples tested.

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SOME INHIBITORY CHARACTERISTICS OF SOILS SUPPRESSIVE TO PSEUDOMONAS SOLANACEARUM. W. C. Ho[1], L. L. Chern[2] and W. H. Ko[2]. [1]Taiwan Seed Service, Shinshieh, Taichung, Taiwan and [2]Department of Plant Pathology, University of Hawaii, Hilo 96720

There was a direct relationship between the recovery rate of Pseudomonas solanacearum and soil pH when 46 soil samples collected in Taiwan were analyzed. Twelve soil samples which were strongly suppressive to P. solanacearum have pH values ranging from 3.8 to 5.7. The inhibitory effect of these soils decreased or disappeared when the pH was adjusted to 6. Sterilization by autoclaving did not affect the inhibitory effect of 8 suppressive soils. However, 2 suppressive soils became conducive after autoclaving. Aqueous extracts (pH 3.8 to 4.8) of 6 suppressive soils were also inhibitory to P. solanacearum. The extract of the conducive soil did not inhibit P. solanacearum even when its pH was adjusted to 3.5. This indicates that the inhibitory effect of these suppressive soil extracts was not due to low pH.

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PSEUDOMONAS SYRINGAE PV. TOMATO STRAINS ARE HOMOGENEOUS. T. P. Denny and C. P. Benner, Dept. of Plant Pathology, University of Georgia, Athens, GA 30602.

In preparation for cloning pathogenicity genes of Pseudomonas syringae pv. tomato (PST), we felt it was important to assess the genetic diversity of this pathovar. We evaluated 28 strains of PST isolated during the last 45 years by various workers in North and South America, Europe, New Zealand and Australia. The strains were tested for pathogenicity on tomato, carbohydrate utilization, fatty acid profile, phage sensitivity, plasmid profile, total DNA homology and DNA restriction fragment length polymorphism (RFLP). Seven P. syringae pv. syringae (PSS) strains, two of which are weak tomato pathogens, were included for comparison. The results indicated that the PST strains examined comprise a homogenous group that is clearly different from PSS. Furthermore, the results of the RFLP analysis may be the first step in reclassifying PST as a species separate from P. syringae, and suggest that PST specific DNA probes could be developed.

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ARIZONA PLANT DISEASE DATABASE. J.D. Mihail and M.R. Nelson. Dept. Plant Pathology, Univ. Arizona, Tucson, AZ, 85721.

A relational database has been constructed to hold disease and weather data for the state of Arizona, using RBASE 4000 relational software. Disease observations are recorded from several sources including University diagnositic clinics loacted in Phoenix, Tucson and Yuma. Additional records come from Cooperative Extension Agents in the counties as well as from historical documents. The database is composed of three relations (tables) which are related by a specific accession number for each record. One relation (BASIC) holds primary information including host plant, sample date, county, community, and up to three pathogens. In the relation BASIC, non-numeric data are numerically encoded using a heirarchical system. The relation DETAILS contains information concerning cultural practices and the relation, PEST contains data on pesticide usage and recommendations. The database will be used in the detection of new diseases and in monitoring the severity and distribution of established diseases.

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A SURVEY OF THE MICROORGANISMS PRESENT ON THE PHYLLOPLANE OF COMMON WATERPLANTAIN IN CULTIVATED WILD RICE. C.M. Huot and J.A. Percich. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Common waterplantain (Alisma triviale Pursh) is the most destructive weed pest of cultivated wild rice (Zizania palustris L.). A survey of the microorganisms present on the phylloplane of this weed was made through leaf prints, leaf washings and direct isolations. Leaves of common waterplantain were sampled from three grower's fields at five stages of plant development. A Septoria sp. was consistently isolated (100%) by all three techniques, at all fields, from pycnidia in lesions that were circular and brownish-black in color. Detected through leafprints, but not associated with the lesions were Epicoccum, Erwinia, Pseudomonas, and Xanthomonas spp. At seed set, Alternaria, Penicillium and Rhizopus spp. were isolated by all three techniques. Tests are in progress to identify from the microorganisms isolated, a possible biological control agent of common waterplantain in wild rice.

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WALNUT WITCHES'-BROOM DISEASE IN GEORGIA. C. J. Chang, L. K. Impson, and B. M. Cunfer. Dept. of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

Eastern black walnut (Juglans nigra L.) trees with witches'-broom symptoms were observed in Griffin, GA in 1985. Leaves were dwarfed, curled, and chlorotic. Three trees, two showing witches'-broom and one without symptoms were sampled for electron microscopy. Pleomorphic mycoplasma-like organisms (MLO) ranging from 100 to 650 nm in diameter enclosed in a trilaminar unit membrane were observed only in sieve elements of tissues of twigs, petioles, and leaf mid-veins from diseased trees. Spherical or oval shapes predominated, but tubular forms were also evident. This is the first report of an MLO associated with J. nigra L. in Georgia.

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EFFICACY OF NEMATICIDE SEED TREATMENTS IN CONTROLLING SEEDLING BLIGHT IN ALFALFA INCITED BY DITYLENCHUS DIPSACI AND MELOIDOGYNE HAPLA. F.A. Gray and D.H. Soh, Plant Science Department, University of Wyoming, Laramie 82071.

Studies were conducted to evaluate the efficacy of three nematicides (carbofuran, oxamyl and phenamiphos) as seed treatments for control of seedling blight in alfalfa incited by the stem nematode, Ditylenchus dipsaci, and the northern root-knot nematode, Meloidogyne hapla. Plant mortality was measured weekly for 5 wk. All three nematicides were efficacious (.05) in protecting seedlings from D. dipsaci in the resistant cultivar (cv.) Lahontan as well as the susceptible cv. Ranger. Seeding mortality caused by M. hapla was significantly reduced with all three nematicides in the resistant cv. Nevada Synthetic XX and by oxamyl and phenamiphos in the susceptible cv. Lahontan. D. dipsaci was more easily controlled with nematicide seed treatment than M. hapla. Severe seedling mortality occurred from both D. dipsaci and M. hapla even in resistant cultivars unprotected with nematicide seed treatment.

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AN ITERATIVE PROCEDURE FOR DETERMINING LIFE CYCLE PARAMETERS FROM STAGE FREQUENCY DATA. S. M. Schneider, USDA-ARS Tobacco Laboratory, Oxford, NC 27565 and H. Ferris, Division of Nematology, University of California, Davis, CA 95616

Simulation models for pest populations often require information on the duration of each life stage and the proportion of the population which survives from one stage to the next. Direct determination of these values for soil inhabiting organisms may be difficult or impossible. An interative computer procedure was developed to estimate these values from stage frequency data corrected for extraction efficiency. Using this procedure, parameter estimates were obtained that predicted development of a single generation of Callosobruchus chinensis, an insect pest. Application of the procedure to a multi-generation data set for the plant parasitic nematode, Paratrichodorus minor, predicted development adequately, but was more variable then with the insect data. Stage frequency data can be used to estimate life cycle parameters for soil inhabiting organisms.

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PHYTOPARASITIC NEMATODES FOUND IN FRUIT-PRODUCING SOILS OF WESTERN COLORADO. H. J. Larsen, Colo. St. Univ.-Orchard Mesa Res. Center, 3168 B 1/2 Rd., Grand Junction, CO 81503; and E. A. Nigh, Arizona Ag-Con, Inc., 1365 W. 17th Pl., Yuma, AZ 85364.

Eight genera of phytoparasitic nematodes were found in a survey of fruit-producing soils from 27 sites in three western Colorado counties. The survey included stone fruit, pome fruit, cane berry, and fallow sites. Maximum nematode numbers recovered from 150 ml of soil/root fragments ranged from 12 to 371. Helicotylenchus, Meloidogyne, Paratylenchus, Tylenchorhynchus, and Xiphinema spp. were recovered most frequently, while Hoplolaimus, Pratylenchus, and Rotylenchus spp. were found more sporadically and in small numbers. The maximum number of Xiphinema spp. found in a given sample was only 15, but their frequency of occurrence is potential cause for concern because they are known vectors for several tree fruit viruses. Numbers of Meloidogyne spp. from several sites exceeded 300 nematodes per sample, high enough for potentially significant root damage.

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ENDOGENOUS CYTOKININ ACTIVITY IN ROOTS OF PEANUTS SUSCEPTIBLE AND RESISTANT TO MELOIDOGYNE HAPLA. H. H. Fagbenle and A. B. Filonow. Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Roots of six-week-old peanut (Arachis hypogaea L.) lines susceptible (S) or resistant (R) to Meloidogyne hapla were extracted in 80% methanol. Extracts were purified by solvent partitioning and by column chromatography (CC) on polyvinylpolypyrrolidone and cellulose phosphate columns. Cytokinins were separated by CC on Sephadex LH-20 and thin layer chromatography (TLC) on silica gel in butanol:acetic acid:water (12:3:5 v/v). Fractions from LH-20 were analyzed by U.V. and by the radish cotyledon bioassay. Thin layer bands were analyzed by the same bioassay. Extracts of S peanuts yielded peaks of activity with band Rf values of .2-.3, .4-.5, .6-.7 and .75-.95, whereas R roots yielded peaks of activity with Rf values of .3-.5, .6-.7 and .8-.9. Susceptible roots also had higher activity at Rf .1-.2 than R roots. Cytokinin activities of healthy peanut roots and nematode-infected roots were also compared.

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NEMATODE MITOCHONDRIAL DNA FOR MOLECULAR DIAGNOSTICS. T.O. Powers, E.G. Platzer and B.C. Hyman. Department of Plant Pathology, Univ. of Nebraska, Lincoln, NE 68583 and Departments of Nematology and Biology, Univ. of California, Riverside, CA 92521.

We have begun to evaluate nematode mitochondrial DNA (mtDNA) for diagnostic probe construction. MtDNA has been isolated from four species of Meloidogyne, M. incognita host-races, and numerous isolates of the mosquito parasite Romanomermis culicivorax. Genomic DNA of R. culicivorax extracted from single worms and hybridized with a mtDNA probe indicates individuals are monomorphic for mtDNA genotype. Three distinct mtDNA genotypes from a laboratory population of R. culicivorax have been identified. No intrapopulation variation from Meloidogyne spp. has been observed. Numerous restriction fragment length polymorphisms exist between species of Meloidogyne and between populations of M. incognita. Field isolates of Meloidogyne species can be rapidly screened using DNA extracted from squashed root galls and hybridized with a mitochondrial DNA probe. We are currently investigating the correlation between interpopulation variation of mtDNA genotypes and host-races.

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RADIOIMMUNOASSAY OF GLYCEOLLIN I IN SOYBEAN ROOTS INFECTED WITH RACE 1 OF HETERODERA GLYCINES. Jeng-sheng Huang and K. R. Barker, Department of Plant Pathology, North Carolina State University, Raleigh, 27695-7616.

A radioimmunoassay method was employed to detect and quantify glyceollin I in soybean roots infected with soybean cyst nematode (SCN). Soybean root segments containing a single juvenile were dissected from infected susceptible 'Ransom' and resistant 'Centennial' soybeans; each segment was cut proximal to the nematode into 16-um sections at -20C with a cryostat microtome. Radioimmunoassay of these sections indicated accumulation of glyceollin I at the site adjacent to the nematode head 8 hr after inoculation in 'Centennial' but not 'Ransom' roots. These results suggest that glyceollin I may be an important early response of resistant soybeans to SCN infection.

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INOCULATION AND SURVIVAL OF XANTHOMONAS CAMPESTRIS PV. PRUNI ON PEACH LEAVES IN SOUTH GEORGIA. R. D. Gitaitis and P. Bertrand, Dept. of Plant Pathology, University of Georgia, Tifton, GA 31793 and Cooperative Extension Service, University of Georgia, Tifton, GA 31793.

Effects of inoculation date on disease development and epiphytic survival of Xanthomonas campestris pv. pruni (XPR) were evaluated. Different peach trees were inoculated with XPR every two weeks from 8 March to 17 May, 1985. Leaves from each inoculation date were assayed for XPR every two weeks until harvest. Early inoculation dates resulted in the highest disease levels. Trees that were inoculated on 22 March were used to monitor XPR survival throughout the summer. Bacterial populations became more homogeneous over time as XPR spread and colonized leaf surfaces. A sudden drop in XPR populations was detected on 28 October. A six-leaf bulk sample was superior to a 25-leaf bulk sample for estimating leaf surface populations of XPR determined from 25 single leaf washings.

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ENDOPHYTIC ERWINIA AMYLOVORA NOT RECOVERED FROM CORE TISSUES OF APPLES FROM APPARENTLY HEALTHY TREES. T. van der Zwet, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430; S. V. Thomson, Utah State University, Logan, UT 84327; R. P. Covey, Tree Fruit Research Center, Wenatchee, WA 98801 and W. G. Bonn, Agriculture Canada, Harrow, Ontario NOR 1GO.

Mature, apparently healthy apples of Red Delicious and other varieties were collected from orchard trees with and without visible symptoms of fire blight in West Virginia, Utah, Washington, and Ontario. Ten fruit each were surface sterilized and cores (1 cm diam.) were removed and cut into 3 sections between stem and calyx end. Finely chopped core tissue was placed in 2.0 ml sterile saline. After 1 min. vortexing, 0.05 ml of saline were spotted in triplicate on Miller-Schroth and CCT selective media and plates were incubated for 3 days at 26 C. Erwinia amylovora was not recovered from symptomless Red Delicious orchards, nor from York (WV), Rome Beauty (UT) and Idared (Ont) varieties. Only in Utah, was E. amylovora recovered from 5 of 10 Red Delicious and 2 of 10 Rome Beauty apples from severely blighted trees.

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ELICITATION OF PHYTOALEXIN ACCUMULATION IN COTTON COTYLEDONS. M. Pierce, A. Mort, and M. Essenberg. Dept. of Biochem., Oklahoma State U., Okla. Agricultural Expt. Station, Stillwater, OK 74078

We have begun an investigation of the mechanism of phytoalexin accumulation in the cotton/Xanthomonas campestris pv. malvacearum (Xcm) host/pathogen system using cotton line OK 1.2, an outstanding producer of cotton's phytoalexins. We are bioassaying for elicitation of necrosis, yellow-green autofluorescence characteristic of the phytoalexins lacinilene C and its methyl ether, as well as phytoalexin content. Cotyledons are infiltrated through open stomata with a test solution applied with a needle-less syringe to the abaxial surface. Application of water by this method causes no wounding. Culture filtrates of Xcm grown in a minimal medium using cotton cell walls as the carbon source were found to contain a heat-stable elicitor of phytoalexin accumulation. Fragments released from citrus pectin by acid hydrolysis also elicited phytoalexin accumulation. At effective concentrations the hydrolysate also caused patches of necrosis. Strong elicitation of yellow-green autofluorescence and phytoalexin accumulation was found with other treatments which also caused limited but macroscopically visible necrosis, such as 0.1 mM cupric sulfate and wounding with a hemostat.

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POPULATIONS OF XANTHOMONAS CAMPESTRIS PV. ORYZAE IN DIFFERENTIAL RICE (ORYZA SATIVA L.) CULTIVARS. P. A. Barton-Willis, M. L. Rhoads, and J. E. Leach. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Bacterial populations were monitored in race-specific interactions of Xanthomonas campestris pv. oryzae and rice. Incompatibility in this interaction is not hypersensitive but rather results in reduced lesion length. Compatible and incompatible populations increased equally for 2-4 days (to 10^5-10^7 cfu/leaf), but thereafter the growth rate of incompatible bacteria slowed in comparison to compatible. The time at which the differential growth rate occurred was dependent on the initial inoculum; when heavier inoculum was applied, the time period was shorter. After populations at the inoculation point reached 10^5-10^6 cfu/2 cm section, bacteria advanced out from that point at a rate of approximately 2 cm/day in the compatible and 0.8 cm/day in incompatible interactions. No symptoms were observed in leaf sections containing less than 10^7 cfu/2 cm section. Mixed population experiments are in progress.

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An Extracellular Protease-Deficient Mutant of *Xanthomonas campestris* pv. *malvacearum* Maintains Its Pathogenicity to Cotton. R. K. Gholson and M. Essenberg. Dept. of Biochemistry, Oklahoma State University, Oklahoma Agricultural Experiment Station, Stillwater, OK 74078

Daniels *et al.* (EMBO J. 3:3323, 1984) reported that a pathogenicity negative mutant of *X. campestris* pv. *campestris* is deficient in extracellular protease and pectate lyase. We have prepared a stable, extracellular protease-deficient mutant of *X. campestris* pv. *malvacearum* (Xcm) by treating race 3 with NTG and screening for colonies not forming clear haloes on nutrient agar plates supplemented with 1% skim milk. This mutant (3PM1) and its race 3 parent were spray-inoculated into leaves of susceptible cotton line Ac44 at 5 X 10^6 CFU/ml. Both strains produced water soaked lesions typical of the disease and both reached about 5 X 10^9 CFU/sq cm at day 11. The mutant reisolated from leaves completely retained its haloless phenotype. 3PM1 grows more slowly than race 3 in defined liquid medium with 0.1% bovine serum albumin as nitrogen source. Culture filtrates from race 3 have about 4-5 times higher protease activity on azocasein than culture filtrates of 3PM1 at the same growth stage.

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CONTROL OF MYCENA CITRICOLOR, THE CAUSAL AGENT OF THE AMERICAN LEAF SPOT OF COFFEE, BY LIME SPRAYING. J.P. Tewari and D.V. Rao, Dept. of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

Detached leaves of coffee were scratched and sprayed with aqueous solutions of lime at the rates equivalent to 4, 8 and 10 kg/ha (containing 0.2% spreader sticker, Citowett Plus) in a RIC spray chamber. Each scratched site on the leaf was inoculated with three gemmae of Mycena citricolor. Leaf spot development was completely inhibited in inoculated leaves sprayed with lime at the rate equivalent to 10 kg/ha. In the controls about 95% of the inoculations developed into spots. These results indicate that lime has the potential of controlling this pathogen in the field. Secretion of oxalic acid by the pathogen is the major cause of symptom developmnent in this disease. Scanning electron microscopy and energy dispersive X-ray microanalysis revealed that application of lime counteracts this process by neutralizing the acid secreted by the pathogen.

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PRELIMINARY STUDIES OF POLYSSACCHARIDES COMPOSITION OF THE INFECTION STRUCTURES OF HEMILEIA VASTATRIX USING FITC-LABELED LECTINS A. CORRÊA JR. and W.B.C. MORAES. Plant Biochemistry Dept., I. Biológico, CP. 7119, 01000 SP - BRASIL.

Fluorescence microscopy studies were undertaken to show the carbohydrates distribution on the cell walls of infection structures of H. vastatrix. Fluorescein-isothiocyanate(FITC) labeled lectins of Canavalia ensiformis(Con A), Lens culinaris(LLA), Triticum vulgaris(WGA) and Glycine max(SBA) were tested for binding to the infection structures of the pathogen. FITC-labeled ungerminated urediniospores and germlings at different stages of development were examined under fluorescence microscopy. Thin sections of inoculated leaves were treated with FITC-labeled lectin. Inoculated leaves after infiltrating with FITC-labelled lectins were sectioned in thin cuts which were also observed under fluorescence microscopy. Presence of -mannose, -glucose, N-acetylglucosamine and N-acetylgalactosamine was evidentiated in the cell walls of urediniospores and their infection structures.

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INFLUENCE OF THE WASHING OF INTERCELLULAR SPACES OF COFFEE LEAVES INDUCED TO RESISTANCE ON THE SYMPTOMS EXPRESSION OF COFFEE LEAF RUST. D.M.M.M. CAVICHIOLI and W.B.C. MORAES. Plant Biochemistry Dept., I. Biológico, CP. 7119, 01000 SP - BRASIL.

Infection rate in detached coffee leaves (Coffea arabica cv. "Mundo Novo") decreased of ca.80% when leaves were submitted to water washing of the intercellular spaces followed by inoculation with a spore suspension of Hemileia vastatrix. However, when yeast extract was applied to the leaves 72 hr before the washing treatment and inoculation with the pathogen, an increase of ca. 50% in the number of infection sites was observed in induced leaves submitted to the preinoculation washing treatment when compared with the non induced controls.

Chemical tests indicate significant changes in the total content of neutral sugars and proteins in the two intercellular washing fluids (IWF).

674 Withdrawn

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DISEASES OF MAJOR FOOD CROPS GROWN IN THE BAY REGION OF SOUTHERN SOMALIA, EAST AFRICA. F.A. Gray, B.J. Kolp and M.A. Mohamed, Plant Science Department, University of Wyoming, Laramie, WY 82071.

A disease survey of major food crops was conducted in 1984 in the semi-arid Bay Region of southern Somalia, East Africa. Diseases of sorghum included sooty stripe (Ramulispora sorghi), covered kernel smut (Sphacelotheca sorghi), anthracnose (Colletotrichum graminicola), downy mildew (Sclerospora sorghi), head smut (Sphacelotheca reiliana) and head mold (Cladosporium herbarum). Major diseases of cowpeas included ashy stem blight (Macrophomina phaseoli), powdery mildew (Erysiphe polygoni) and bacterial blight (Xanthomonas sp.). Crown and pod rots, caused by Rhizoctonia solani and Aspergillus niger, were the major diseases of groundnut while ashy stem blight and angular leaf spot (Phaeoisariopsis griseola) were the major diseases of mungbeans.

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SPACE GREENHOUSES COULD OPERATE EFFICIENTLY AT LOW PRESSURES IF FUNGI ARE CONTROLLED. C. H. Walkinshaw, Southern Forest Experiment Station, P. O. Box 2008 GMF, Gulfport, MS 39505.

Bean, corn, cotton, lettuce, sorghum and wheat seeds germinated and grew at 105 mm Hg pressure with a 12 hour photoperiod of 5,000 to 8,000 lux of lighting. Adjustment of air to contain 10% carbon dioxide and 10% oxygen (v/v) at this low pressure permitted growth of additional cultivars. In other tests, soybean callus produced identical amounts of chlorophyll a and b, protein, and dry weight at 250 and 760 mm Hg pressure. Carbon monoxide and methane increased in 105 mm Hg pressure chambers while sulfur dioxide remained low. Fungal growth on media and seedlings at 105 mm Hg pressure was extensive with cowpea, okra, endive, pole bean, sorghum, spinach, and wheat. However, contamination was not a problem in chambers at 250 mm Hg pressure. Results from these chamber tests indicate that space greenhouses could have minimal structure and could operate at low pressures. This research was supported by the National Aeronautics and Space Administration and USDA, Forest Service.

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METHODS FOR DETAILED STUDY OF THE SPLASH DISPERSAL PROCESS. K. M. Reynolds, L. V. Madden, D. L. Reichard, and M. A. Ellis, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

A drop-generating and photographic system has been developed to record detailed information on splash dispersal events. Uniform drops, 0.18-1.88 mm diameter, are produced by a piezoelectric crystal which is activated by a pulse generator. Uniform drops of larger diameter are produced by a solenoid pump with a similar activation mechanism. Both drop generators produce single drops on demand. The drop generator activation signal is used to activate a microprocessor-controlled timing circuit that can control up to seven strobe lamps for use in multiple exposure photography. A primary programmable delay controls the start of the flash sequence. Delay between flashes is controlled by an oscillator signal input to the timer. The system is being used to study splash dispersal of sporangia of Phytophthora cactorum from the surface of infected strawberry fruit. Data is being acquired on the size of splash zone as well as the number, size, and velocities of droplets produced.

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CYTOLOGICAL EFFECTS OF BACILLUS SUBTILIS AGAINST MONILINIA FRUCTICOLA ON POST-HARVEST PEACHES. B.E. Hazen and J.R. Aist. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

To examine how Bacillus subtilis (Bs) controls Monilinia fructicola (Mf), fruit wounds (4 mm deep) were treated with either 20 µl of nutrient yeast dextrose broth (NYDB), ca. 10^8 cfu of Bs/ml NYDB (Bs/N) or Bs cells suspended in water (Bs/W), then challenged one hour later by inoculating the wounds with 20 µl of an aqueous suspension of ca. 10^5 Mf conidia/ml. Sections were excised between 0 and 48 h after inoculation and processed for electron and light microscopy. Conidia and hyphae were often associated with Bs cells in a fibrillar matrix. Germination was prevented or delayed by Bs/N and Bs/W. Mf colonized wounds, but only Bs/W-treated fruit developed lesions. The lesions were about one-fifth the size of those in NYDB-treated fruit. Hyphal elongation was reduced by Bs/N and Bs/W compared to NYDB treatment. Some hyphal tips were lysed by Bs/N. Control by Bs includes inhibition of spore germination and hyphal growth.

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FORMATION AND REGENERATION OF PROTOPLASTS OF GLIOCLADIUM ROSEUM, G. CATENULATUM, AND G. VIRENS. M. L. Seh and C. M. Kenerley. Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843

Protoplasts were prepared from germinated, but not ungerminated, conidia of three Gliocladium species by cell wall digestion using commercially available enzymes: chitinase (5mg/ml), lyticase (5mg/ml), and cellulase "onuzaku" (20mg/ml) in 0.5M mannitol, pH 5.2. The number of protoplasts formed, assayed against the duration of incubation and concentration of germinating conidia in the lytic solution, was constant over 2-6 hrs; optimum production at 200, 300, and 400 mg/ml for G. catenulatum, G. roseum, and G. virens, respectively, was 6.1, 1.0, and 0.1 x 10^8 protoplasts/ml of lytic solution. Protoplasts were incubated on 9 different media with variable C and N sources and osmoticums to determine conditions of optimal regeneration. Regeneration was 6-40% depending on the medium and species. This protocol for protoplast formation and regeneration will be used in protoplast fusion and transformation studies of these candidate biocontrol agents.

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PATHOGENIC VARIATION IN ISARIOPSIS GRISEOLA, CAUSE OF ANGULAR LEAF SPOT OF BEAN. F.J. Correa and A.W. Saettler, ARS/U.S. Department of Agriculture, Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824-1312.

Angular leaf spot, caused by Isariopsis griseola, is a major worldwide disease of dry edible beans. To assist efforts in breeding resistant beans, we examined pathogenic variation in 70 isolates of the pathogen from Latin America and Africa. Monosporic cultures were inoculated to 21-days-old plants of a series of 8 bean differentials in the greenhouse. Inoculum was prepared from V-8 juice agar plates to contain 10^4 spores/ml. Plants were maintained at 100% RH for 4 days, 22-26 C, followed by an additional 10 days in the greenhouse. Results suggest that the pathogen has six major pathogenicity groups. Differences were noted between isolates relative to virulence characters such as size and number of lesions, sporulation density, and amount of chlorosis in infected leaves.

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ARMILLARIA MELLEA Quelet. INFECTED BY BACTERIA AND RICKETTSIAE-LIKE ORGANISMS. W. WELVAERT, G. SAMYN. Rijksuniversiteit-Gent,Coupure,653,B-9000 Gent (Bel.)

When pieces of rhizomorphs of Armillaria mellea s. str. Romagn. were plated out on agar medium in order to obtain pure fungus cultures in many cases bacteria were present, some of them could even be identified as pathogenic.
By E.M. observations of thin sections it appeared that high concentrations of bacteria were visible in the mycelial cells next to organisms with a typical morphology: elongated forms with a crisped cell wall, analogous to those of Rickettsiae. These forms were not found in pure cultures on agar medium.

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ANASTOMOSIS GROUPS AMONG BINUCLEATE RHIZOCTONIA ASSOCIATED WITH STRAWBERRY ROOT ROT IN CONNECTICUT. S. B. Martin, Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT 06504.

Rhizoctonia spp. were isolated from lesions on strawberry roots from five commercial fields in Connecticut in the spring and early summer of 1985. Isolates were identified as R. solani or binucleate Rhizoctonia spp. based on staining of nuclei with DAPI. Rhizoctonia solani was isolated very infrequently (4/149) in comparison with binucleate Rhizoctonia spp. Binucleate Rhizoctonia were grouped into at least four anastomosis groups by pairing with tester isolates from Japan (Ogoshi, et al. 1983. J. Fac. Agr. Hokkaido Univ., Vol. 61:244-260). Isolates were assigned to AG A (39/149), AG G (64/149), AG I (23/149), and the remainder (19/149) which has not been grouped. Pathogenicity to strawberry was confirmed for representative isolates within each anastomosis group, although virulence was extremely variable. These results indicate heterogeneity among binucleate Rhizoctonia spp. associated with strawberries.

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EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON LONGEVITY OF PLASMOPARA HALSTEDII ZOOSPORANGIA. T. J. Gulya, USDA-ARS and Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105

Detached zoosporangia of Plasmopara halstedii, the causal agent of sunflower downy mildew, were stored at temperature-relative humidity (RH) regimes of 7 and 18C, and 35 and 75% RH. Sporangial viability, measured as infectivity, was determined bi-weekly for 4 wk by inoculating 200 seedlings/treatment with 10^4 spores per ml. The use of Evans blue, phenosafranin, and fluorescein diacetate to stain viable sporangia was unsuccessful. The greatest longevity was observed with sporangia stored at 7C and 35% RH. After 3 days infectivity dropped to 53% and gradually declined to 22% at 28 days. Longevity was poorest at 18C and 75% RH. Infectivity at 3 days was 23%, dropping to 3% after 7 days where it remained constant through 28 days. Under temperature and RH conditions normally encountered during sunflower planting in North Dakota zoosporangia remain infective for up to 4 wk and may therefore play an important role in disease dispersal over long distances.

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RELATIONSHIP OF YIELD LOSS WITH FOLIAR DISEASES OF SORGHUM LAND RACES UNDER SUBSISTENCE FARMING IN HONDURAS. G.C.Wall, M.J.Jeger and R.A.Frederiksen, Dept. of Plant Path. and Microbiol., Texas Agr. Exp. Sta., College Station, TX, 77843.

Data were collected in farmers' sorghum fields where gray leaf spot, smut, rust, and oval spot epidemics occurred in 1985 to develop a multivariate model for predicting yield. Several models were tested to explain variation in yield based on various parameters measured on individual plants. Data were first subjected to principal component analysis, and the major principal components were used to develop a multiple regression model to explain variations in yield. The best model included the following parameters: percentage severity of the 4 diseases, panicle length and plant height. Yield differences of up to 7% were estimated between the minimum and maximum disease severities observed by holding plant height and panicle length at mean values. Using the observed plant height and panicle length corresponding to actual disease severities, yield differences estimated were 14.6%, 3.6%, and 5.5% for gray leaf spot, rust and oval spot, respectively. Losses to smut were insignificant.

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SPATIAL PATTERN OF MACROPHOMINA PHASEOLINA IN A CULTIVATED FIELD. J.D. Mihail and S.M. Alcorn. Dept. Plant Pathology, Univ. of Arizona, Tucson, AZ, 85721.

The spatial pattern of Macrophomina phaseolina sclerotia in an Arizona field soil was characterized using population data from three blocks each containing 750 (31 x 102 cm) quadrats. Observed frequency distributions did not fit the Poisson, Negative Binomial, or Neyman Type A distributions. The values of variance to mean ratios (1.5-6.2) and of Morisita's Index of Dispersion (1.2-1.8) indicated an aggregated spatial pattern in the field. Repeated computer simulations of the simple random sample and cluster sampling approaches showed that selection of four to eight clusters of three adjacent quadrats per block was most efficient for estimating the population means. Number of clusters selected was dependent on the degree of positive skewing in the population frequency distribution. Comparison of the random sampling and cluster sampling simulations with traditional sampling methods will be discussed.

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Factors affecting the development of leaf spots in Croton
Einat Cohen, A. Dinoor and Nava Eshed
Faculty of Agriculture, Rehovot, Israel

Colletotrichum variegatum in croton causes leaf spots with reddish halos, wilting and shedding of very young leaves, resulting in severe damage to potted plants. Factors enhancing disease development are: 1. Young age of leaves. 2. No need for abrasing or wounding. 3. Load of inoculum may be very low. 4. Lack of sanitation. 5. Free water on leaves. 6. Temperature -opt. 25°C. 7. Season - in Israel, more severe in summer. 8. Varietal response- America and No.16 most susceptible. Bravo and Norma are more resistant. 9. Latency on older leaves, was found even one year after inoculation. Latency occurred also naturally. 10. Chemical control of the disease (visible and latent) was limited to prophylactic sprays. Eradication was not possible. Benlate, bravo, captan and maneb were effective. Integrated control may succeed without chemicals. Additional pathogens, differing in important characteristics from the above, were isolated from dry necrotic spots: two types of Glomerella, a Phoma - like fungus and Alternaria.

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HOMOTHALLIC AND HETEROTHALLIC ISOLATES OF *FUSARIUM SUBGLUTINANS*. **John F. Leslie[1], Fred Hwang[2], and Frank J. Doe[2]. [1]Dept. of Plant Pathology, Kansas State University, Manhattan 66506. [2]Dept. of Biology, University of Dallas, Irving, Texas 75061.**

Heterothallic and homothallic progeny were recovered in a 1:1 ratio from a cross of two naturally occurring heterothallic *F. subglutinans* isolates. Among the heterothallic progeny, both mating types were recovered. Homothallic progeny could serve as male parents in crosses with both heterothallic mating types. Cultures originating from single ascospores from homothallic perithecia were also homothallic. Cultures originating from uninucleate microconidia from homothallic isolates were homothallic, indicating that homothallism was of a primary rather than a secondary nature.

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ANTIBIOTIC RESISTANT AND DEPENDENT MUTANTS OF CROSS-INDUCING SPECIES OF PHYTOPHTHORA. P. J. Ann and W. H. Ko, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI 96720.

Culture blocks of Phytophthora parasitica, P. palmivora, P. capsici and P. cinnamomi were placed on 5% V-8 agar containing 300 ppm streptomycin or 100 ppm chloramphenicol, and incubated at 24 C for one month. All except P. cinnamomi were inhibited by streptomycin and chloramphenicol. Streptomycin resistant mutants obtained include 17 isolates (6 A^1:11 A^2) of P. parasitica, 2 isolates (1 A^1:1 A^2) of P. palmivora and 1 isolate (A^1) of P. capsici. Four isolates (A^2) of P. parasitica and 1 isolate (A^1) of P. palmivora became streptomycin dependent requiring the presence of streptomycin for growth on V-8 agar. Six isolates (1 A^1:5 A^2) of P. parasitica and 1 isolate (A^2) of P. capsici were resistant to chloramphenicol. Three isolates (A^2) of P. parasitica were resistant to both streptomycin and chloramphenicol. These mutants appeared to be suitable for genetic studies because normal oospores were produced when opposite mating types of antibiotic resistant mutants were paired.

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VARIATION IN ENZYME PRODUCTION AMONG SPECIES AND ISOLATES OF THE SAME SPECIES OF CROSS-INDUCING PHYTOPHTHORA. P. J. Ann and W. H. Ko, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI 96720.

Solid media for assaying the activity of different extra-cellular enzymes were used to determine the variation in enzyme production among 19 isolates of Phytophthora parasitica, 18 isolates of P. palmivora, 9 isolates of P. cinnamomi and 2 isolates of P. capsici. None of the isolates tested produced protease, RNAase, chitinase or phosphatidase. All isolates tested were very uniform in their ability to produce phosphatase, urease and lipase. They were weak phosphatase and urease producers and strong lipase producers. Ability to produce pectin depolymerase, pectate transeliminase, amylase, and DNAase varied greatly among species and among isolates of P. parasitica and P. palmivora, but not P. cinnamomi and P. capsici. The variation in enzyme production among species and isolates of certain species of Phytophthora suggests the possibility for using activities of enzymes as markers for genetic studies

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SPONTANEOUS CHLORATE-RESISTANT MUTANTS OF *GIBBERELLA FUJIKUROI* (*FUSARIUM MONILIFORME*). **C.J.R. Klittich, J.F. Leslie, and J.D. Wilson, Department of Plant Pathology, Kansas State University, Manhattan, 66506.**

The physiological phenotypes of mutants generated spontaneously on minimal medium containing 1.5% chlorate were determined for 14 vegetatively-incompatible isolates of *Gibberella fujikuroi* (*Fusarium moniliforme*). Chlorate-resistant sectors, most of which could not utilize nitrate as a sole nitrogen source (*nit* mutants), were readily isolated. At least 50 *nit* mutants from each strain were analyzed. Mutations at different loci were distinguished using physiological complementation for mutants originating from the same parent and genetic complementation for mutants originating from different parents. At least five *nit* loci were identified in each strain. Up to 48 percent of the *nit* mutants from a single strain had mutations at the nitrate reductase structural locus, and up to 38 percent had mutations at a pathway-specific regulatory locus. Mutants lacking an active molybdenum cofactor were less frequently isolated; at least four loci controlled this phenotype.

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CELL-SUBSTRATUM ADHESIVE PROTEIN INVOLVED IN SURFACE CONTACT RESPONSES OF THE BEAN RUST FUNGUS. Epstein, L.[1], Laccetti, L.[1], Staples, R.C.[1] and Hoch, H.C.[2] Boyce Thompson Institute, Ithaca, NY 14853[1] and NY State Agricultural Experiment Station, Cornell University, Geneva, NY 14456[2].

Uromyces appendiculatus, the bean rust fungus, displays two contact-sensitive responses on leaves or certain synthetic

surfaces: a specific orientation of germ tubes and an induction of appressoria at stomates or scratches. Germ tube orientation, nuclear division (used as a marker for appressorium formation) and germling adhesion on scratched "Parafilm" was reduced by similar concentrations of Pronase E (ED_{50} = 20-50 µg/ml), but not by the heat denatured proteolytic enzyme. Germlings adhered to hydrophobic and hydrophilic surfaces. Pronase E reduced adhesion on either type of surface regardless of whether the enzyme was applied during growth or after attachment had occurred. We used ^{125}I to identify five extracellular proteins which might be involved in germling adhesion to a substratum; we have removed some of these proteins from the germlings for characterization.

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CRYPTIC RESISTANCE AND SUSCEPTIBILITY OF MEDICAGO SATIVA TO PERONOSPORA TRIFOLIORUM. D.Z. Skinner and D.L. Stuteville, Dept. of Plant Pathol., Kansas State Univ., Manhattan 66506.

Plants P1 and P2, whose S_1 populations were 0% and 7% resistant (supported no conidial production) to P. trifoliorum isolate I-7 were crossed and the progeny selected for resistance. Two F_1s, whose S_1 populations were 36% and 66% resistant, were crossed and their progeny selected for resistance. S_1 population resistance of selected plants ranged from 77% to 96%. Two of these plants, whose S_1 populations were 88% and 77% resistant, were crossed and their progeny selected for susceptibility. S_1s of one of the selected plants were 0% resistant. Successful bidirectional selection in these increasingly inbred populations indicated that many, initially heterozygous, genes were involved whose effects were additive in both directions, or that some gene combinations conditioning susceptibility masked gene combinations conditioning resistance and vice-versa.

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EFFECTS OF INOCULUM CONCENTRATION AND VIRULENCE OF COLLETOTRICHUM TRIFOLII RACE 1 AND 2 ISOLATES ON INDUCED PROTECTION IN MEDICAGO SATIVA. N.R. O'Neill, J.H. Elgin, Jr., and C.J. Baker. USDA, Agricultural Research Service, Beltsville, MD 20705.

Two-week-old seedlings of Medicago sativa 'Arc' spray-inoculated with spores of Colletotrichum trifolii race 1 (incompatible interaction) were protected against anthracnose caused by race 2 (compatible interaction). Induced protection, determined by % seedling survival, ranged from 0 to 65%, correlating with increasing race 1 inoculum concentrations from 1.1 to 4.0 x 10^6 spores/ml. Induced protection against anthracnose was not broken down by challenge inoculation with race 2 at concentrations of 0.25 to 4.0 x 10^6 spores/ml applied 24 hours after induction with race 1. Virulence among 13 race 1 isolates on a susceptible cultivar was similar but the level of induced protection in Arc varied significantly. Induced protection in spray-inoculated Arc seedlings was associated with elevated levels of the phytoalexin medicarpin.

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CRYOGENIC STORAGE OF PHYTOPHTHORA INFESTANS USING UNCONTROLLED FREEZING. P. W. Tooley, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701

Simple, inexpensive procedures were developed to provide slow-freezing rates that allow survival of P. infestans at cryogenic temperatures. No. 2 cork borer plugs of four P. infestans isolates were cut from the margins of colonies growing on V8-juice agar and placed into 1 ml of 10% glycerol in 2 cc Nunc cryotubes. Cryotubes were placed at 4 C for 30 min, then snapped into aluminum canes which were placed either in styrofoam boxes or in stainless steel pipet cannisters in a -80 C ultrafreezer. At intervals of 0, 15, 30, 45, 60, and 84 min the canes were removed from the boxes or cannisters and plunged into liquid nitrogen. P. infestans grew only from plugs which had received a -80 C pretreatment of at least 30 min, and rates of recovery were close to 100%. The success of this technique with P. infestans should allow more widespread use of cryogenic storage for Phytophthora species.

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V-9 AGAR: AN INNOVATIVE, GENERAL PURPOSE FUNGAL MEDIUM FOR PHYTOPATHOLOGISTS. Dillavou, C.L. and Calvert, O.H. Mycology Ref. Lab., Inc., Columbia, MO 65205, and Dept. of Plant Path. Univ. of Missouri, Columbia, MO 65211.

V-9 agar was developed by the senior author for screening fungi for the production of antibiotics. It was formulated to induce early and characteristic sporulation. This medium has been found to be useful in the isolation and growth of fungi from man, animals, plants, and soil. It combines potato agar with non-autoclaved V-8 juice (Campbell Soup Co., Camden, NJ 08101) and no glucose. Alternaria, Fusarium, Aureobasidium, Cryptococus, Sporothrix, Trichoderma, Acremonium, Aspergillus, Penicillium spp. are some genera that sporulate better on V-9 agar than on PDA and/or all published versions of V-8 A and with less vegetative and pleomorphic growth. V-9 agar consists of 305 ml of distilled or deionized water (61%), 10 g purified powdered agar (2%), and 10 g dried potato flakes (2%). Blend on low speed in a Waring blender for 30 sec. Place all ingredients in a one liter Erlenmeyer or similar flask and include a 4-5 cm magnetic stirrer bar. Autoclave 20 min. at 15 psi and let flask cool for 5 min. at room temp. Aseptically add one 6 oz. (177 ml) can of V-8 juice (35%). Mix gently with a magnetic stirrer to avoid introducing bubbles. Pour V-9 agar while still hot into sterile petri dishes or tubes.

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RAPID PRODUCTION OF ZOOSPORES OF PHYTOPHTHORA PARASITICA FOR CITRUS GERMPLASM SCREENING. C. T. Henderson*, R. Cohen**, D. Hutchison*, and S. M. Garnsey*, *U.S. Department of Agriculture, ARS, Orlando, FL 32803, **Plant Protection Institute, ARO, Volcani Center, Bet Dagan 50-250, Israel.

A rapid procedure was developed for regulated production of zoospores of P. parasitica for screening citrus rootstocks' susceptibility to foot rot. Isolates of P. parasitica were grown at 28 C on carrot agar (5% finely grated carrots, 1.8% water agar; w/w) for 72 to 120 hr. To produce sporangia, twenty 0.5 cm culture discs were cut and transferred to 60 mm petri dishes containing 10 ml of sterile deionized water. Dishes were covered and incubated at 28 C for 72 hr. Regulated and abundant zoospore release occurred when plates were uncovered and incubated sequentially at 28 C for 60 min, 6 C for 15 min, and 40 C for 15 min. Zoospores produced were active and pathogenic to stems of inoculated citrus seedlings.

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STORAGE OF PYTHIUM SPECIES ON A WHEAT LEAF:WATER MEDIUM. L. L. Singleton, Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-0285.

Medium was prepared by adding 9ml of tap water to six air dried wheat leaf segments(2cm) in test tubes(18X150mm), and autoclaving for 15 min at 1.1 kg/cm^2. Wheat leaf pieces were collected from lab grown 10 day old plants, air dried, and cut into 2cm lengths. Medium was inoculated with 5mm mycelial plugs of Pythium isolates grown on 0.2X corn meal agar(CMA), incubated for 3-4 days at 15C, and stored at 4C after observed growth. Isolates were recovered as needed by placing single leaf segments on CMA. Growth was usually observed within 2-7 days. Using this procedure, wheat isolates of Pythium are maintained, and used for up to a year without loss in viability. Some isolates have maintained their viability for 27 months. P. arrhenomanes and other spp. of Pythium that typically produce lobulate sporangia, and P. irregulare have survived for this length of time. Other Pythium spp. having oogonial walls with spiney or blunt projections did not survive the 27 month period of storage.

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ENNIANTIN PRODUCTION BY FUSARIUM TRICINCTUM. Harland R. Burmeister, NRRC, ARS, Northern Regional Research Center, 1815 North University Street, Peoria, IL 61604

Enniantins are cyclodepsipeptide metabolites known to be produced by at least seven species in the genus Fusarium. All seven species contain strains that parasitize plants, and the enniantins have been suspected of contributing to fungal pathogenicity. In addition to the seven Fusarium species recorded as enniantin producers, F. tricinctum sensu stricto has now also been found to produce about a g/kg of enniantin-B and a trace of enniantin-A on a corn medium. Fusarium tricinctum strains parasitize winter wheat, resulting in crown and root rot, a slow developing disease causing stunting, reduced tillering, and prematurely senescent heads. When 40 µg/ml of enniantin was added to the solution in which wheat seeds germinate, growth of the developing plant was reduced about 50 percent, with root elongation being inhibited more than leaf development.

699

A SIMPLE, VERSATILE METHOD OF DETERMINING EXTRACELLULAR ENZYMES IN SNOW MOLDS. J. H. McBeath and L. Wenko. Agricultural & Forestry Experiment Station, University of Alaska, Fairbanks, AK 99701 and Molecular Genetics Laboratory, Agricultural Research Center, Beltsville, MD 20705

A method has been developed to detect the extracellular enzymes of snow molds: Sclerotinia borealis and sclerotial low temperature basidiomycete (sLTB). S. borealis and sLTB were cultured on a mineral salts liquid medium containing 1% wheat straw and incubated at 7C for 6 weeks. Minute samples of

culture filtrates were resolved by nondenaturing polyacrylamide gel electrophoresis. Cellulytic enzymes were assayed with cellulose-agarose overlay stained with Congo red and pectolytic enzymes were assayed with pectin-agarose overlay stained with ruthenium red. By sandwiching a gel between two overlays and then staining with respective dyes, cellulytic and pectolytic enzymes were detected from a single preparation. Cellulytic enzyme activity was detected in sLTB. Pectolytic and cellulytic enzyme activities were noted in S. borealis.

700

A TECHNIQUE FOR QUANTITATING TILLETIA INDICA TELIOSPORES IN SOIL. L. E. Datnoff, M. R. Bonde, and M. H. Royer. USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701

Teliospores of T. indica, causal agent of Karnal bunt of wheat, are introduced naturally into the soil by infected seeds or debris at harvest. A method is needed for quantifying teliospores to predict potential crop loss. A modified flotation-bubbling system using glycerol was tested to determine potential recovery of teliospores from soil. The procedure involved placement of a 2-g sample of soil artificially infested with 10^2 or 10^3 teliospores/g into a 20 cm x 2 cm fritted disc glass column containing 16 ml of a 50% glycerol solution through which air was bubbled for 5 min. The suspension was allowed to settle for 10 min. and removed by suction at 5 mm above the soil. This procedure was repeated two times. The supernatant was poured through a 20-um sieve; concentrated teliospores were resuspended in water and enumerated on a Hawksley eelworm cell. We recovered 75-90% of the teliospores using this technique.

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PREHARVEST AFLATOXIN CONTAMINATION: EFFECT OF WATER AVAILABILITY IN DEVELOPING CORN KERNELS AND COTTONSEED. E. B. Lillehoj, J. H. Wall, and E. J. Bowers, Southern Regional Research Center, USDA/ARS, P. O. Box 19687, New Orleans, LA 70179

Associations were identified between availability of moisture in preharvest corn kernels/cottonseed and ability of Aspergillus parasiticus to develop and produce aflatoxin. Osmotic pressure and moisture content (MC) of developing seed were determined at varied maturity stages. Seeds were inoculated with A. parasiticus spores and left on the plant or removed for in vitro studies. From 21 to 49 days post-flowering MC values decreased from 58 to 28% in corn and 83 to 64% in cottonseed. Osmotic pressure increased from 450 to 683 kPa in corn and decreased from 437 to 368 kPa in cottonseed. Aflatoxin B_1 levels were highest (141 ppb) in corn kernels inoculated 28 days after flowering and retained on the plants, and >10,000 ppb in seed removed from the plant and used as fungal substrate. Toxin concentrations in cottonseed peaked at 35 days post-flowering in seed removed from the plant, but levels remained high in seed inoculated at 35 to 49 days post-flowering in the in vitro tests.

705

INHIBITION OF PROTEIN SYNTHESIS IN MAIZE BY TRICHOTHECENE MYCOTOXINS. W.L. Casale and L.P. Hart. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Maize leaf discs were floated on buffer solutions containing selected trichothecene mycotoxins, and the effect on incorporation of ^{3}H-leucine into acetone:ethanol insoluble material was determined. ID_{50} values (concentration inhibiting 50% of ^{3}H-leucine incorporation) for T-2 toxin, nivalenol and deoxynivalenol were 0.2, 0.5 and 2.0 ug/ml, respectively. If toxin-treated leaf discs were placed in fresh buffer, inhibition diminished only slightly 2 hr after removal from toxin solution. Maize lines highly susceptible and resistant to ear rot (caused by toxigenic Fusarium spp.) were equally sensitive to these toxins. In another set of experiments, susceptible maize discs or seedlings were inoculated with toxigenic or non-toxigenic strains of Fusarium, and after 24 hr protein synthesis was assayed as above. In all cases, tissues inoculated with toxin-producing strains had 5-fold less incorporation of ^{3}H-leucine than did tissues inoculated with non-toxigenic strains, and virulence was associated with toxigenicity.

706

IDENTIFICATION AND PLACEMENT OF AVERUFANIN AS A PRECURSOR IN THE BIOSYNTHESIS OF AFLATOXIN B_1. D. Bhatnagar, S. P. McCormick, and L. S. Lee, USDA/ARS, Southern Regional Research Center, P. O. Box 19687, New Orleans, Louisiana 70179.

A static culture of Aspergillus parasiticus was observed to accumulate an 8 to 10-fold greater amount of a yellow pigment as compared to a shake culture. This pigment was identified as the anthraquinone, averufanin. Radiotracer studies with [14C] averufanin showed that 23% of the label from averufanin was incorporated into aflatoxin B_1. Feeding [14C]averufanin to mutants of A. parasiticus that do not produce aflatoxins but accumulate either averufin (ATCC 15517), or both versicolorin A and averantin (SRRC 163), or O-methylsterigmatocystin (CP 461), resulted in the accumulation of the [14C] label in averufin (72%), versicolorin A (54%) and O-methylsterigmatocystin (31%), respectively, but not in averantin. Averufanin was also observed, by mass spectrometry, to autooxidize to averufin (>75%) in 10 days. Averufanin can, therefore, be placed after averantin and before averufin in the aflatoxin B_1 biosynthetic pathway.

707

POLYPEPTIDE AND ENZYMATIC CHARACTERISTICS OF FUSARIUM SOLANI ISOLATES PATHOGENIC AND NONPATHOGENIC ON CITRUS ROOTSTOCK SEEDLINGS. S. Nemec, U.S. Department of Agriculture, 2120 Camden Road, Orlando, FL 32803.

Hyphal tip cultures of Fusarium solani pathogenic and nonpathogenic on citrus rootstock seedlings were grown 48-72 hr in liquid Fries medium on a shaker. Polypeptides were extracted in TRIS-HCL buffer, precipitated with nine volumes of acetone and separated by SDS-PAGE on a slab in a single dimension. Enzymes of both isolate types were extracted in phosphate buffer pre-

cipitated by 5 volumes of acetone, partially clarified by centrifugation and detected by starch gel electrophoresis. Up to 37 polypeptides were resolved, 10 to 11 major bands were present, most had Mw of 14,000-94,000 Daltons, with the densest band having an Mw of about 40,000. Nine of 15 enzymes were detected on starch gels. No obvious quantitative differences in polypeptides were detected visually and by densitometry. No enzymatic differences were apparent between pathogenic and nonpathogenic isolates.

708

DIFFERENTIAL GROWTH RESPONSE OF FUSARIUM OXYSPORUM AND FUSARIUM SOLANI ON A MEDIUM CONTAINING POTASSIUM CHLORATE. J. C. Correll, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

The growth rates of 17 strains of F. oxysporum (Fo) and 26 strains of F. solani (Fs) were measured on potato sucrose agar (PSA) and PSA amended with $KClO_3$ (15g/L). Chlorate, a nitrate analog, restricted the growth of all Fo strains tested; colony diameters were restricted 61.5-82.1% after 120 hrs. Fs strains were much less sensitive to chlorate. Three strains of Fs were not restricted by chlorate while 22 were restricted 5.1-36.6% after 120 hrs. One exceptional isolate of Fs was restricted 65.3%. Nitrate non-utilizing mutants were recovered from all of the Fo strains tested and from 8 of the Fs strains. In addition, several strains of Fo and Fs were tested for their growth response to chlorate on a defined medium when either NO_3 or L-asparagine was used as the nitrogen source. All isolates tested were more restricted by chlorate on the defined medium when NO_3 was used as the nitrogen source.

709

TIME-TEMPERATURE RELATIONSHIPS FOR THE INACTIVATION OF SCLEROTIA OF MACROPHOMINA PHASEOLINA. A. H. Shaikh and A. Ghaffar, Department of Botany, University of Karachi, Karachi-32, Pakistan.

The viability of sclerotia of Macrophomina phaseolina was tested in both wet and dry soil under simulated elevated temperatures in either constant, cyclic or short temperature regimes. In wet soil viability was reduced to zero by a constant temperature of 55 C and above for 1 day or a minimum oven temperature of 50 C for 3 days. Viability of sclerotia were destroyed by 2-h temperature cycle for 7 days at 60 C or 14 days at 55 C. Below 40 C sclerotia were not affected. In dry soil at 60 C, a 7-day constant temperature or a 21 days 2-h temperature cycle reduced sclerotial viability by 88 and 54%, respectively. Data on time and temperature relationships on loss in viability of sclerotia in soil could thus be used to predict efficacy of soil solarization.

710

DIFFERENTIAL CATABOLISM OF NITROGEN BY CHLORATE RESISTANT AND SENSITIVE ISOLATES OF MACROPHOMINA PHASEOLINA. C.A.S. Pearson, J.F. Leslie, and F.W. Schwenk, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

M. phaseolina from corn and soybean differ in chlorate (ClO_3^-) sensitivity. Corn isolates are ClO_3^R and grow normally on a 1.5% ClO_3^- medium. Soybean isolates show two ClO_3^S phenotypes, termed feathery and restricted. An isolate from each phenotypic class was evaluated for its ability to catabolize 56 nitrogenous compounds. All isolates grew well when supplied with Arg, Asn, Gln, Glu, and Pro, compounds commonly found at elevated levels in plants under environmental stress. Differential growth was obtained when these isolates were supplied with KNO_2 or KNO_3. While the two soybean isolates grew well when supplied these compounds, the corn isolate did not. Such differences in NO_2^- and NO_3^- usage are correlated with ClO_3^- sensitivity. The ability of all of the isolates to use stress-related nitrogenous compounds may reflect the opportunistic nature of this pathogen.

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PECTOLYTIC ENZYMES IN COTTON BOLLS WOUND-INOCULATED WITH ASPERGILLUS FLAVUS. T. E. Cleveland and S. P. McCormick, USDA, ARS, SRRC, P. O. Box 19687, New Orleans, LA 70179

Pectolytic enzymes from A. flavus liquid cultures or cotton bolls wound-inoculated with the same fungus were analyzed by isoelectric focusing (IEF). At least three A. flavus pectolytic enzymes (pI 3.2, 3.8, and 5.1, respectively) were detected in both liquid cultures containing pectin and in the lint and seed of inoculated bolls. Buffer extracts of lint and seed were made at various times following inoculation and assayed for ability to reduce the viscosity of a 1% pectin solution. Pectolytic activity was first detected 2 days after inoculation and reached maximum levels in 3 to 5 days. Incubation of the extracts on pectin-agarose plates and subsequent staining with ruthenium red to detect pectolytic activity, confirmed the results of vicosimetric assays. Only A. flavus pectolytic enzymes were present in bolls 2 to 9 days after inoculation based on enzyme pI values after IEF of boll extracts. The early accumulation and presence of pectolytic enzymes throughout the infection process suggests they are involved in establishment of A. flavus in plant tissues.

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CHARACTERIZATION OF ENZYMES INVOLVED IN AFLATOXIN B_1 BIOSYNTHESIS BY ASPERGILLUS PARASITICUS. T. E. Cleveland and D. Bhatnagar, USDA, ARS, SRRC, P. O. Box 19687, New Orleans, LA 70179.

Two enzyme activities were discovered in cell-free mycelial extracts of A. parasiticus, which together are required to convert sterigmatocystin (ST) to aflatoxin (B_1). A soluble methyl transferase (MT) converts ST to O-methylsterigmatocystin (OMST). The presence of a particulate enzyme complex (PEC) containing an oxidoreductase component is required to complete the conversion of OMST to B_1. PEC and MT were separated by ultra-high speed centrifugation and characterization of the individual activities was initiated. PEC requires NADPH for activity and MT is stimulated about 6-fold by S-adenosylmethionine. PEC converts OMST to B_1 at a rate of about 15 pmoles (min. mg protein)$^{-1}$ at 30°C and optimum pH (7.1-7.5). PEC rapidly loses activity above 40°C. At optimum pH (8.1-8.5), MT converts ST to OMST at a rate of about 38 pmoles (min. mg protein)$^{-1}$ at 30°C. This enzyme system is being characterized for substrate specificity to gain a better understanding of the enzymatic regulation of aflatoxin biosynthesis.

713

CONIDIUM GERMINATION OF BIPOLARIS MAYDIS RACE T: EFFECTS OF PH AND AMMONIUM. T. W. Bischoff and M. O. Garraway. Department of Plant Pathology and OARDC, The Ohio State University, Columbus, OH 43210.

Conidia from 6 day cultures of Bipolaris maydis race T were incubated for 3 hr in fluid recovered from 48, 72 or 96 hr liquid cultures (2 g/l glucose, 4 g/l L-asparagine and mineral salts) of the fungus. Germination was >80% in 48 hr fluid and <10% in 72 or 96 hr fluid. These trends coincided with no NH_4^+, a pH of 5.8 and the presence of glucose in 48 hr fluid and 20-30 µmoles NH_4^+/ml, a pH of 7.9-8.3 and the absence of glucose in 72 or 96 hr fluid. To test the inhibitory effect of NH_4^+ and pH, conidia were incubated with 1 mg/ml glucose, 0.4 mg/ml L-asparagine and mineral salts. At pH 6.0 germination was >90% without and <40% with 30 µmoles NH_4^+/ml and at pH 8.0 was <20% without and <2% with 30 µmoles NH_4^+/ml. Thus it appears that the ammonium and pH levels seen in cultures of B. maydis race T can impede conidium germination.

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A MECHANISM FOR ASCOSPORE DISCHARGE IN UNCINULA NECATOR. D. M. Gadoury and R. C. Pearson, Plant Pathology Dept. N.Y.S. Agr. Expt. Sta., Cornell Univ., Geneva, NY 14456.

Although frequently described as spherical structures, we observed that ascocarps of Uncinula necator are concavo-convex unless in contact with free water. The ascocarp wall is freely permeable to water and dry ascocarps swell to become spherical within 5 minutes of wetting. The osmotic potential of ascospore and ascus cytoplasm decreased during maturation, resulting in increased turgor pressures when ascocarps were wet. Internal turgor pressure increased from 6.7 bars in 8-wk-old ascocarps to 29.9 bars in 5-month-old ascocarps, as determined by distention or plasmolysis of spores and asci in NaCl solutions. The increase in turgor pressure was accompanied by a decrease in extracellular lipid. Ascospore discharge occurred when the ascocarp either dehisced circumscissilely near the junction of the concave and convex surfaces, possibly due to fatigue from flexing of the wall during cycles of wetting and drying, or split longitudinally over the apicies of the asci.

715

WATER RELATIONS OF GEOTRICHUM CANDIDUM: GROWTH ON SEMI-PERMEABLE MEMBRANES. A.B.A.M. Baudoin and L.L. Davis, Department of Plant Pathology, Physiology & Weed Science, VPI & SU, Blacksburg, VA 24061

The physiological basis for the reduced susceptibility to decay by Geotrichum candidum in lemons at lower water potentials was studied. Conditions in non-waterlogged intercellular spaces were simulated using semipermeable membranes (perfluoro polymer, molecular weight cut-off 5000) floating on asparagine glucose medium amended with polyethylene glycol 20,000 to achieve water potentials from -0.5 to -2.5 MPa. Each membrane was inoculated with a tiny agar plug containing 10^5 spores of

G. candidum, and incubated at 25 C. The colony surface area on membranes at -2.5 MPa was about 8% of that at -0.5 MPa. The effect of water potential on fungal dry weight was similar to the effect on colony surface area. Our findings suggest that the ability of G. candidum to grow rapidly from the inoculation site, before defense mechanisms of the host become operative, is important in the development of sour rot.

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COMPARISON OF SEEDLING ROOT ROT CAUSED BY APHANOMYCES EUTEICHES AND PHYTOPHTHORA MEGASPERMA f.sp. MEDICAGINIS ON ALFALFA. E.B. Holub and C.R. Grau. Univ. of Wisconsin, Madison, WI 53706

Three day old seedlings of Vernal and Apollo II alfalfa (5 and 54% Phytophthora resistant, respectively) were inoculated with 0, 10, 100, 1000 zoospores/seedling of Aphanomyces euteiches (Ae) or Phytophthora megasperma f.sp. medicaginis (Pmm) and incubated at 16, 20, 24, 28, or 32 C. Seedling survival was recorded at three day intervals for nine days and disease ratings (1=no symptoms to 4=dead seedling) were taken the final day. Disease was compared using percent incidence (PI) of seedlings with symptoms, a disease severity index (DSI), and area under the progress of mortality curve (AUPMC). Temperatures from 16-28 C did not affect PI for either pathogen with an ED_{50} of ca. 2-5 and 40 zoospores/seedling for Ae and Pmm, respectively. Pmm was more aggressive at 16-20 C than Ae as measured by DSI and AUPMC. However, Ae was more aggressive at 24-28 C. Vernal was more susceptible to both pathogens than Apollo II, but the difference in cultivar susceptibility was greater when inoculated with Pmm.

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EVALUATION OF BARLEY CULTIVARS FOR RATE-REDUCING RESISTANCE TO PYRENOPHORA TERES. B. J. Steffenson and R. K. Webster. Department of Plant Pathology, University of California, Davis, CA 95616.

Barley entries from Western Australia, CIMMYT, and Davis were evaluated for rate-reducing resistance to net blotch (Pyrenophora teres) in a field nursery at Davis. Infection response (based on lesion size and chlorosis) and the area under the disease progress curve (AUDPC) were determined for each entry. The entries varied greatly with respect to their infection response and AUDPC. Net blotch developed slower on UC 603 (AUDPC=285) and Prato (AUDPC=703) than on the susceptible check, Kombar (AUDPC=1303). These three cultivars all had similar susceptible infection responses to P. teres. The resistant check, Tifang (which possesses the resistance gene Rptla) had a resistant infection response and an AUDPC of 4. The results indicate that UC 603 may be a useful parent in programs breeding for resistance to P. teres in barley.

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ANALYSIS OF RESTRICTION FRAGMENT POLYMORPHISM OF THE mtDNA OF THE FORMAE SPECIALES OF PHYTOPHTHORA MEGASPERMA. Helga Förster, T. G. Kinscherf, S. A. Leong and D. P. Maxwell, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

The taxonomic relationship between P. megasperma f. sp. glycinea (Pmg) and P. megasperma f. sp. medicaginis (Pmm) was studied by comparing DNA restriction patterns. DNA was extracted from fungal isolates and separated by bisbenzimide-CsCl centrifugation into two fractions, corresponding to the nuclear and mitochondrial DNA. The mtDNA appeared to be circular and 42-52 kb in size. Pmg isolates were placed into two major groups and several minor groups by the restriction enzyme fragmentation patterns obtained with 10 endonucleases. Restriction patterns were more similar among isolates of Pmg than between isolates of Pmg and Pmm. The mtDNA from a Pmg race 1 isolate was further characterized by restriction enzyme and gene mapping.

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ANTHER CULTURE AND PLANT REGENERATION FROM EARLY MATURING, COLD HARDY, HARD RED WINTER WHEAT. J. H. McBeath and G.W. Schaeffer, Agricultural & Forestry Experiment Station, University of Alaska, Fairbanks, AK 99701 and Molecular Genetics Laboratory, Agricultural Research Center, USDA-ARS, Beltsville, MD 20705

Anther cultures of early-maturing, cold-hardy, hard-red winter wheat cultivars--Capitan, Norstar and Roughrider--have been established in an effort to expand the range of their snow mold tolerance. Anthers at the uninucleate stage of pollen (microspore) development were excised after cold shock treatment and cultured on boiled potato extract medium at 25C, 60% RH and low light intensity. After 5 weeks, embryoids produced from microspores were transferred to an increase medium containing MS basal salts, 2,4-D and NAA for callus proliferation. Large calli were later transferred to a step-down medium without 2,4-D and NAA but containing IAA and kinetin to promote the differentiation of roots and shoots. Embryoids and small microspore calli have been observed from all three cultivars. Green plantlets were obtained from the cultivar Capitan.

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HOST RANGE OF TILLETIA INDICA ON SEVERAL GRASSES. M. H. Royer[1], J. L. Rytter[1], and T. T. Matsumoto[2]. [1]USDA-ARS, Frederick, MD 21701 and [2]CDFA, Sacramento, CA 95814

The distribution of Karnal bunt, caused by Tilletia indica, is inferred from reports of the pathogen on wheat. This study was initiated to determine the susceptibility of grass species that naturally occur in areas that may be environmentally conducive to Karnal bunt development. Plants of seven grass tribes were artificially inoculated with sporidia of T. indica by injection into or drenching onto wheat spikes at heading. Several species that were tested had one or more accessions susceptible to T. indica: Bromus ciliatus, Lolium multiflorum, L. perenne, Triticum monococcum, T. timopheevi, Aegilops triunciale, A. cylindrica, A. bicornis, A. comosa, A. searsii, A. tauschii, and A. triaristata. Several of these species occur in the United States.

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TESTING PROPOSED MECHANISMS OPERATING IN MULTILINES BY EPIMUL. I.S. Hoang, P.S. Teng, and A.P. Roelfs. Dept. of Pl. Path., Cereal Rust Lab., USDA, ARS, Univ. of MN., St. Paul, MN 55108.

Two possible mechanisms, 'reduced density effect' and 'barrier effect', were tested with EPIMUL, a disease simulator, to explain how disease is reduced in multilines. When disease severities in mixtures composed of 2, 3, and 4 components were compared with that of monoculture at the same plant density, significant differences were found, showing that mixing resistant with susceptible plants reduces the simulated severity of disease. Disease severities in the mixtures were compared with that of monocultures at a lower plant density, but with the same number of susceptible plants. The two-component mixture did not provide a significant difference in disease severity, showing that the effect of multiline resulted chiefly from the 'reduced density effect'. However, mixtures of 3 and 4 components provided significant differences, and the 'barrier effect' was considered to be more important in these mixtures.

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COUPLING A DISEASE MODEL TO A WHEAT GROWTH MODEL FOR YIELD LOSS ASSESSMENT. I.S. Hoang, P.S. Teng, A.P. Roelfs. Dept. of Pl. Path., Cereal Rust Lab., USDA, Univ. of MN., St. Paul, MN 55108.

A wheat growth model, TAMW, was coupled to a disease simulator, EPIMUL, for yield loss assessment. TAMW and EPIMUL were coupled at the grain-filling stage. To achieve this coupling, a loss prediction model was developed by regressing reduction in caryopsis dry matter, YL (expressed as a fraction of healthy), with percent disease severity of stem rust, X, to give the equation : $YL = 0.1689 + 0.0351 * \ln(X)$. YL was calculated with an input of disease severity simulated from EPIMUL. The grain filling rate of TAMW then was adjusted with multiplying with the value of YL. The coupled models predicted yield loss acceptable when compared to published data.

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DISEASE LEVELS OF BARLEY SCALD ON PURELINE CULTIVARS AND ON COMPOSITE CROSS POPULATIONS FROM EITHER CALIFORNIA OR MONTANA. B. A. Crandall and L. F. Jackson, Department of Agronomy and Range Science, University of California, Davis.

Disease levels of barley scald, caused by Rhynchosporium secalis, originating from natural infection in the field and from artificial inoculation in the greenhouse were measured on pureline cultivars (Prato, UC 566, CM 72) or composite cross populations in which generations had been advanced in either California (CCII F29 and F48, CCV F24) or Montana (CCII F29 and F44, CCV F19). Based on past observations the California environment favors disease development more than the Montana environment. Preliminary field results suggest that although there are differences between the level of disease in composite cross populations and pureline cultivars, the differences between composite cross populations grown in California and Montana are not significant. The responses of these populations to artificial inoculation with three selected California races of R. secalis were similar to the results found in the field.

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GROWTH STAGE AND LEAF POSITION EFFECTS ON RESISTANCE OF WHEAT TO LEAF RUST. Z. A. Pretorius, F. H. J. Rijkenberg, and R. D. Wilcoxson. Small Grain Centre, South Africa; Department of Plant Pathology, University of Natal, Pietermaritzburg, S.A.; Department of Plant Pathology. University of Minnesota, St. Paul, MN 55108, USA.

Wheats with adult plant resistance to Puccinia recondita f. sp. tritici 'Era', 'Glenlea', 'Sinton' and line RL 6044 were inoculated at growth stages 4 to 18 on Romig's scale and latent period (LP) and uredinial numbers (UN) recorded. UN decreased and LP increased as stage increased for each wheat. LP in the flag leaf and flag leaf-2 was greater in RL 6044 and Era than in Sinton and Glenlea. In the latter two cultivars, LP was greater in the flag leaf of Glenlea than Sinton but the order of the two cultivars was reversed on leaf-2. UN on three cultivars was greater on flag leaves than on leaf-2 but on RL 6044 UN was greater on leaf-2. Therefore, both age of plant and leaf position must be considered in accessing adult plant resistance to wheat leaf rust.

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Spectroradiometric readings of barley canopies grown from fungicide treated seed. Vernyl D. Pederson, Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105.

Seed of 'Morex' barley was treated with several different fungicide formulations and combinations and was planted at Langdon, ND. At the soft dough stage of kernel development (July 30, 1985) reflection of solar radiation from the barley canopies was measured in 8 discrete wavelength bands ranging from 500 to 850 nm. Highly significant differences (P=.01) among treatments were obtained with each of the 8 wavelengths. Plots from the seed treatment combination containing 10% Imazalil (1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxylethyl)-1H-imidazole)) and Carboxin (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide=Vitavax) reflected significantly less visible (500-700 nm) and significantly more infrared light (750-800 nm), indicating healthier plants from treated seed. Mean yield from this seed treatment was 6% greater than was yield from the untreated check. Seed treatment may have suppressed common root rot or early development of scald.

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TOXIGENIC FUSARIUM MONILIFORME FROM EQUINE FEED. Ronald F. Vesonder, Northern Regional Research Center, USDA-ARS, 1815 N. University Street, Peoria, Illinois 61604

Four feed samples from North Carolina associated with equine leukoencephalmalacia (ELEM) were examined mycologically and for mycotoxins. Dilution plating technique and surface-sterilized corn kernels and oats revealed a high incidence of Fusarium moniliforme. The relative fungal density of F. moniliforme in the feed samples was 66, 58, 66.6, and 53%. Thirty-three F. moniliforme strains were isolated from the equine feed which contained corn and oats produced in North Carolina. The strains were cultured on autoclaved corn at 25°C for 2 weeks. Methanol extract of each fermented corn was acutely toxic to mice. No moniliformin was detected in the corn fermentation. In some mice, a hypertensive reaction was observed 48 hours after intraperitoneal administering of the extract, followed by death 12 hours later. Aflatoxin at <10 ppb was detected in the equine feed and no vomitoxin or moniliformin was detected.

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VIRULENCE COMBINATIONS IN WHEAT LEAF RUST AND LOSSES IN 1985. D. L. Long and J. F. Schafer, Cereal Rust Laboratory, USDA-ARS, Dept. of Plant Path., U. of Minn., St. Paul 55108.

The 1985 United States survey of Puccinia recondita f. sp. tritici differed from the 1978-84 surveys in two significant ways. In each instance one wheat cultivar was primarily involved. 1) Virulence to Lr 16 occurred frequently and was always combined with virulence to Lr 1. Seventy-four percent of the rust collections from Pro Brand 812 were virulent to Lr 1 and 16. Virulence to Lr 16 had been present in Canada during the late 1960s and early 1970s when Selkirk was grown, but was not found from 1977-83. 2) A new phenotype of UN race 13, virulent to Lr 9, also occurred, providing the first identification of combined virulence to Lr 2a and 9. Seventy-five percent of the collections virulent to both Lr 2a and 9 were from Coker 762 which was previously resistant. Total loss of wheat from leaf rust in the United States was estimated to be 143 million bushels, the largest recorded national, annual limitation of the crop from this disease.

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CROWN AND ROOT ROT OF WILD RICE IN CALIFORNIA CAUSED BY A PHYTOPHTHORA SPECIES. P. S. Gunnell and R. K. Webster, Department of Plant Pathology, University of California, Davis, CA 95616.

A Phytophthora species was isolated from necrotic roots and crowns of cultivated wild rice plants (Zizania palustris L.) exhibiting drought symptoms in flooded paddies in California. Foliar symptoms consisted of wilting followed by desiccation. Leaf sheaths of diseased plants were rotted at the base and separated easily from the crown. The crowns of diseased plants were severely rotted whereas the roots showed a milder rot. Koch's postulates were completed using seedlings and mature plants of wild rice grown in flooded pots in the greenhouse. This is the first report of a Phytophthora species pathogenic on wild rice.

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RESISTANCE TO STEM RUST IN Avena fatua L. D. R. Johnson and P. G. Rothman. Department of Plant Pathology, University of Minnesota and USDA Cereal Rust Laboratory, St. Paul, MN 55108.

There is little effective resistance to stem rust (Puccinia graminis f. sp. avenae) in cultivated oats, however the discovery of new resistances in Avena sterilis suggested additional resistance might be found in other wild hexaploids. Over 6000 samples of A. fatua collected in Mexico were screened for seedling resistance. Among the resistant seedlings observed, were three with highly resistant infection types. Descendants of these selections were highly resistant to many races, including those currently prevalent in North America. The resistance was effective in adult plants as well as seedlings, but was thermolabile, changing to full susceptibility at temperatures of 29 C and above. F2 progenies of crosses between resistant A. fatua and susceptible lines segregated for susceptibility, moderate resistance and high resistance to races NA 30 and NA 39, suggesting the resistance is conferred by two or more factors.

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TIMING OF FOLIAR FUNGICIDES ON SOYBEAN IN LOUISIANA - A THREE YEAR SUMMARY. J.S. Gershey, G.T. Berggren, J.P. Snow, E.C. McGawley, and M.E. Pace. Dept. of Plant Pathology and Crop Physiology, La. State Univ. Agric. Ctr., Baton Rouge, LA 70803

A three year study was initiated in 1982 to identify the most cost-effective rates and timings of fungicides for the control of foliar, pod and stem diseases of soybean caused by species of Diaporthe, Phomopsis, Colletotrichum, Cercospora, and Septoria. Four commercially labeled fungicides, Benlate 50WP (benomyl), Mertect 340F (thiabendazole), Bravo 500 (chlorothalonil), and Topsin M 70 WP (thiophanate-methyl), were applied in single and multiple applications at R3 to R6 growth stages. Results indicate that a single appliation of benomyl applied at R3 did not significantly differ from the recommended application timings (R3 plus R5) at the p=0.05 level.

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EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON INFECTION, LESION DEVELOPMENT, AND SPORULATION BY CERCOSPORIDIUM PERSONATUM ON PEANUT. B. B. Shew, J. C. Wynne, and M. K. Beute, Departments of Crop Science and Plant Pathology, North Carolina State University, Raleigh, 27695.

Leaves were detached from peanut plants having low, moderate, or high resistance to late leafspot and maintained in moist sand. Leaves were inoculated with Cercosporidium personatum, held at high RH for 3, 12, 18 or 24 hr/da at 20, 24, 28 or 32C for 6 da, and then incubated in the greenhouse 14 da. RH >96% for ≥12 hr/da promoted infection; infection increased with decreasing temperature. High RH for ≤12 hr/da at 28 and 32C greatly inhibited infection. Rankings of geno-types were similar at all temperatures and RHs. Inoculated leaves were also held for 4 da at high RH and 20C and then incubated at 20, 24, 28 or 32C for 28 da. Lesion development and sporulation were greatest at 24C; higher temperatures inhibited disease development, especially on resistant genotypes.

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A study of the incompatibility system of Laccaria bicolor in relation to its symbiotic ability. Bradley R. Kropp, Brenda J. McAfee, and J. Andre Fortin. Centre en Recherche en Biologie Forestiere, Faculte de Foresterie et de Geodesie, Universite Laval, Quebec, Canada G1K 7P4.

The relative ability of sibling monokaryotic and reconstituted dikaryotic cultures of Laccaria bicolor to colonize short roots of Pinus banksiana was studied 4, 8, and 12 months after isolation. Monokaryons were capable of ectomycorrhiza formation but were variable. One nonmycorrhizal monokaryon was found, while others declined in ability after 12 months in culture. Dikaryons reconstituted soon after isolation were vigorous colonizers, but dikaryons reconstituted after 12 months in culture using monokaryons which had lost their mycorrhizal ability showed reduced symbiotic ability. However, a dikaryon formed after 12 months by pairing two monokaryons that had retained their mycorrhizal ability formed abundant mycorrhizae. The mycorrhizal capability of dikaryons formed by crossing compatible monokaryons from Europe and Canada is being evaluated.

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EFFECT OF HIGH TEMPERATURE STRESS ON APPRESSORIUM FORMATION AND SPORULATION OF BIPOLARIS MAYDIS RACE T ON MAIZE LEAVES. E.C. Wokoma, M.O. Garraway, and R.E. Whitmoyer. Dept. of Plant Pathology, OARDC/The Ohio State University, Columbus, Ohio 43210

Leaf sections ($2cm^2$) from maize plants were exposed to high temperature stress (41C for 6 h)(HTS), before or after inoculation with 2×10^4 conidia/ml of Bipolaris maydis race T (BMT). Controls were similarly inoculated but not exposed to HTS. After 6 h incubation at 28 C, the number of conidia that had germinated on leaves exposed to HTS before inoculation was similar to the control, but was significantly less if the leaves were exposed to HTS after inoculation. Up to 70% more appressoria were formed on leaves exposed to HTS before inoculation than on controls. If the HTS was after inoculation, fewer appressoria were formed on leaves. After 96 h of incubation at 28C, sporulation on leaves exposed to HTS prior to inoculation was higher than the control, but less on leaves exposed to HTS after inoculation. HTS prior to inoculation, but not after, promotes appressorium formation and sporulation of BMT.