ABSTRACTS OF PRESENTATIONS

1

THE ASSOCIATION OF DSRNA WITH DECREASED VIRULENCE AND SPORULATION IN LEUCOSTOMA CINCTA, THE CAUSAL AGENT OF CYTOSPORA CANKER OF PEACH. S.A. Hammar, G.C. Adams, D.W. Fulbright, Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

A nonsporulating avirulent strain of Leucostoma cincta (14.4A) contains dsRNA and virus-like particles. This strain was subjected to various treatments in attempts to recover virulent, sporulating, dsRNA-free clones ("cured strains"). Partially cured strains of the infected isolate were obtained by hyphal tipping, treatment with Ribivirin, and reisolation from susceptible plant tissue. The derived strains had fewer bands in gel electrophoresis and increased virulence. They did not, however, sporulate or have normal virulence. Selection of regenerated 14.4A protoplasts resulted in normal virulent, sporulating strains. DsRNA is absent or at very low levels in these "cured" strains. Heat and cycloheximide treatments were not effective in eliminating dsRNA.

2

ULTRASTRUCTURE OF THE INFECTION OF SCLEROTIA OF SCLEROTINIA SCLERCTIORUM BY TALAROMYCES FLAVUS. D.L. McLaren[1], H.C. Huang[1], S.R. Rimmer[2] and E.G. Kokko[1]. [1] Research Station, Agriculture Canada, Lethbridge, Alberta T1J 4B1. [2] Plant Science Department, University of Manitoba, Winnipeg, Manitoba, R3T 2N2.

Talaromyces flavus (Klöcker) Stolk and Sam is a destructive hyperparasite of Sclerotinia sclerotiorum (Lib.) de Bary. Sclerotia inoculated with spores of T. flavus were colonized by the hyperparasite after 3, 7 or 12 days and often the tissue became soft and decayed. Results of investigation using transmission electron microscopy showed that hyphae of T. flavus penetrated the walls of rind cells and grew both inter- and intra-cellularly within the sclerotia. Degradation and destruction of the host cell walls were evident in the process of ramification of T. flavus hyphae inside the sclerotium tissue. This study suggests that infection of sclerotia by T. flavus may be an important factor affecting the survival of S. sclerotiorum.

3

BACILLUS SUBTILIS AS A POTENTIAL BIOLOGICAL CONTROL AGENT FOR RHIZOCTONIA ROOT ROT OF SOYBEANS. Z. Liu and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801

A spore suspension of isolate CA-8 of B. subtilis from Canada and IL 153-2-2 of Bacillus sp. from Illinois-grown soybeans were introduced onto soybean seedlings and into soil by seed treatment. Both isolates were nonpathogenic and had generation times of 28-38 min at 25C and 21-28 min at 30C. Cells of the same isolates were recovered 3 weeks after treatment on soybean hypocotyls over 5cm above and on roots to 20cm below the soil line with a population of $2x10^3$ to $2.5x10^6$ cfu/ml associated with stem and root tissues. Seedlings from treated seeds grown in soil infested with isolate L65-2 of R. solani had a significantly (P=0.05) lower disease index and number of lesions/seedling, and higher root volume and plant dry weight than those from untreated seeds in the greenhouse. R. solani was recovered significantly less often from soil planted with treated than with untreated seeds. Concentrated cell-free culture extracts of Bacillus sp. were antagonistic to R. solani in culture.

4

EFFECTS OF CRUCIFER AMENDMENTS ON APHANOMYCES ROOT ROT OF PEAS. A. M. Muehlchen and J. L. Parke, Dept. of Plant Pathology, University of Wisconsin-Madison 53706.

Crucifer amendments were evaluated for their potential to control pea root rot caused by Aphanomyces euteiches in greenhouse and growth chamber experiments. Peas were planted in naturally infested soil amended with nine crucifers with different glucosinolate profiles. All amendments significantly increased shoot dry weight and reduced disease severity. Curled mustard was the most effective, increasing pea dry weight by 159% compared to the non-amended treatment. Crucifers grown in infested soil and then removed had no residual effect on peas planted subsequently. To determine the role of volatiles released from decomposing crucifers, small bags of infested soil were suspended for 1 wk over curled mustard slurry, water, slurry plus soil, or water plus soil, or were partially submerged in slurry or water. Subsequent pea growth in treated soils indicated that both volatiles and direct contact between soil and crucifer slurry contributed to disease reduction.

5

EFFECT OF CATIONS ON LYSIS OF PHYTOPHTHORA CACTORUM ZOOSPORES BY BACILLUS CEREUS. G. S. Gilbert, J. L. Parke, and J. Handelsman. Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Culture filtrates of Bacillus cereus isolate UW85 lyse zoospores of Phytophthora spp. in vitro. Lysis of P. cactorum zoospores by culture filtrate of B. cereus was quantified in solutions containing cations at concentrations typical of soil solution. Cl^- or NO_3^- salts of Ca^{2+} (0-20 meq), Mg^{2+} (0-12 meq), Na^+ (0-16 meq), and K^+ (0-2 meq) were tested individually. After 20 minutes, 12 meq of Ca^{2+} or Mg^{2+} reduced lysis to 43% or 64%, respectively, of that in filtrate with no added salts. The percentage of zoospores that did not lyse was exponentially dependent on the concentration of Ca^{2+} and Mg^{2+}. K^+ and Na^+ did not affect lysis. Zoospore lysis by Bacillus culture filtrate also decreased with increasing concentrations of filter-sterilized soil extracts. These results suggest possible soil-dependent limitations on the effective use of B. cereus for biological control of Phytophthora root rots.

6

STUDIES ON THE BIOLOGICAL CONTROL OF PYTHIUM ULTIMUM ROOT ROT OF RED KIDNEY BEAN WITH BACTERIAL AND FUNGAL SEED TREATMENTS. S. M. BISSONNETTE, B. J. JACOBSEN. DEPT. OF PLANT PATHOLOGY, UNIVERSITY OF ILLINOIS, URBANA, IL 61801

Potential biocontrol agents were isolated from the rhizoplane/rhizosphere of kidney bean roots. Of 28 candidates, 6 exhibited

significant control of P. ultimum root rot of Phaseolus vulgaris in glasshouse screening, as exhibited by reduced oospores/g dry root weight, and increased plant weight and length. These isolates were utilized in both glasshouse and field studies. Biocontrol treatments were 2 Streptomyces sp., 2 nematode trapping fungi, Fusarium merisimoides, and an unidentified bacterium. These were compared with metalaxyl seed and soil treatments. At 10% bloom and full pod all biocontrol treatments had significantly fewer oospores/g dry root weight than the untreated control. The F. merisimoides seed treatment resulted in significantly higher yield/plant than the untreated control. All biocontrol treatments yielded as well or better than the metalaxyl treatments. In vitro studies suggest that antibiosis is not the mode of action for control by these biocontrol agents.

7

PHENOTYPIC CHARACTERIZATION OF FLUORESCENT PSEUDOMONAD POPULATIONS IN SOIL AND ON PLANT PARTS. J. B. Bahme, M. N. Schroth, O. C. Huisman, D. C. Hildebrand, and S. D. Van Gundy, Dept. of Plant Pathology, Univ. of California, Berkeley, CA 94720 and Dept. of Nematology, Univ. of California, Riverside, CA 92521.

Analysis of three-dimensional models based on phenotypic profiles derived from nutritional tests enabled an assessment of the population composition of fluorescent pseudomonads in soil, and on potato plant parts. Great phenotypic diversity was detected among the 1430 strains with 114 of a possible 144 phenotypic profiles represented. Placement of the 65 predominant profiles in a three-dimensional similarity matrix revealed 15 phenon clusters. Only approximately 14% of the strains exhibited phenotypic profiles identical to named fluorescent pseudomonad strains. Distinct differences were detected in the composition of populations isolated at two field sites and among strains from non-rhizosphere soil and the rhizosphere.

8

COLONIZATION OF WHEAT ROOTS BY BACTERIA SUPPRESSIVE OR NON-SUPPRESSIVE TO WHEAT ROOT PATHOGENS. C. T. Bull and D. M. Weller, WSU and USDA-ARS, Pullman, WA 99164-6430.

Bacteria, suppressive (Pseudomonas fluorescens 2-79 and Q72a-80) or nonsuppressive (Bacillus subtilis, Escherichia coli, Erwinia herbicola, E. carotovora, P. syringae and Xanthomonas campestris pv. phaseoli) to wheat pathogens (Gaeumannomyces graminis and Pythium spp.) were compared for ability to colonize wheat roots. Each bacterium was introduced on seed and its population subsequently determined on root sections 3-5 and 5-7 cm below the seed. All bacteria were recovered from roots of greenhouse (15 C) and field-grown plants 14 and 30 days after planting, respectively, but the populations of suppressive bacteria were greatest. In the greenhouse at 30 C, populations of X. campestris, 2-79 and Q72a-80 were the same. Only 50-60% of individual seminal roots from greenhouse (15 C) and field-grown plants were colonized by 2-79 or Q72a-80. There was no correlation between when a root emerged from the seed and whether or not it was colonized.

10

EFFECT OF RHIZOBACTERIA ON YIELD AND RHIZOPLANE FUNGI OF CELERY. J. O. Becker, S. Van Gundy, J. G. Hancock, A. R. Weinhold, C. Hepfer, and M. N. Schroth. Dept. of Nematology, Univ. of California, Riverside, CA 92521 and Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Thirty-five strains of rhizobacteria were field tested for their effect on celery (Apium graveolens L. 'Fordhook'). Bacterial strains were applied as a drench (ca. $5x10^9$ cfu/ml to seedlings grown in vermiculite. The transplants were planted into a field infested with Fusarium oxysporum f. sp. apii. Four bacterial strains significantly increased yields from 16 to 32%, whereas metham-sodium fumigation improved yields by 75%. Root isolations indicated that the bacterial treatments resulted in a qualitative change in rhizoplane fungi.

11

BINUCLEATE RHIZOCTONIA SPP. AND LAETISARIA ARVALIS, BIOCONTROL AGENTS FOR RHIZOCTONIA CROWN ROT OF SUGAR BEET. L. J. Herr, Dept. of Plant Pathology, OSU/OARDC, WOOSTER, OH 44691

Five binucleate Rhizoctonia spp. (BN-1, -2, -3, -9 and CAG-7) were screened in greenhouse tests. Agent inoculum (colonized barley) was applied to 6-wk-old beets and R. solani inoculum 3 d later. Disease was rated (DR) (0=healthy to 5= dead) 4 wk later. BN-1 and BN-3 gave low (0.2-0.4), CAG-7 intermediate (3.5) and BN-2 and BN-9 high DR similar to the R. solani only control (3.6-4.7). Application of BN-1 and L. arvalis (La7) at planting and to 6-wk-old beets was compared. La7 DR did not differ from the R. solani control (4.9-5.0). DR of BN-1 applied at planting was 3.1 vs 1.1 for 6-wk-old beets. A field test of BN-1, BN-3 and La7 applied at 3 rates (1, 0.5 and 0.1X) and at 2 times (thining, layby) was evaluated for control by % plant loss, DR and yield/plot (R. solani applied after layby). BN-1 (8.8%, 0.31 DR, 53.3 kg) and BN-3 (5.7%, 0.20 DR, 53.4 kg) gave control; whereas, La7 (29.8%, 1.32 DR, 43.6 kg) did not.

12

Biological control of Phytophthora root rot of azalea with Penicillium oxalicum. B. Ownley Gintis and D. M. Benson. Dept. of Plant Pathology, N.C. State Univ., Raleigh, NC 27695-7616.

Mycelial germlings of P. oxalicum on bran, added to pine bark (1% w/w), suppressed ($P = 0.05$) Phytophthora root rot of azalea caused by Phytophthora cinnamomi in two of three greenhouse experiments. Fresh shoot weights were increased and mortality reduced by 30 - 50% for azaleas planted in P. oxalicum-treated media compared to controls. Addition of P. oxalicum to pine bark limited total fungal counts but the relative population density of P. oxalicum remained stable. In the nursery, P. oxalicum was not as effective as metalaxyl ($P = 0.05$) in suppression of Phytophthora root rot of azalea under conditions of low disease pressure. The population density of naturally-occurring P. oxalicum was as much as 50% of the total fungal population density in container media treated with metalaxyl. P. oxalicum, a naturally-occurring inhabitant of pine bark media, shows potential as a biological control of Phytophthora root rot of ornamentals.

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BIOLOGICAL CONTROL OF APHANOMYCES EUTEICHES F. SP. PISI BY BACTERIA APPLIED TO PEA SEEDS. J. L. Parke, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Two hundred bacterial isolates from pea rhizospheres in Wisconsin were coated onto pea seeds and evaluated in a growth chamber bioassay for their ability to increase plant dry weight and reduce disease severity of seedlings inoculated with Aphanomyces euteiches zoospores. Thirteen organisms were selected for further testing in a naturally infested field. In the field experiment, plant emergence was significantly increased by five bacteria. The best strain also increased shoot dry weight by 70% compared to seeds coated with captan alone. Effective biocontrol strains include a species of Bacillus, Pseudomonas fluorescens, and three unidentified bacteria. The ability of bacteria to inhibit the growth of Aphanomyces euteiches in culture was poorly correlated with effective biological control of root rot.

14

ZOOSPORE LYSIS IN BIOCONTROL OF PHYTOPHTHORA MEGASPERMA BY BACILLUS CEREUS UW85. J. Handelsman, E. H. Mester, L.

Wunderlich, and C. R. Grau. Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Bacillus cereus strain UW85, isolated from the alfalfa rhizosphere, reduced mortality of alfalfa seedlings when the seedlings were inoculated with the bacterium and then with zoospores of Phytophthora megasperma f.sp. medicaginis (Pmm). Sterile filtrate of a fully sporulated culture of UW85 grown in tryptic soy broth protected alfalfa and lysed zoospores of Pmm in vitro. The zoospore-lysing activity and plant-protecting activity were both present in a methanol soluble fraction, were abolished by protease treatment, and both were present in a fraction that crossed a dialysis membrane with a cut-off of 1000 mw. In preliminary experiments, a mutant of UW85 that did not protect alfalfa seedlings from damping off did not have zoospore-lysing activity in its culture filtrate. These data suggest that the zoospore-lysing activity is involved in plant protection.

15

METALAXYL CONCENTRATIONS REQUIRED FOR INHIBITION OF PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA IN SOYBEAN CULTIVARS DIFFERING IN PHYTOPHTHORA ROOT ROT TOLERANCE. A. F. Olah and A. F. Schmitthenner, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Phytophthora megasperma f.sp. glycinea (Pmg), races 1, 4 or 7, were grown for 96 hr on lima bean agar plus 0 to 1000 ppb metalaxyl. The same rates of metalaxyl were applied to Pmg-infected high, moderate or low tolerant 6-day-old infected soybean plants and incubated for a further 7 days using a slant board culture system. Pmg growth inhibition was not significantly different in culture as compared to in planta, suggesting that its action within the plant, at the concentrations used, was solely to inhibit Pmg. Results of a bioassay that utilized Phytophthora boehmeriae plus Pmg to quantify metalaxyl concentrations from 0 to 500 ppb within infected roots prior to and during disease suppression for the 7 day incubation period will be presented.

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REACTIONS OF NAVY OR SMALL WHITE AND PINTO PHASEOLUS VULGARIS CULTIVARS TO UROMYCES APPENDICULATUS. J. R. Stavely, USDA, ARS, Microbiol. and Plant Pathology Lab., Beltsville, MD 20705.

The largest acreages of dry edible beans in the United States are in the navy or small white (SW) and pinto classes. For each class, the reactions of 30 cvs. were tested to 31 races of the rust pathogen, U. appendiculatus. Many cvs. were susceptible to most races. The most broadly resistant cvs. (and no. of races to which resistant) were California SW 59 and 643 (22), Neptune and Swan Valley (21), C-20 (19), and Fleetwood (17) navies; and Olathe (23), Scout (21), Luna and Pinto 5 (20), Nodak (17), Agate, Ouray, and Topaz (16), Navajo (15), and Colorado (14) pintos. Specific reactions had significant similarities or complementarities among cultivars. The identical resistances of C-20, Neptune, Swan Valley, and other cvs. to 13 races suggest they contain the same gene or gene complex. Optimal combination of the resistances of C-20 and Fleetwood navies or Colorado and Olathe pintos would supply resistance to 94% or 100% of these races, respectively.

17

EFFECT OF SOYBEAN CULTIVARS AND PLANTING DATE ON DEVELOPMENT OF SOYBEAN SUDDEN DEATH SYNDROME. D. E. Hershman, J. W. Hendrix, R. E. Stuckey, P. R. Bachi, and J. H. Grove, University of Kentucky, P.O. Box 469, Princeton, KY 42445.

Eight soybean cultivars from maturity groups III, IV, or V, with and without soybean cyst nematode (SCN) resistance, were planted on three dates in a field with a history of sudden death syndrome (SDS) and SCN. Planting date had a pronounced affect on SDS development in certain cultivars. Generally, SCN-resistant cultivars showed less SDS than SCN-susceptible cultivars. Overall, there was no correlation between cultivar maturity group and susceptibility to SDS. There was a weak positive correlation (r=0.65) between the incidence of SDS and maximum SCN population under SCN-susceptible cultivars.

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LEPTOSPHAERULINA LEAF SPOT OF SOYBEANS IN MISSOURI. S.M. Rosenbrock and T.D. Wyllie, Dept. of Plant Pathology, Univ. of MO, Columbia, MO 65211.

In July 1986, symptoms similar to those described for soybean sudden death syndrome (SDS) were seen on four soybean (Glycine max) cultivars (Williams 82, Asgrow 3427 & 3966, and Challenger. The infected areas were located in five northeast Missouri fields. Perithecia containing asci and ascospores of a species of Leptosphaerulina were consistently isolated from necrotic leaf tissue. Koch's postulates were fully satisfied in the greenhouse. Lesions are purplish circular spots with eventual gray-tan necrotic centers containing black perithecia. Leptosphaerulina leaf spot caused by L. trifolii and L. briosiana have only been reported on soybean from India (1972) and Maryland (1986). Analysis of variance of mycelial growth rates between the Missouri isolate, the Maryland isolate, and a L. trifolii isolate indicated a significant difference ($P > F$ 0.0001, R^2 0.97) between L. trifolii and the Missouri isolate, but no difference between the Maryland isolate. Investigation thus far indicates the Missouri isolate to be L. briosiana. This is the first observation of the disease west of the Mississippi River. Future investigation will attempt to identify the association of the fungus with SDS.

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HOST PREFERENCE BY CORN AND SOYBEAN ISOLATES OF MACROPHOMINA PHASEOLINA. C. A. S. Pearson, J. F. Leslie, and F. W. Schwenk, Dept. of Plant Path., Kansas State Univ., Manhattan, KS 66506.

Over 2100 isolates of M. phaseolina were collected from 13 states in the central and eastern United States. These isolates were evaluated for chlorate sensitivity by growing cultures on a defined medium containing 120 mM $KClO_3$. Soybean field soils averaged 2.1% dense isolates (chlorate resistant), 49.3% feathery isolates (chlorate tolerant), and 48.6% restricted isolates (chlorate sensitive). Phenotypic frequencies of soybean-tissue isolates did not differ significantly from soybean-soil isolate frequencies. Phenotypic frequencies of corn-soil isolates averaged 29.5% dense, 57.9% feathery, and 12.6% restricted. Corn-tissue isolate frequencies differed from corn-soil isolate frequencies, averaging 83.0% dense, 17.0% feathery, and 0.0% restricted. Crop rotation may also influence M. phaseolina populations. In a field infested with feathery and restricted isolates, soybean-soybean cropping resulted in higher soil and tissue fungal populations than did corn-corn cropping.

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PATHOGENICITY OF THREE FUNGI ON SOYBEANS AND CULTURAL CHARACTERISTICS OF CYLINDROCLADIUM CROTALARIAE. Graydon Kingsland, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Cylindrocladium crotalariae, a microsclerotia-producing fungus (MSF), and Neocosmospora vasinfecta were isolated from roots of Hampton 266A soybeans (Glycine max) from three fields in which ca. 60% of the plants expressed severe wilt, chlorosis, dwarfing, necrosis and root rot. Severe symptoms developed on wounded Hampton and PI 299358 plants root-inoculated in pot culture with C. crotalariae. Incipient symptoms developed on these hosts when inoculated without wounding. Wounded uninoculated control plants and wounded plants inoculated with N. vasinfecta or with the MSF were symptomless. A degree of cross protection by MSF was demonstrated when plants were inoculated with both C. crotalariae and the MSF. Illuminated cultures of C. crotalariae produced more conidia on malt agar and potato dextrose agar than on Czapek agar. Conidia were not produced on corn meal agar. Perithecia were produced only on corn meal agar.

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OCCURRENCE AND PATHOGENICITY OF FUSARIUM SOLANI RECOVERED FROM SOYBEANS WITH SUDDEN DEATH SYNDROME. J. C. Rupe, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701

Fusarium solani was isolated from roots of soybeans with sudden death syndrome (SDS) grown in 9 fields in Arkansas, 1 field in Illinois, and 1 field in Kentucky. One cm sections of the roots were incubated on water agar. F. solani was recovered from 8% of the lateral root sections, 16% of outer tap root sections, 9% of the inner tap root sections, and 4% of the inner lower stem sections. Eleven isolates representing 9 locations were used to inoculate 2-week-old Lee 74 soybean seedlings by placing a plug of mycelium next to the stem just below the soil line. All isolates produced reddish-brown lesions at the soil line within a week. SDS-like foliar symptoms began to appear one week after inoculation and increased in frequency and intensity thereafter. All but one isolate produced foliar symptoms; the other isolates varied in symptom intensity. Foliar symptoms ranged from light interveinal chlorotic spots to interveinal necrotic streaks and defoliation.

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Bacterial Blight of Soybean Incidence at Three Row Spacings in No-Till and Conventional Planting Systems. A. O. Anaele, U. R. Bishnoi, and R. P. Pacumbaba. Dept. of Plant and Soil Science, Alabama A&M University, Normal, AL. 35762.

Incidence of bacterial blight of soybean (BBS) at three row spacings (45, 60, and 90 cm) in no-till and conventional planting systems was investigated. Weeds were controlled by use of herbicide (Blazer), mechanical (hoe), and check (no control). Soybean yields obtained at 45 cm spacing were higher for both planting systems. BBS ratings at R4 in no-till was 8 for all treatments. Disease infestations of 78, 73, and 60% with corresponding yields of 3938, 3501, and 2478 kg/ha were obtained in herbicide, mechanical hoeing, and check plots, respectively. BBS ratings in conventional planting were 8, 7, and 7 with 55, 78, and 50% BBS infestations. The corresponding yields were 4829, 4785, and 3835 kg/ha from same treatments. Yields from conventional planting were higher for all the 3 row spacings. Incidence of BBS in 1986 reduced soybean yields from 4 to 40% compared to 1985 yields.

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STUDIES ON THE CAUSAL ORGANISM OF RED LEAF BLOTCH OF SOYBEANS. G. L. Hartman and J. B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

The causal organism of red leaf blotch of soybeans was named by R. B. Stewart as Pyrenochaeta glycines. Stewart's herbarium specimens were examined by us and R. Schneider, who stated that P. glycines was Phoma-like. We agree. However, none of the current Phoma spp. describe our isolates. By comparing other Phoma-like isolates from soybeans with those causing red leaf blotch, we found they could be separated by inoculating fresh soybean leaf disks. Isolates that cause red leaf blotch cause an achlorophyllous condition with abundant pycnidia and sclerotia after 3 wk. On autoclaved soybean leaf disks all isolates produced pycnidia, but sclerotia formed only from isolates that produced symptoms on fresh leaf disks. Growth rates on agar media were two to three times as fast for nonsclerotia-producing isolates. Mean conidial size ranged from 5.4-6 um in length and 2.3-2.8 um in width for sclerotia-producing isolates, while nonsclerotia-producing isolates varied from significantly (P= 0.01) smaller in length and width to the same size.

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EFFECTS OF OZONE ON SOYBEAN RESISTANCE TO THE MEXICAN BEAN BEETLE. A. H. Chappelka, M. E. Kraemer and T. Mebrahtu, Virginia State University, Petersburg, VA 23803.

Two soybean cultivars, "Forrest" and "Essex", were exposed to O_3 in open-top-field chambers receiving carbon-filtered air and non-filtered air with 0.00, 0.03 or 0.06 ppm O_3 added (7 hr/d). After 3 weeks, plants were removed and placed in a carbon-filtered chamber enclosed by an aluminium screen. Mexican bean beetle (MBB) adults were introduced into this chamber and allowed to feed for 1 week. Visible O_3 injury increased significantly with increasing O_3 concentration and Forrest exhibited more damage than Essex. No cultivar differences were found in MBB adult feeding preference, but defoliation increased significantly with increasing O_3 concentration.

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EFFECTS OF INSECT-INDUCED DEFOLIATION, STEM CANKER DISEASE, AND SOYBEAN CYST NEMATODE ON SOYBEAN GROWTH AND N_2 FIXATION. M.B. Layton, J.S. Russin, E.C. McGawley, D.J. Boethel, G.T. Berggren, and J.P. Snow. Depts. of Entomology and Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, 70803

Individual and interactive effects of soybean looper (Psuedoplusia includens) defoliation, stem canker (Diaporthe phaseolorum var. caulivora) (DPC), and Race 3 soybean cyst nematode (Heterodera glycines) (SCN) on growth, development, and $N_2(C_2H_2)$ fixation in 'Bragg' soybean were examined in greenhouse studies. Stem canker and defoliation caused additive reductions in leaf area, stem and root dry weights, and N_2-fixing ability, but number and dry weight of N_2-fixing nodules were reduced only by defoliation. DPC and SCN caused additive reductions in leaf area and stem dry weight. However, only 68 days elapsed between SCN inoculation (1500 eggs/pot) and experiment completion, which resulted in no effect of SCN on nodulation, root development, or N_2 fixation.

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INTERACTIONS BETWEEN SOYBEAN LOOPER, STEM CANKER FUNGUS, AND SOYBEAN CYST NEMATODE ON SOYBEAN. J.S. Russin, M.B. Layton, E.C. McGawley, D.J. Boethel, G.T. Berggren, and J.P. Snow. Depts. of Entomology and Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, 70803

Interactions of soybean looper (Psuedoplusia includens) (SBL), stem canker fungus (Diaporthe phaseolorum var. caulivora) (DPC), and Race 3 soybean cyst nematode (Heterodera glycines) (SCN) on 'Bragg' soybean were examined in three greenhouse experiments. In Exp. I, 50% SBL defoliation reduced DPC canker lengths 29% and delayed mortality. In Exp. II, DPC canker lengths were reduced 40% on SCN-infected plants (SCN inoculum level=1500 eggs/pot). Also, numbers of both SCN cysts and juveniles were reduced on DPC-cankered plants. In Exp. III, plants inoculated with both DPC and SCN (400 eggs/pot) showed reductions in both DPC canker length and numbers of SCN juveniles, as in Exp. II. In addition, SBL defoliation levels of 30 and 60% increased both numbers of juveniles and total SCN numbers on roots.

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USE OF WOUND RESPONSE PARAMETERS TO ASSESS THE RELATIVE SUSCEPTIBILITY OF PEACH CULTIVARS TO LEUCOSTOMA SPP. A.R. Biggs. Agriculture Canada, Research Station, Vineland Station, Ontario, Canada, LOR 2E0.

Twigs of ten field-grown peach clones were wounded and sampled for histological study after 0, 3, 7, 10, 14, and 17 days. Counts of cells and tissue thickness measurements were used to determine the extent of formation of lignosuberized boundary tissue and suberized wound periderm. Also, suberin autofluorescence intensity was measured using a microscope photometer. The experiment was conducted at one location in 1985 and two in 1986. Experiments were conducted in April, May, June, July, September, October and November in randomized complete block plantings at both sites. There were no consistent relationships between relative susceptibility to disease and cell number or tissue thickness. The rate of increase in suberin autofluorescence intensity during May and June was significantly correlated in both years with the relative susceptibility of the cultivars to the peach canker fungi.

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THE OCCURRENCE, DISTRIBUTION AND CONTROL OF BOTRYODIPLODIA THEOBROMAE ON GRAPES (VITIS VINIFERA) IN CALIFORNIA. G. M. Leavitt and D. E. Munnecke. University of California Cooperative Extension, 328 Madera Avenue, Madera, CA 93637, and University of California, Riverside, CA 92521.

B. theobromae (Botryosphaeria rhodina) was found statewide in cankers on grapes in greater incidence than Eutypa armeniacae. The relative incidence of the two diseases varies according to location. Spurs, arms, cordons and whole vines may be affected and eventually die. Two biotypes have been found. One grows rapidly at 36° C, produces red pigment and is the only type in Coachella Valley. The other grows very slowly at 36° C, produces no red pigment and is the main type (95%) in Northern California. Various materials were used as pruning wound paints to prevent infection. Most fungicides were effective in reducing the incidence when used on fresh wounds. Latex paint reduced infection, but was not acceptable as a control. The natural incidence of the disease on vines 10 years old and older can be as great as 100% with several cankers per vine.

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SURVIVAL OF COLLETOTRICHUM ACUTATUM IN SOIL AND BURIED STRAWBERRY TISSUE. D. M. Eastburn and W. D. Gubler, Department of Plant Pathology, University of California, Davis, CA 95616.

Propagules of Colletotrichum acutatum, the causal agent of strawberry anthracnose in California, were first detected in soil taken from a site which six weeks earlier had supported strawberry plants infected with anthracnose. Continued sampling of this site has revealed that C. acutatum propagules are able to survive in soil, in the absence of a host plant, for at least 7 months. The fungus also has the ability to survive in soil in infected runner tissue for at least 5 months. Thus far, propagules of C. acutatum have not been detected in soils which have been fumigated since the last strawberry crop was removed, but the fungus has been found in soil washed from stored nursery stock. Anthracnose symptoms have been initiated under greenhouse conditions by inoculating

young strawberry plants with a solution of naturally infested soil.

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EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM ON THE SEVERITY OF STRAWBERRY ANTHRACNOSE-CROWN ROT. Barbara J. Smith, USDA, ARS, Small Fruit Research Station, Poplarville, MS 39470

The effect of N, P, and K levels on the severity of anthracnose-crown rot (casual fungus, Colletotrichum fragariae) was evaluated on 3 resistant strawberry selections, grown in 10 cm pots and fertilized 3 times weekly with one of 16 nutrient solution treatments. Each treatment contained a standard level of Ca, S, Mg and micronutrients and one of 8 levels of N (NLEV) (0, 5, 10, 20, 40, 80, 160 and 320 ppm N) with either low P (8.7 ppm) and K (16.6 ppm) or high P (35.8 ppm) and K (66.4 ppm) (PKLEV). After 6 wk of treatment, the leaves were analyzed for %N, and the plants were inoculated with C. fragariae. Thirty days later each plant was rated for disease severity (DS) on a scale of 0 to 6. DS and %N increased on each selection as NLEV increased. Plants became susceptible (DS > 4) when %N was > 2. Predicted values at NLEV=0 to NLEV=320 ranged from 1.3 to 2.9 for %N and 2.3 to 5.9 for DS. PKLEV did not have a significant effect on DS or %N.

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IMPORTANCE OF TREE VIGOR IN REACTION OF PEACH TREES TO INOCULATION WITH BOTRYOSPHAERIA SPP. K. O. Britton, and F. F. Hendrix Dept. of Plant Pathology, Univ. of Georgia, Athens, Ga 30602.

The predictive value of tree vigor prior to inoculation as an indication of tree growth following inoculation with 3 Botryosphaeria spp. was compared with that of 4 commonly used disease severity measures. Tree diam. was measured twice prior to inoculation to measure tree vigor. Four-mm cork borer wounds were inoculated with B. dothidea, B. obtusa, B. rhodina, or sterile PDA. Post-inoculation increase in caliper (PIC) and disease severity were assessed after 9 wk. None of the measures of disease severity explained variance in PIC; the resulting R^2 were: gum exudate weight, 0.02; length of necrotic lesion, 0.04; dye translocation, 0.002; extent of fungal colonization, 0.05. Tree vigor, had an R^2 value of 0.35. Although inoculation with Botryosphaeria spp. significantly increased all disease severity measures, no decrease in growth resulted. This result is probably affected by the low stress conditions of this experiment.

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ISOZYME VARIATION IN XANTHOMONAS CAMPESTRIS PV. CITRI RESOLVED BY STARCH-GEL ELECTROPHORESIS. Q. B. Kubicek, E. L. Civerolo, J. R. Hartung, and M. Bonde, USDA, ARS, HSI, Beltsville, MD 20705

Enzyme electrophoretic variation was studied in strains of Xanthomonas campestris pv. citri. Starch-gel electrophoresis of bacterial lysates using two buffer systems revealed polymorphism in ten of fourteen enzymes. Thirtyone additional enzymes could not be consistently resolved. Strains of the A type group could be differentiated from the B and C type group strains by a single locus. A single strain from Mexico appeared to be more closely related to the B type group strains. Isolates from Florida possessed the greatest amount of variability and had alleles not shared by other isolates or pathovars.

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PHYTOPHTHORA ROOT AND CROWN ROTS OF PEACH IN THE EASTERN GREAT LAKES REGION. W. F. Wilcox and M. A. Ellis, Depts. of Plant Pathology, N.Y.S. Agr. Expt. Station, Cornell Univ., Geneva, NY 14456 and OSU-OARDC, Wooster, OH 44691.

A sudden collapse and death of peach trees was common along Lakes Erie and Ontario in 1986. Isolations from characteristic orange-to red-brown necrotic crown tissue of affected trees in 6 New York and 3 Ohio peach orchards yielded the following Phytophthora species: P. megasperma, all orchards; P. cryptogea, 3 NY orchards; P. cactorum, 1 Ohio orchard; and Phytophthora sp., 1 Ohio orchard. When 'Halford' peach seedlings were transplanted into artificially infested soil mix and flooded for 48 hr periods biweekly, all isolates of P. cryptogea, P. cactorum, and Phytophthora sp. caused complete root rot and an 80-100% incidence of crown rot and seedling mortality; P. megasperma caused variable levels of root and crown rots, depending upon the isolate.

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NATURAL DISTRIBUTION OF PHYTOPHTHORA CACTORUM IN PENNSYLVANIA APPLE ORCHARDS. S. H. Kim and G. Emberger, PA Dept. of Agr., Harrisburg, PA 17110-9408, and Messiah College, Grantham.

Importation of Phytophthora cambivora(Pcb) and P. cactorum(Pct) into Pennsylvania from Oregon and Washington via apple rootstocks led to investigation of the natural occurrence of Pcb and Pct in 52 PA orchards. Composite soil samples from a 20 tree area in each orchard were collected with a 2 cm diam. soil probe for isolation of Pcb and Pct using an apple cotyledon technique (Phytopathology 74:867). Isolation of Pcb was negative; however, Pct was isolated from 18 of 52 orchards, representing 9 growers out of 20. Pct was recovered from orchard soil in 7 of 9 counties sampled. Recovery of Pct from the soil near the trunk was higher (83.3%) than from the soil between trunk and drip line (11.1%), or from the drip line (16.7%). Soil samples from 1 - 25 year-old trees indicated that % recovery of Pct with the apple cotyledon technique from dried soil of older trees was higher than from younger trees ($r = 0.8509$, $y = 5.97 + 2.3x$).

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EFFECT OF SYSTEMIC FUNGICIDES ON PHYTOPHTHORA POPULATIONS, FEEDER ROOT DENSITIES, AND YIELD OF CITRUS. H. A. Sandler, L. W. Timmer, and J. H. Graham, University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850.

Fosetyl-Al was applied by foliar sprays and metalaxyl by soil surface sprays or drenches to four bearing citrus orchards in Florida. Most treatments had no effect on Phytophthora populations or feeder root densities during the first season. In the second season, the increase in root weights were 24, 2, 58, and 27% for four sprays of fosetyl-Al and 39, 43, 114, and 27% for three applications of metalaxyl in orchards of sweet orange on Cleopatra mandarin, sour orange, and sweet orange rootstocks, and grapefruit on sweet orange rootstock, respectively. Decreases in fungal populations following metalaxyl treatments were 50, 71, 38, and 50%, respectively. Fosetyl-Al applications did not usually affect Phytophthora populations. Four sprays of fosetyl-Al increased yield compared to the control in one orchard.

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PHYTOPHTHORA ROOT ROT/DIEBACK OF CRANBERRY IN MASSACHUSETTS. F.L. Caruso, C.C. Kusek, and W.F. Wilcox*, Cranberry Experiment Station, Univ. of Mass., East Wareham 02538 and Cornell Univ., New York State Agr. Exp. Station, Geneva 14456*.

During the fall of 1986, Phytophthora cinnamomi(mating type A2) was isolated from discolored underground runners sampled from dying "Early Black" and "Howe" cranberry plants in 18 bogs. Each bog typically had large patches of plants which were dead or dying and stressed plants were associated with low/wet spots in the bog. When affected areas were sanded and replanted with new vines, the replacement vines also died. Underground runners lack feeder roots, show discrete lesions and a distinctive red to olive-brown discoloration. Observations of the disease during the 1987 growing season, plant inoculations, and control strategies will be discussed. This is the first report of this fungus causing damage to the cranberry plant.

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AN EXPERT SYSTEM TO AID IN THE APPLICATION OF A STEROL-INHIBITING FUNGICIDE IN CONTROLLING APPLE SCAB. J. W. Travis and K. D. Hickey, Dept. of Plant Pathology, and E. J. Rajotte, Dept. of Entomology, The Pennsylvania State University, University Park, PA.

Sterol-inhibiting fungicides increase the growers' ability to control apple scab, but also increase the complexity of the disease management decision-making process. An expert system, a type of artificial intelligence where the logical processes of a human expert are captured in a computer program, was developed to aid apple growers in determining the effective use of

fenarimol. The system considers crop phenology, cultivar, tree size, canopy density, disease incidence, infection periods, fungicide history, and environmental conditions before recommending a fungicide rate and application timing, and develops recommendations for regular application intervals, post-infection applications or a combination of both. The expert system was evaluated in Pennsylvania during the 1987 growing season.

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CO_2 EXCHANGE AND PHOTOSYNTHATE TRANSLOCATION IN CORN LEAVES INFECTED WITH Exserohilum turcicum. Y. Levy and K. J. Leonard, Dept. Life Sci., Bar Ilan Univ., 52 100 Ramat Gan, Israel, and USDA,ARS N. C. State Univ., Raleigh, NC 27695.

Sixth leaves of Seneca 60 sweet corn were inoculated with 15-ul droplets containing ca. 15, 75, and 150 conidia of Exserohilum turcicum. One day after inoculation CO_2 uptake in infection sites decreased by 10, 39, and 49%, respectively. CO_2 exchange approached 0 at 11, 9, and 6 days after inoculation in these lesions. By 13 days after inoculation, CO_2 evolution occurred at a high rate. Increased carbon assimilation was observed in tissue surrounding infection sites 1 and 2 days after inoculation, but carbon assimilation decreased drastically by the third day and dropped to near 0 by 15 days after inoculation. From tissue adjacent to the distal edge of the infected area, 89% of the labled photosynthate was translocated into the lesion within 1 hr after assimilation. Significantly less photosynthate was translocated to lesions from tissue adjacent to the proximal edge of the lesion. Translocation from lesions to healthy tissue was not observed.

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A STATISTICAL ANALYSIS OF FIELD POPULATIONS OF COLLETOTRICHUM GRAMINCOLA. K. Cardwell de Castillo, and R. A. Frederiksen, Dept. Plant Path. & Micro., Tex. Agri. Exp. Sta., College Station 77843.

A quantitative relationship among populations of Colletotrichum graminicola on sorghum was sought using statistical techniques. The Spearman correlation method was used to compare data on disease ratings of sorghum differential varieties among sites and years. Disease ratings on a scale of 1-5 (1-resistant to 5-susceptible) were taken on the International Anthracnose Virulence Nursery in Texas, Puerto Rico, and Brazil in 1986 and in Georgia from 1980-1986. The ratings within each site/year were ranked to standardize the data. The correlation coefficients for the among-site data averaged .55. The correlation coefficient for between years 1984 and 1986 in Georgia was .84. The populations from geographically isolated regions have approximately .50 homology with respect to virulence capabilities of C. graminicola on sorghum. Populations of the fungus within a site may vary .20-.30 between years as measured by ratings on the differential varieties. A correlation coefficient of .75 or more indicates a high degree of relationship between two populations.

40

NON-SPECIFIC ADHESION OF PERONOSPORA TABACINA SPORES. M. L. Menetrez 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 ability of spores to adhere to the surface of a host is a key step in the initiation of disease. Peronospora tabacina spores adhere tenaciously to tobacco leaves suggesting the possibility that adhesion is involved in specificity of the pathogen for tobacco. P. tabacina spores were placed on glass and on tobacco, tomato, petunia, and bean leaf discs and tested for adhesion. The spores adhered to all surfaces. Treating the spores with a protease resulted in a significant decrease in binding to tobacco leaf surfaces. Therefore, a protein may be involved in adhesion of these fungal spores. We conclude that adhesion is a general property of P. tabacina spores and not related to specificity or host recognition.

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TOXICITY OF DIHYDROXYCADALENE TO VERTICILLIUM DAHLIAE. M. E. Mace, R. D. Stipanovic, and A. A. Bell. USDA, ARS, SCRL, P. O. Drawer JF, College Station, TX 77841.

The sesquiterpenoid 2,7-dihydroxycadalene (DHC), previously shown to be a phytoalexin in bacterial blight of cotton, also is toxic to Verticillium dahliae, incitant of verticillium wilt of cotton. The toxicity of DHC to a nondefoliating and a defoliating isolate was determined in a buffered liquid medium. DHC killed conidia and mycelia of the nondefolitating isolate at 70 and 80 μg/ml, respectively; and of the defoliating isolate at 80 and 100 μg/ml, respectively. Studies are underway to determine the concentrations of DHC formed in V. dahliae-infected verticillium-susceptible and -resistant cottons.

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IMMUNIZATION OF TOBACCO AGAINST PERONOSPORA TABACINA ADAM PRIMARILY AFFECTS COLONIZATION BY THIS FUNGUS. K. Stolle, L. Shain, F. Hebard, and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Nicotiana tabacum, 'Kentucky 14', plants were stem-injected with Peronospora tabacina (immunized) or with water (control). For challenge inoculation, filter paper discs soaked with a sporangiospore suspension of this fungus were placed on upper leaf surfaces. Nearly all (>99%) germinated spores penetrated both immunized and control tissue, as shown after staining with trypan blue. Light microscopy of cleared leaf tissue revealed that colonization of immunized tissue ceased within 5 days post inoculation (dpi); mycelium was confined within the lesion area (ca. 4 mm^2). Colonization continued in control tissue for ca. 10 days, lesions measuring ca. 310 mm^2 7 dpi; hyphae extended ca. 1 mm beyond the lesion margins. The density of sporangiophores and sporangiospores, but not the ratio between them, was reduced >65% in colonized immunized as compared to colonized control tissue.

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ASPERGILLUS NIDULANS: A POTENTIAL PLANT PATHOGEN? R. A. Dean and W. E. Timberlake, Dept. of Plant Pathology, University of Georgia, Athens, GA 30602

We are investigating the potential of A. nidulans as a plant pathogen. Inoculation of wounded excised cucumber, bean and pea tissues results in limited water soaking and host cell death. Sporulation occurs within several days. Inoculation of orange fruits causes a firm, dark, discolored rot. In liquid culture, growth on polypectate occurs after a lag of 24h. Polygalacturonase (pH optimum = 4) activity increases thereafter and reaches maximum at 50h. Pectate transeliminase (pH optimum = 8-9) is first detected at 40h and continues to increase for at least 72h. They are glucose repressed, and of the substrates tested, only induced by polygalacturonic acid. The levels of these enzymes are of the same order as those produced by the pathogens Erwinia carotovora (EC14) and E. chrysanthemi (EC16) grown in liquid culture. This is the first report, to our knowledge, of the production of pectolytic enzymes by A. nidulans and its ability to cause at least limited plant disease.

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CORRELATED LIGHT/TEM STUDIES OF CALLOSE-CONTAINING DEPOSITS IN VASCULAR TISSUE OF TOMATO INFECTED WITH FUSARIUM OXYSPORUM f. sp. LYCOPERSICI. W. C. Mueller and C. H. Beckman, University of Rhode Island, Kingston, R.I. 02881.

Vascular parenchyma cells of tomato respond strongly to vascular parasites, including Fusarium oxysporum f. sp. lycopersici, by depositing callose-containing papillae and apposition layers at pits and along walls adjacent to infected vessels. Alternate thick (1μm) and a series of thin sections of plastic-embedded tissues were cut and labelled in a sequential manner. The thick sections were placed on glass microscope slides, treated with sodium ethoxide to etch the plastic from the sections, stained with periodic acid Schiff's reagent followed by alkaline aniline blue, and examined microscopically using incident u.v. light. Deposits that were characteristic in their location and appearance were photographed. Corresponding deposits in thin sections were examined by TEM and photographed. Callose-containing deposits were characteristically marbled or layered in appearance under TEM.

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RESPONSE OF XYLEM PARENCHYMA CELLS IN TOMATO TO VASCULAR INFECTION BY FUSARIUM OXYSPORUM f. sp. LYCOPERSICI. C. H. Beckman and W. C. Mueller, University of Rhode Island, Kingston, R.I. 02881

Spores introduced into vascular elements are carried upward

to a variety of trapping sites where foci of infection are established. Many contact parenchyma cells adjacent to these sites are penetrated, colonized, and degraded. Other contact cells respond with callose deposition and osmiophilic "lignification" (CDO), or with a hypersensitive response (HR) that prevents invasion. When contact cells are invaded, many of the next adjacent (1^{o}adj) or second adjacent (2^{o}adj) cells respond with CDO or HR. By the third day, the infection appears to be contained in a lateral direction by these responses that constitute a defense in depth. As hyphae grow upward through the vessels, host cells above the trapping sites are challenged, but the infection pressure is less and a higher rate of containment is achieved in the contact, 1^{o}adj, and 2^{o}adj cells.

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INTERFERANCE BY AGROBACTERIUM TUMEFACIENS WITH THE DEVELOPMENT OF A HYPERSENSITIVE RESPONSE ELICITED BY PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA. Ann G. Matthysse and David Robinette, Dept. of Biology, University of North Carolina, Chapel Hill, NC 27514.

Prior injection into tobacco leaves of wild type A. tumefaciens inhibited the development of an HR in response to subsequent injection of P. s. phaseolicola. This interferance with the HR was not seen with prior injection of bacterial growth medium, E. coli, or Rhizobium meliloti. Live A. tumefaciens was required for the inhibitory effect. Various mutants of A. tumefaciens were examined in order to determine the genes involved. The presence of the Ti plasmid was required for HR interferance by A. tumefaciens. Neither bacterial virulence, motility, cellulose synthesis, nor ability to attach to plant cells were required for the inhibition of the HR.

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TWO GENES CONTROLLING MAACKIAIN METABOLISM IN NECTRIA HAEMATOCOCCA ARE ASSOCIATED WITH MAACKIAIN TOLERANCE AND ENHANCED VIRULENCE ON CHICKPEA. V. Miao and H. D. VanEtten, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

We have used the fungus Nectria haematococca (MP VI) to investigate the role of phytoalexin tolerance in pathogenesis. Enzymatic detoxification of the pea phytoalexin pisatin is controlled at several independently sufficient loci, but alleles conferring high activity are required for virulence on pea. Some isolates can also metabolize the chickpea phytoalexin maackiain. Previously, detoxification of maackiain among field isolates was correlated with maackiain tolerance and high virulence on chickpea. Conventional genetic analyses suggest that multiple loci control metabolism of maackiain by field isolate T234. Progeny from crosses segregating for two of these loci were tested for maackiain tolerance and virulence. Isolates able to metabolize maackiain were more tolerant of maackiain and more virulent on chickpea seedlings than siblings which were unable to metabolize maackiain.

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VIRULENCE AND DEOXYNIVALENOL (DON) PRODUCTION OF PROTOPLAST FUSIONS FORMED BETWEEN TOXIGENIC VIRULENT AND NON-TOXIGENIC AVIRULENT STRAINS OF GIBBERELLA ZEAE. G.C. Adams, N. Johnson, and L.P. Hart. Michigan State Univ., East Lansing, MI 48824.

Pathogenicity in vivo and toxin production in vivo and in vitro were measured in regenerated products of protoplast fusions. Protoplast fusions between toxigenic virulent (type A) and non-toxigenic avirulent (type B) strains were compared to wildtype parents, auxotrophs and forced heterokaryons of G. zeae. Protoplast fusion products were stable on inoculated maize ears and were readily reisolated. Protoplast fusion products varied in the amount of DON produced from none to low, moderate, and high concentrations. They also varied from avirulent to low, moderate, and high levels of virulence. Several fusion products that did not form detectable levels of DON were virulent. However, higher levels of virulence were often correlated to production of higher concentrations of DON. DON might be a virulence factor in pathogenesis of maize.

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RECURRENT SELECTION FOR COMPATIBILITY IN RICE-BLAST FUNGUS INTERACTION. Hei Leung and E. S. Borromeo, The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

The low level of pathogenicity exhibited by sexual progenies of Magnaporthe grisea on rice has hindered genetic analysis of cultivar specificity. We aim to develop compatible interactions through recurrent selection in both the host and the pathogen. Of over a thousand asci derived from various backcross generations of rice isolates, 2-3% produced susceptible lesions on rice lines. The fungus was reisolated from these lesions and intercrossed to improve fertility and pathogenicity. A high percentage of pathogenic progenies was recovered from crosses with a hermaphroditic rice isolate Guy-11 (gift from J. L. Notteghem). These progenies showed distinct differential pathogenicity on four rice lines. Concurrently, susceptible rice lines were intercrossed to generate F_2 populations to select for increased susceptibility. From such intermating populations, homozygous lines of different levels of susceptibility are being extracted for genetic analysis.

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DIFFERENCES IN THE GERMINATION OF MACROPHOMINA PHASEOLINA SCLEROTIA IN THE PRESENCE OF SOYBEAN AND SORGHUM SEED. G. L. Cloud and J. C. Rupe. Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Pathogenic, metabolic and morphological differences within M. phaseolina are associated with host specificity. Different germination rates of M. phaseolina sclerotia exposed to germinating seeds of different hosts might also allow detection of host specificity. To test this hypothesis, surface sterilized soybean and sorghum seeds were placed on nylon mesh disks atop field soil in petri dishes surrounded by artificially produced sclerotia. Percent germination of sclerotia after 6, 10, and 30 hrs in the presence of soybean and sorghum seed was 8.2, 5.3; 18.3, 13.0; and 41.5, 15.5 percent, respectively. Differences in germination in the presence of soybean and sorghum seed were not significantly different at 6 hr but were at 10 and 30 hr.

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CHEMOTAXIS OF AGROBACTERIUM TUMEFACIENS TO ISOLATED ROOT CAP CELLS. M. C. Hawes, Departments of Plant Pathology and Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.

Root cap cells produce a large part of the polysaccharide exudate that surrounds roots of crop plants. Because the cells can be isolated nondestructively, and can be cultured, it was possible to use them to measure chemotaxis of A. tumefaciens to a defined population of developmentally uniform root cells. Isolated root cap cells (ca. 10^4) in 100 µl semisoft agar were added to wells at the edges of swarm plates, and A. tumefaciens was added as a droplet at the center of the plate. Chemotaxis in wide host range strains was expressed as expanding rings that moved only in the direction of root cap cells; nonmotile mutants did not move from the origin. It should be possible to exploit the assay to select mutants that can be used to determine if chemotaxis to root exudates is important in rhizosphere colonization.

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EVALUATION OF A CLONED SEQUENCE FROM CORYNEBACTERIUM SEPEDONICUM FOR USE AS A PATHOGEN-SPECIFIC DNA HYBRIDIZATION PROBE. B.D. Mogen, N.C. Gudmested, and A.E. Oleson. Depts. of Biochemistry and Plant Pathology, North Dakota State Univ., Fargo, ND 58105.

In an attempt to enhance our ability to detect Corynebacterium sepedonicum through DNA hybridization procedures, we have cloned a 1.3 kb repeated sequence (RS) that is present at a high copy number in all C. sepedonicum strains that have been tested. The specificity of this cloned fragment has been studied by using it as a hybridization probe against immobilized total DNA purified from known nonpathogenic potato endophytes and ectophytes. Substantial cross-hybridization occurred between some of the nonpathogenic potato-associated bacteria and the recombinant probe, which contained the RS in an M13 cloning vector. However, much of the cross-reaction was due to the vector sequences. Minimal cross-hybridization was observed when the purified 1.3 kb insert was used as a probe, indicating the potential utility of the RS for detection of the ring rot pathogen.

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DECAY OF GRAPE ROOTS BY AGROBACTERIUM TUMEFACIENS BIOVAR 3 IS NOT ASSOCIATED WITH Ti PLASMID. A. L. Bishop, L. M. Blanchard, and T. J. Burr, Dept. Plant Pathology, Cornell University, New York State Agric. Exp. Station, Geneva, NY 14456.

Nine strains of Agrobacterium tumefaciens biovar 3 from the United States and Europe were assayed for their ability to decay grape roots, tumorigenicity on 5 hosts, and homology between indigenous plasmids and a DNA probe (pTHE17) containing the T-DNA from pTiC58. All strains decayed grape roots; 6 were tumorigenic and 3 were nontumorigenic. Five of the tumorigenic strains contained a 95 or 120 Mdal plasmid with homology to pTHE17. Plasmids from the remaining tumorigenic strain did not hybridize with pTHE17; this strain produced only very small tumors on Nicotiana glauca. No homology was detected between plasmids of nontumorigenic strains and pTHE17. Thus, pTHE17 may be useful for in vitro diagnosis of tumorigenicity in A. tumefaciens biovar 3. The root decay phenotype of A. tumefaciens biovar 3 appears to be independent of the Ti plasmid.

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STREPTOMYCIN RESISTANCE OF PSEUDOMONAS SYRINGAE PV. PAPULANS (PSP) ASSOCIATED WITH A CONJUGATIVE PLASMID. J. L. Norelli and T. J. Burr, Depart. of Plant Pathology, N.Y.S. Agr. Expt. Sta., Cornell Univ., Geneva, NY 14456.

A 68 Md plasmid present in 7 streptomycin-resistant field strains of PSP was not observed in 4 streptomycin-sensitive field strains. Streptomycin resistance was transferred from resistant donor strains Psp 34 and Psp 36 to sensitive recipient strains in matings on nitrocellulose membranes at frequencies of 4.7 X10-3 and 2.0 X10-2 /recipient cell (RC), respectively. Transconjugants contained all the plasmids of recipient strains plus the 68 Md plasmid of the donor strain. Transfer of streptomycin resistance from resistant donor strain Psp 33 occurred at much lower frequency, 2.0 X10-6 /RC, and resulted in the transfer of a 75 Md plasmid and the loss of some recipient plasmids. This is the first report of the field occurrence of a streptomycin-resistant plant pathogenic bacterium associated with a conjugative plasmid. Its potential spread to other bacteria may pose economic and health risks.

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COMPARISON OF SELECTIVE MEDIA FOR ISOLATION OF CLAVIBACTER MICHIGANENSE PV MICHIGANENSE. B. N. Dhanvantari, Agriculture Canada, Research Station, Harrow, Ontario N0R 1G0

The selective media CNS, CMS, KBT (King's medium B with 10 ug/ml of potassium tellurite and nalidixic acid) were tested using 6 strains of Clavibacter michiganense pv michiganense and 6 strains of phytopathogenic and soil bacteria. The KBT medium was mostly selective for the tomato canker bacteria. It permitted faster (6 days) development and a 10-fold higher colony count of pv. michiganense than CMS medium; the colonies developed mustard yellow pigmentation characteristic of the tomato canker bacteria and tellurite was reduced to a deep-grey deposit within. In plating canker-infected tomato seeds on KBT, only about 1% were found to be contaminated by other bacteria and fungi. Three of the 6 strains of pv. michiganense showed variation for colony morphology as large (2-3 mm) fluidal and small (1-1.5 mm) mucoid colonies on CMS and KBT but not on King's medium B.

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RAIN-TRIGGERED MULTIPLICATION OF PSEUDOMONAS SYRINGAE ON SNAP BEAN LEAFLETS. S. S. Hirano, C. B. Tanner+, and C. D. Upper*, Departments of Plant Pathology and +Soil Sciences, and *ARS USDA, University of Wisconsin, Madison, WI 53706.

Rain triggers the onset of rapid multiplication of P. syringae (Ps) on snap bean leaflets. Increases in population sizes of Ps as large as 2300-fold followed rainfalls. To determine whether the momentum of raindrops is an important factor in the growth-triggering phenomenon, 12-mesh fiberglass screens (1.8 m x 0.9 m) were placed ca. 15 cm above bean plants in field plots prior to rain. After each of two separate rains, population sizes of Ps were about 1,000-fold less on leaflets from plants under the screens than on plants on which rain fell directly. The screens decreased the velocity and thus, the momentum and impact of the raindrops as they fell onto the bean leaves. All of the water associated with these rains dripped through the screens onto the plants, but with greatly reduced momentum. Thus, the momentum of raindrops appears to be associated with the growth-triggering effect of rain on populations of Ps.

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ISOLATES OF PSEUDOMONAS SYRINGAE PV. SYRINGAE GIVING A NULL REACTION SUPPRESS THE PATHOGENIC REACTION OF OTHER ISOLATES. D. L. McCoy and J. V. Leary, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Fifty different hosts from six different cultivated plant families were surveyed for their reaction to four strains of Pseudomonas syringae pv. syringae isolated from four different hosts. Inoculum levels which clearly produced the optimum responses on differential hosts to the four isolates were determined. Coinoculation of all possible combinations of the four isolates revealed an ability of some isolates to block the normal reaction of another isolate. A narrow host range isolate, which produced no response in many hosts (null reaction), prevented the development of disease symptoms by a moderately virulent isolate. However, one highly virulent isolate produced disease symptoms, even when coinoculated with strains which produced the null reaction on that same host. Isolates which produced the hypersensitive response did not completely block the pathogenic response of other isolates.

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VIRULENCE OF ERWINIA AMYLOVORA ALTERED BY PLASMID DNA. J.L. Norelli, H.S. Aldwinckle, E.M. Steinberger, and S.V. Beer, Departments of Plant Pathology, N.Y.S. Agr. Expt. Sta., Cornell Univ., Geneva 14456 and Ithaca, NY 14853.

Erwinia amylovora strain Ea 266 is virulent to Malus sp. cv. Novole and harbors a 30 kb plasmid. Strains Ea 322 and Ea 273 are avirulent to Novole and harbor 60 kb and 30 kb plasmids. A transconjugant of Ea 266 containing a Tn5-labelled 60 kb plasmid of Ea 322 was avirulent to Novole. Ea 266 was complemented with a library of Ea 322 plasmid DNA cloned in pBR322. Many clones did not affect the virulence of Ea 266 to Novole, but a clone containing a 4 kb and a 1.5 kb EcoRl fragment of the 30 kb plasmid was avirulent to Novole. The 4 kb fragments from Ea 273 and Ea 266 were cloned in both orientations in the stable, low copy number vector pCPP8. The four chimeric plasmids did not affect doubling time in vitro of Ea 266, or its pathogenicity to immature pear fruits. However, Ea 266 complemented with any of the four plasmids, but not with the vector alone, was avirulent to Novole.

60

A METHOD FOR THE RAPID IDENTIFICATION OF CLAVIBACTER MICHIGANENSE SUBSP. MICHIGANENSE. R. D. Gitaitis and R. W. Beaver. Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793.

Bacteria with colony characteristics similar to those of Clavibacter michiganense subsp. michiganense (CMM) were selected from CNS and SCM agar media for further analysis. Fatty acid methyl esters prepared from whole-cell bacteria were analyzed by gas-liquid chromatography. The identification of CMM was possible within the range of fatty acids containing 12 to 18 carbon atoms. If the content of a fatty acid with the equivalent chain length of 14.49 exceeded 2.56 percent of the total, then the confidence level for identification of CMM was fairly high ($P = 0.10$). Bacterial identities were confirmed by pathogenicity tests on tomato

(Lycopersicon esculentum). Bacterial suspensions were also infiltrated into leaves of Mirabilis jalapa, a plant that exhibits a hypersensitive response when challenged with CMM.

61

A PLANT INDUCIBLE LOCUS CONTROLLING PATHOGENICITY AND HYPERSENSITIVITY IN PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA. P. B. Lindgren, R. Frederick, and N. J. Panopoulos. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

We have previously described a gene cluster, designated hrp, which controls the pathogenicity of P. s. phaseolicola on bean and its ability to elicit the hypersensitive reactions on non-host plants. We have analyzed this cluster by constructing gene fusions with a promoterless ice nucleation gene (inaZ) and have identified a plant inducible locus. Activation of this locus occurred both on the susceptible host as well as on non-host plants beginning at 2 to 2.5 hr post-inoculation suggesting that this locus controls a very early step(s) in the bacterium/plant interaction.

62

CLONING OF AN ICE NUCLEATION GENE FROM XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS. Valerie N. Hall and Donald A. Cooksey, Department of Plant Pathology, University of California, Riverside, CA 92521.

A genomic library of the ice nucleation active (INA+) cereal grain pathogen Xanthomonas campestris pv. translucens was prepared using the cosmid vector pLAFR3. Of 1500 E. coli clones screened for ice nucleation activity, two were INA+. Subcloning was done in the high copy vector pUC18. Restriction maps of the subcloned fragment will be compared to maps of ice nucleation genes described in other bacterial species. Homology studies with other pathovars of Xanthomonas campestris and with Pseudomonas and Erwinia spp. will be presented.

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PREDICTION OF RECOMBINANT PLASMID TRANSFER IN THE PHYLLOSPHERE WITH A SIMULATION MODEL. G. R. Knudsen and J. L. Armstrong, U.S. Environmental Protection Agency, Corvallis, OR 97333.

A computer simulation model predicted dynamics of survival and conjugation on leaf surfaces for donor (D) strain Pseudomonas cepacia (pR388:Tn1721) and a non-recombinant recipient (R) strain. Plasmid transfer rates for a mass action model were estimated using D+R strains incubated on excised radish or bean leaves in petri dishes. Survival was modeled as exponential growth and/or death depending on ambient humidity. Radish and bean plants growing in microcosms were then inoculated with D and R strains, and bacteria were enumerated over 14 days using a most probable number (MPN) technique with selective media in microtiter plates. The simulation model predicted hourly D, R and transconjugant populations. When initial D and R populations were $>10^7$ cfu/g, 10^3 or more transconjugants were predicted and observed. The model effectively used laboratory data to predict dynamics of a more complex system, and may be useful in assessing environmental risk of releasing recombinant bacteria.

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SOYBEAN COTYLEDON BIOASSAY FOR DETECTING NONPATHOGENIC MUTANTS OF XANTHOMONAS CAMPESTRIS PV. GLYCINES. I. Hwang, S. M. Lim, P. D. Shaw. Department of Plant Pathology, University of Illinois, IL 61801.

A rapid bioassay technique was developed to detect nonpathogenic mutants of X. campestris pv. glycines on inoculated soybean cotyledons within 48 hrs. The IncP type plasmid RP4 was mobilized into the recipient strain Xcg8ra(pathogenic) of the bacterium, but transposition of Tn5 from pSUP1011 did not occur at a detectable frequency. Attemps are being made to generate nonpathogenic mutants using other transposable elements. Strain Xcg8ra was highly resistant to ampicillin, pipericillin, and carbencillin up to 1,000 ug/ml, but it was sensitive to tetracycline and chloramphenicol at 50ug/ml. A genomic library was constructed in E. coli from partially digested DNA of Xcg8ra with endonuclease Sau3a using the mobilizable broad host range cosmid vector pLAFR3.

65

BENEFICIAL RESPONSE OF SUGARCANE CROP TO INOCULATION WITH NITROGEN FIXING MICROORGANISMS. C.LAKSHMINARASIMHAM, R.KUMAR, R.MUTHUKUMARASAMY & N.THAMILZHSERAN DEPT. OF BOTANY, AVVM SRI PUSHPAM COLLEGE, POONDI, THANJAVUR DISTRICT 613 503 , TAMILNADU, INDIA.

Strains of Azotobacter and Azospirillum were isolated first time from different sugarcane rhizosphere soils of 60 K.M radius around the college in India. The trials were conducted in farmers' fields in collaboration with three sugar factories, Kothari sugars, Arooran sugars, and Anna sugarsand found that Azotobacter strains were effective in clay loamy soilswhile Azospirillum strains were effective in sandy soils. Quantitative and qualitative increase along with soil structure improvement in sugarcane fields were recorded. Based on these results the programme is extended to 1500 acres to benefit farmers and factories.

66

DISEASE RESISTANCE RESPONSE GENES (DRRG) INDUCED IN PEAS BY FUNGAL WALL CHITOSAN: MODE OF INDUCTION LA Hadwiger, DF Kendra and BW Fristensky, Washington State University, Department of Plant Pathology, Pullman, WA 99164-6430

Fusarium solani f. sp. phaseoli is inhibited by pea endocarp tissue. Resistance is associated with changes in nuclear conformation, mRNA (of both DRRG and resistance response proteins) chitinase, β-glucanase, PAL, pisatin and lignin. We propose that pea tissue β-glucanase and chitinase degrade phaseoli walls and release chitosan, a polycationic polymer that induces the same responses as phaseoli. Chitosan induces immunity to the pathogen F. solani f. sp. pisi and directly inhibits fungal growth. Chitosan derived from walls of pisi differed from that of phaseoli. Pisi contained smaller oligomers and required greater quantities to inhibit fungi and induce immunity. Evidence related to chitosan's binding to specific DNAs, influence on cell viability, affects on Ca++ and other cellular processes will be discussed as well as predictions of DRRG function.

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DISEASE RESISTANCE RESPONSE GENES IN PEAS, CH Daniels, YS Cody and LA Hadwiger, Washington State University, Department of Plant Pathology, Pullman, WA 99164-6430

Pea varieties express differential race-specific resistance to Races 1, 2 and 3 of Pseudomonas syringae pv. pisi (Psp). Enhanced accumulation of disease resistance response mRNAs (DRRG) homologous to five cDNA probes is seen in five different pea cultivars. Cell walls and heat killed cells of Psp Races 1, 2, and 3 also induce mRNA accumulation but in a non-specific manner. Presence of live cells confers specificity to the interaction. Peas express a non-host resistance response against Fusarium solani f. sp. phaseoli (Fsp). A heat shock (HS) of 40°C for 1h breaks resistance and prevents formation of resistance associated proteins. Nine cDNA clones were hybridized to total RNA from HS tissue. mRNA accumulation in HS tissues was greatly depressed. High mRNA levels and resistance are expressed if Fsp is inoculated 6h prior to HS or after a 9h recovery period. HS and recovery prior to inoculation with F. solani f. sp. pisi temporarily enhanced resistance to this pathogen.

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REDUCED HOST-RANGE MUTANTS OF SCLEROTINIA SCLEROTIORUM. R. V. Miller, E. J. Ford and D. C. Sands, Department of Plant Pathology, Montana State University, Bozeman, MT 59717-0002.

Sclerotinia sclerotiorum was subjected to mutagenesis to alter the host-range of this non-specific pathogen. Mutants, generated by exposure of ascospores to ultraviolet light, expressed reduced host-ranges compared to the wild-type fungal parent. These reduced host-range phenotypes apparently existed as heterokaryons or heteroplasmons as evidenced by instability of the reduced host-range phenotype. Segregation of phenotypes, differing in host-range, was observed after sexual reproduction of this homothallic fungus. This segregation provided further evidence of the presence of different nuclei in the first generation mutant isolates. The reduced host-range isolates, if stabilized, will provide a new approach to biological control of weeds.

69. Withdrawn

70

COMPLEMENTATION FOR PATHOGENICITY OF THREE TN5-INDUCED MUTANTS OF ERWINIA AMYLOVORA. E. M. Steinberger, D. W. Bauer, and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Several non-pathogenic mutants of E. amylovora strains Ea 321 and Ea 322 were induced with Tn5. They retained ability to induce the hypersensitive reaction in tobacco and produced wild-type levels of extracellular polysaccharide. Members of a genomic library of Ea 321 constructed in the cosmid pCPP9 were mobilized from Escherichia coli to mutants of E. amylovora. Clones that complemented were selected based on their ability to restore pathogenicity to mutants on immature pear fruit. Cosmid pCPP420 restored full parental virulence to mutants Ea 321-T101 and Ea 321-T104. Cosmid pCPP410 restored full virulence to Ea 321-T101 and partial virulence to Ea 322-T104. Restriction analysis of the cosmids with EcoRI, BamHI, and SalI revealed inserts of 32 kb and 40 kb, respectively. The complementing fragments are being mapped and subcloned to determine the location and order of the pathogenicity genes.

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SITE-DIRECTED MUTAGENESIS OF CAPSULAR POLYSACCHARIDE GENES IN ERWINIA STEWARTII. D. L. Coplin, D. R. Majerczak, and M. R. Pastian. Dept of Plant Pathology, OSU/OARDC, Wooster, OH 44691

The genes for capsular polysaccharide synthesis (cps) and UDP-galactose-4-epimerase (galE) are clustered in a 19 kb region of the Erwinia stewartii chromosome and this locus has been cloned in plasmid pES2144. Tn3HoHoI and Tn5lac transposon mutagenesis of pES2144 have identified five cps regions (cpsA-E) that result in a butyrous colony-type in Δcps strains. When transposon-induced mutations in each of these regions were crossed into the chromosome of a wild-type strain, insertions in cpsB-E, but not cpsA or galE, greatly decreased the ability of the bacterium to cause watersoaked lesions on young corn seedlings. Complementation tests between chromosomal and plasmid borne cps mutations were done to determine the number of cps operons. Results to date indicate that cpsA, cpsB-D, cpsE, and galE are separate operons. Unlike other enterobacteria, the galE gene in E. stewartii is not part of a galETK operon.

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A COMPARISON OF SEVERAL METHODS FOR EVALUATING FITNESS IN PHYTOPHTHORA INFESTANS. L. J. Spielman and W. E. Fry, Cornell University, Ithaca, NY 14853.

The fitness of Phytophthora infestans isolates was estimated using three methods: a) AUDPC from epidemics in field plots, b) three parameters (infection frequency, lesion area, and sporulation capacity) measured under controlled conditions, and c) in competitive experiments (field plots inoculated with two isolates). AUDPC values were poorly correlated with the parameters measured under controlled conditions. In AUDPC values from two successive years, large differences among isolates remained stable, but rankings of similar isolates changed. In the competitive experiments isolates were identified by their enzyme profiles, and fitness was estimated from the proportion of each isolate recovered. There was very good agreement between the relative fitness of the weaker isolate calculated from the competitive experiments (0.70) and from AUDPC in the singly inoculated field plots (0.71).

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GENETIC ANALYSIS OF ESTERASE POLYMORPHISMS IN HETERODERA GLYCINES. P.R. Esbenshade and A.C. Triantaphyllou. Dept. of Genetics, North Carolina State University, Raleigh, NC. 27695.

The inheritance of esterase isoenzymes in the soybean cyst nematode, Heterodera glycines, was investigated through controlled matings and analysis of F1 and F2 progeny. Three homozygous nematode lines, fixed for different esterase phenotypes were developed following selection and inbreeding. Reciprocal crosses were performed in all combinations. Single male-single female matings were conducted on agar plates. Each parental phenotype consisted of a pair of closely spaced bands. F1 progeny were heterozygous, exhibiting both parental phenotypes, with no hybrid bands. F2 progeny expressed the isozyme phenotypes of the female parental line, the heterozygote and the male parental line in a 1:2:1 ratio. No maternal effects were observed. Apparently, the three esterase phenotypes correspond to three independent, codominant alleles of a single esterase locus. Methods used in this research are applicable to genetic isozyme analyses in other plant-parasitic nematodes.

74

FUSARIUM OXYSPORUM TRANSFORMATION. W. A. Powell, U. K. Benny, and H. C. Kistler, Plant Pathology Department, University of Florida, Gainesville 32611.

A transformation system has been developed for the fungal plant-pathogen, Fusarium oxysporum. This system utilizes the plasmid vector pDH25 which confers resistance to hygromycin B. The transformation frequency was calculated to be approximately one transformant per microgram of vector. The procedures used were able to transform three different F. oxysporum strains tested. We have found that incubation temperature, presence of nuclease inhibitors, sugar concentration, and vector modification all influence transformation frequency. Optimization of the procedures is now being carried out.

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VEGETATIVE COMPATIBILITY AND SELF-INCOMPATIBILITY WITHIN FUSARIUM OXYSPORUM F. SP. MELONIS. D. J. Jacobson and T. R. Gordon, University of California, Berkeley, 94720.

In a world wide collection of Fusarium oxysporum f. sp. melonis (Fom) isolates, five distinct vegetative compatibility groups (VCG) were identified by complementation of nitrate non-utilizing (nit) mutants. There was no consistent correlation between VCG and race within Fom. One VCG contained races 0, 1, 2, and 1.2, while race 2 was included in four VCGs and race 1.2 was included in two VCGs. In the San Joaquin Valley of California nearly all isolates of race 2 belong to a single VCG. A second race 2 VCG was found less frequently in California, although both VCGs may be present in the same field. Isolates that would not form intra-isolate heterokaryons with known complementary nit mutants were identified as vegetatively self-incompatible (VSI). VSI is correlated with the inability of an isolate to initiate anastamoses.

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COMPLEMENTATION OF ETHYL METHANESULFONATE TREATED TOX⁻ MUTANTS OF PSEUDOMONAS CORRUGATA WITH A COSMID LIBRARY. W. Chun, and J. V. Leary, Department of Plant Pathology, University of California, Riverside, CA 92521.

Pseudomonas corrugata, the causal agent of tomato pith necrosis, produces a phytotoxic compound that induces a rapid necrosis in tomato leaves. Prototrophic Tox⁻ mutants were obtained by treatment with ethylmethanesulfonate (EMS). A chromosomal library of P. corrugata strain 0782-6 was constructed in the cosmid vector pLAFR5. The cosmids were mobilized into EMS generated Tox⁻ mutants by triparental matings with E. coli containing helper plasmid pRK2013. Four cosmid clones were found which restored toxin production in three independently isolated EMS mutants. One cloned fragment (561-9-2B) restored toxin production in all three mutants; two others (561-2-5C and 561-10-11F) restored toxin production to EMS1-1. Another cloned fragment (561-12-9D) restored toxin production to EMS1-3. EMS1-4 was complemented only by 561-9-2B. Analysis of restriction enzyme digests revealed the presence of fragments of similar size.

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THE COTTON/BACTERIAL BLIGHT SYSTEM: GENE-FOR-GENE OR GENE-FOR GENES? K. McNally[1], M. Essenberg[1], and D. Gabriel[2], Dept. of Biochemistry, Oklahoma State Univ., Okla. Agricultural Expt. Station, Stillwater OK 74078[1] and Dept. of Plant Pathology, Univ. of Florida, Gainesville FL 32611[2].

D. Gabriel *et al.* (*PNAS* **83**:6415-6419) have isolated four avr genes [conferring avirulence against the cotton line AcB_5 J4265] from *Xanthomonas campestris* pv. *malvacearum* strain H, a widely avirulent strain. J4265 has been shown to segregate into two levels of resistance when tested with race 1, which is phenotypically identical to strain H. Spontaneous mutant KM1 of strain H was virulent on 51B, a uniformly high resistant

segregant from J4265, but not virulent on 51A, a separate uniformly high resistant segregant from J4265. KM1 was complemented to avirulence on 51B by cosmid pUFA809, which carries the AcB_5d gene. pUFA809 was restriction mapped and was found to be colinear to strain H and noncolinear to widely virulent strain N. A 3Kb region of pUFA809 insert DNA was missing in strain N.

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BIOMETRICAL ANALYSIS OF PATHOGENICITY IN THE COVERED SMUT-BARLEY (USTILAGO HORDEI-HORDEUM VULGARE) HOST-PATHOGEN SYSTEM. D.D. Pope, C.F. Wehrhahn and C.O. Person. Department of Botany, 2075 Wesbrook Mall, University of British Columbia, Vancouver, B.C., CANADA V6T 2B1

An F2 population of Ustilago hordei, segregating for an unknown number of genes affecting pathogenicity, was studied quantitatively on two barley cultivars, Trebi and Odessa. Disease reactions, as measured by the percentage of treated plants smutted, were highly variable in the F2 population. This variability was partitioned by analysis of variance into source subcomponents, several of which had a significant F value. Significant contributions to disease level variability were made by subcomponents corresponding to at least one segregating host specific factor and several other segregating factors. The total number of genes affecting disease readings was estimated to be 6.

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PHENOTYPIC EFFECTS OF A *recA* MUTATION IN AN ISOLATE OF *Pseudomonas syringae* pv. *syringae* PATHOGENIC ON BEAN. E. M. Hrabak and D. K. Willis, USDA/ARS and Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706.

We have constructed a *recA4*::Tn*5* derivative of the phytopathogenic *Pseudomonas syringae* pv. *syringae* isolate B728a (bacterial brown spot of bean, *Phaseolus vulgaris*) by site-directed mutagenesis. The plasmid used for this exchange (pEMH001) consisted of pLAFR3 containing the *recA4*::Tn*5* mutation in a 13.5 kb *Eco*RI fragment, originally isolated from the non-pathogenic *Pseudomonas syringae* strain Cit7. The B728a *recA4*::Tn*5* mutant is UV-sensitive, a sensitivity similar to that shown by this mutation in the Cit7 genetic background. However, the presence of this mutation does not compromise the phytopathogenic phenotype of B728a in either pods or leaves of *Phaseolus vulgaris*. This suggests that the *recA*$^-$ background can be a useful tool for genetic studies within *Pseudomonas syringae* pathovars.

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AGROBACTERIUM RHIZOGENES: ROLE OF TL-DNA GENES IN ROOT FORMATION. F. Shaheen and F. F. White, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Agrobacterium rhizogenes forms rooty tumors at the site of inoculation in many plants. TR- and TL-DNA together produce roots on Kalanchoe leaves. The TR-DNA contains genes for auxin biosynthesis. Four genetic loci (rol A, B, C, and D) on the TL-DNA have been reported to affect root morphology on Kalanchoe leaves. To understand the interaction or contribution of TL-DNA genes on the rooty phenotype on Kalanchoe leaves, we have used two approaches: 1) created multiple mutations in the TL-DNA by site-directed mutagenesis, and 2) cloned segments of TL-DNA containing single or multiple genes on a binary vector. These recombinant plasmids were introduced into various Agrobacterium mutants possessing TR-DNA and bioassayed. TR-DNA does not induce roots on Kalanchoe leaves, but TR-DNA in trans with rol B alone results in root formation. The contribution of rol B to root formation will be discussed.

81. Withdrawn

82

EFFECT OF pH ON EFFICACY OF CHLORINATED WATER FOR CONTROL OF POSTHARVEST DISEASE IN TOMATO FRUIT. J. A. Bartz, Plant Pathology Dept., University of Florida, Gainesville, 32611.

The pH of chlorinated water in dump tanks and flumes, controls the ratio of hypochlorous acid to hypochlorite ion in the solution. In laboratory tests, solutions which contained mostly the acid form (pH <7) were more effective against exposed cells of Erwinia carotovora than were solutions which contained mostly the 80-fold less reactive ion form (pH >9). Disease severity among fruit with peeled areas that had been rinsed in 5 X 10^6 cfu/ml, treated with 250 mg Cl_2/L for 2 min and then stored at 24 C increased linearly with solution pH. In contrast, when fruits had pin prick wounds or were infiltrated slowly with chlorinated water, then those treated at pH 9-10 had equivalent or significantly less disease in several different tests than did those treated at pH 7. Presumably, the high pH solutions penetrated more deeply into infection courts before the hypochlorite was lost to reaction with pathogens or plant tissues.

83

FUSARIA AND MYCOTOXINS ASSOCIATED WITH WHITE OR PINK MOLD IN MINNESOTA FIELD CORN. H. K. Abbas, C. J. Mirocha, R. A. Meronuck, J. Pokorny, S. Gould, T. Kommedahl, and P. O. Larsen, University of Minnesota, Department of Plant Pathology, St. Paul, MN 55108.

Ears of field corn exposed to hail and wet weather in 1986 became infected with a white or pink fungus especially on the ear tips. Of 32 fields examined in 10 counties in Minnesota, ears were collected from 19 fields where damage was extensive; they were assayed for Fusarium and mycotoxins. The following mycotoxins were found and expressed as average ppm and ranges: aflatoxin B1, 0; zearalenone (ZEA), 0.57(0-4.1); deoxynivalenol (DON, 3.80(0-18.7); and 15-acetyl-deoxynivalenol (15-ADON), 0.31(0-2.5). Screenings from one farm contained the following mycotoxins: aflatoxins, 0; ZEA, 3.3(2.1-4.1); DON, 10(8.0-13.1); and 15 ADON, 0. Ninety-eight cultures selected at random from field isolates of Fusarium were identified as F. oxysporum (31%), F. graminearum (30%), F. moniliforme (20%), F. proliferatum (12%), and F. subglutinans (7%).

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THE EFFECTS OF MACROCYCLIC TRICHOTHECENES ON IN VITRO AND IN VIVO GROWTH OF MYROTHECIUM RORIDUM PATHOGENIC TO MUSKMELON. Kuti, J.O., T.J Ng, and G.A. Bean. Departments of Horticulture and Botany, University of Maryland, College Park, MD 20742.

Myrothecium roridum (ATCC# 52485), a pathogen of muskmelon (Cucumis melo L.) produced a macrocyclic trichothecene roridin E in culture. Comparison of roridin E effects with those of the trichothecenes roridin A and myrotoxin A+B produced by non-pathogenic strains of M. roridum revealed that muskmelon cultivar responses to roridin E were consistent with cultivar reaction to the pathogen while responses to roridin A and myrotoxin A+B were not. Roridin A and myrotoxin A+B inhibited in vitro growth of the pathogenic strain but roridin E did not. Addition of roridin E to conidial suspension at inoculation enhanced ability of the pathogenic strain to develop larger lesions and to sporulate rapidly on inoculated muskmelon leaves. Although there is no evidence of a direct involvement of the trichothecenes in pathogenicity, the present study indicates that roridin E may play a secondary role in pathogenicity by acting as a virulence factor in the invasion of host tissue.

85

MONOCLONAL ANTIBODIES AGAINST THE MYCOTOXIN DEOXYNIVALENOL AND AN ENZYME IMMUNOASSAY FOR ITS DETECTION IN CONTAMINATED GRAIN. W.L. Casale[1], J.J. Pestka[2], and L.P. Hart[1]. [1]Dept. of Plant Pathology and [2]Dept. of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824.

A hybridoma line was developed which secretes monoclonal antibodies with affinity for deoxynivalenol (DON) and several analogs. To facilitate protein conjugation for production of immunogen and immunoassay reagents, DON was converted to 3-O-hemisuccinyl-DON after protection of C7 and C15 hydroxyls with a cyclic boronate ester. Derivatization was confirmed by thin-layer chromatography, mass spectrometry and proton magnetic resonance spectrometry. Direct- and indirect-competitive enzyme immunoassays utilizing monoclonal antibodies detected DON at 0.2-5.0 ug/ml (0.2-5.0 ug/g grain) in extracts of ground maize kernels.

86

THE EFFECT OF ULTRAVIOLET RADIATION ON THE CONTROL OF STORAGE ROTS OF SWEET POTATOES. C. Stevens, V. Khan, J. Lu and A. Y. Tang, Tuskegee University, Tuskegee, Alabama 36088.

Jewel, Carver and Georgia Jet sweet potatoes were irradiated with ultraviolet radiation (UV) with an average irradiance dose value of 1.2 mw/cm^2. These sweet potatoes were previously predisposed to storage rots. The application of UV stimulated an increase in the resistance of sweet potatoes to storage rots that included softrot (Rhizopus stolonifer), rootrot (Fusarium solani) and charcoal rot (Macrophomina phaseoli). For example, the percent rots of Jewel in 1986 in storage for two months when exposed to UV for 5 min and the controls were 19 and 88, respectively. Percent rots of Jewel in storage in 1987 test were 24, 22 and 52 for UV, botran fungicide treated and control, respectively.

87

EFFECT OF IONIZING AND ULTRAVIOLET RADIATION ON THE RESISTANCE OF ONIONS TO STORAGE ROTS. C. Stevens, P. Loretan, J. Lu, A. Y. Tang and C. Bonsi. Tuskegee University, Tuskegee, Alabama 36088 and D. Eakin, Battelle Pacific Northwest, Laboratories, Richland, WA 99352.

Walla Walla sweet onions, produced in the state of Washington, are high moisture onions making them susceptible to various kinds of storage rots within 30 days. Freshly harvested onions were irradiated with gamma (0.1 to 3.0 kGy), electron bean (0.1 to 5 kGy) and ultraviolet (0.44 to 19.1 x 10^4 ergs/mm^2) radiation. After two months, onions irradiated at UV dose levels between 1.3 to 19.1 x 10^4 ergs/mm^2 were less susceptible to black mold (Aspergillus niger), blue mold (Penicillium spp.), and softrot (Erwinia carotovora). For gamma and electron beam irradiated onions, the dose that appeared to be most effective in reducing storage rots was 0.1 kGy.

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METHOD OF ANALYSIS FOR FUSAROCHROMANONE IN CORN AND CHICKEN FEED AND SCREENING Fusarium ISOLATES FOR THIS TOXIN. Weiping Xie and Chester J. Mirocha, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Fusarochromanone caused tibial dyschondroplasia in poultry and reduced hatchability of fertile eggs. The toxin was extracted by methanol/water/ammonia hydroxide (45:5:1) and cleaned up by partitioning with dichloromethane and a Sep-Pak silica column. The toxin was quantified by using high performance liquid chromatography with a fluorescence detector. The detection limit of the method was 25 ppb. Recoveries from ground corn and chicken feed supplemented at 500 ppb with fusarochromanone averaged 70% and 73%, respectively. Forty of 65 Fusarium isolates from barley, oat and wheat collected in Alaska produced this toxin when fungi were cultured on liquid media for 48 hr at 24 C. Fusarochromanone fluoresces in liquid culture when illuminated with UV light; its presence is confirmed by thin layer chromatography and mass spectrometry.

89

EQUINE LEUCOENCEPHALOMALACIA IN MARYLAND HORSES ASSOCIATED WITH CONTAMINATED CORN CONSUMPTION. G. A. Bean and C. La Grenade, Dept. of Botany, Univ. of Maryland, College Park, MD 20742.

Equine leucoencephalomalacia (ELEM) is a fatal disorder of horses reportedly caused by consuming corn contaminated with Fusarium moniliforme. In Maryland, 14 cases of ELEM were verified by autopsies during 1987. All horses had consumed feed rations with high levels, 35% or more, of corn. The corn was locally produced and did not appear moldy although Fusarium moniliforme was the most frequently isolated fungus from 85-95% of surface disinfected corn kernels. High levels of F. moniliforme were found in Maryland corn during 1986, but no cases of ELEM were reported. Analyses of feed rations by thin layer chromatography for the Fusarium mycotoxins, zearalenone, moniliformin and fusarin C were negative. Culture filtrates of F. moniliforme isolates from corn that were grown on steamed rice for 28 da were also negative for Fusarium mycotoxins previously reported responsible for ELEM. These studies suggest that the mycotoxin(s) responsible for ELEM in the U.S. are not those reported in other countries.

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MYCOTOXIN PRODUCTION BY FOUR ISOLATES OF ALTERNARIA ALTERNATA AND SOYBEAN SEED QUALITY ANALYSES OF ASYMPTOMATIC AND SYMPTOMATIC SEEDS COLONIZED BY A. ALTERNATA. K.J. Cavanaugh, and J.B. Sinclair, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Four isolates of A. alternata from field-damaged soybean seeds, grown in modified-Czapek liquid media at 20 and 25 C for 21 days, were analyzed by thin layer (TLC) and gas chromatography (GC) for toxin production. Alternariol monomethyl ether, alternariol, altenuene, and altertoxin I were observed as present in culture extracts and one 50 g seed extract of symptomatic seeds when analyzed by TLC. GC analysis showed that higher amounts of toxins were produced at 25 than at 20 C. Seed weight, volume, and density measured on symptomatic, asymptomatic, and mixtures of both was significantly greater for asymptomatic seeds. Mean number of splits/100 seeds was significantly (P=0.05) greater for symptomatic than asymptomatic seeds. Percent protein of derived flour of symptomatic seeds was significantly greater than that of asymptomatic ones.

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TOXICITY OF FUSARIUM ISOLATES IN RATS OBTAINED FROM NEW ZEALAND AND CORRELATED WITH EXTRACTED TOXINS. U. Bosch, C.J. Mirocha and H. K. Abbas, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Sixty - two isolates of Fusarium spp. were obtained from pasture leaves and soil from various areas of New Zealand and identified as F. avenaceum (11), F. culmorum (12), F. graminearum (2), F. moniliforme (1), F. nivale (3), F. oxysporum (15) and F. poae (1). These isolates were grown on autoclaved rice and tested for toxicity in rat feeding tests. Eighty two percent of the isolates were toxic but 24% were severely toxic and caused hemorrhage of stomach and intestine, hematuria and finally death. Extracts of the most toxic isolates contained 0 to 271 ppm of hemorrhagic factor (H-1), 0 to 569 ppm of HM-8 (uncharacterized cytotoxic factor), 0 to 1021 ppm of zearalenone, 0 to 103 ppm of deoxynivalenol (DON) and 0 to 13000 ppm of moniliformin.

92

Effect of relative humidity and leaf age on sporulation of *Alternaria porri* on onion leaves. K. L. Everts and M. L. Lacy, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Onion plants were inoculated with dry conidia of *Alternaria porri*, incitant of onion purple blotch, and placed in a dew chamber at 24C for 24 hours. Plants were then incubated in a growth chamber at 24C for 6 days to obtain lesion development. Leaf segments containing lesions of uniform size were removed, placed under near-ultraviolet light (peak wavelength = 360nm) for 3 hours to induce conidial formation, then incubated in sealed glass jars containing water or saturated salt solutions at 24C. These jars maintained humidities at 100, 97.5, 85.5 or 75%. Spores formed at all humidities used, but the number of conidia per cm^2 increased with increasing relative humidities. Leaves of different ages were exposed to near-ultraviolet light and placed in a dew chamber at 24C and 100% RH for 3 days. Leaf age did not affect numbers of conidia formed per cm^2 of leaf.

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THE DYNAMICS AND ROLE OF FUNGAL IMMIGRATION IN PHYLLOPLANE COMMUNITY DEVELOPMENT. L. Kinkel and J. H. Andrews, Dept. of Plant Pathology, UW-Madison, Madison, WI 53706.

Immigration of fungi to apple leaves was estimated by sampling phylloplane communities 12 hours after leaf-surface disinfestation in the field. Numbers of individuals and community composition were evaluated for sequential 12-hour immigration periods. Composition and size of communities developing after exposure for 2-14 immigration periods were compared with corresponding immigration estimates. Although there were significant differences among 12-hour immigration periods in total numbers of individuals as well as filamentous fungi and yeasts, no diurnal patterns were evident. Leaf area had a significant influence on numbers of immigrants per leaf. Developing communities supported progressively fewer individuals than the estimated sums of immigrants. Contrasts between immigrants and develop-

ing communities may provide insight into the relative roles of immigration, growth, and emigration or death in community development.

94

PROGRESS OF ANGULAR LEAF SPOT OF BEAN GROWN IN MONOCULTURES AND IN BEAN-MAIZE INTERCROPS. J.M. Lanter, M.A. Pastor Corrales*, and J.G. Hancock. Plant Pathology, University of California, Berkeley, CA 94720; *CIAT, AA 6713, Cali, Colombia.

Detailed information on disease progress in different cropping systems is lacking. Angular leaf spot (ALS), caused by Phacoisariopsis griseola, is an important disease of bean (Phaseolus vulgaris) occurring in Latin America and Africa where bean intercropping is a common practice. Two lines, BAT 76 and RAB 206, were grown in monocultures and in bean-maize intercrops in field trials conducted near Cali, Colombia. ALS severity was recorded at weekly or semiweekly intervals by evaluating leaflets of 12 plants in each of the above treatments and in the fungicide-treated controls. In the first season, ALS severity differed significantly among the treatments with the fungicide effect being most significant ($P>.0001$), followed by that of genotype ($P > .0001$) and cropping system ($P>.02$). Severity of ALS was higher on RAB 206 grown in monoculture than in intercrop, but the reverse was true for the more resistant line, BAT 76. ALS reduced seed yield/plant by 13-34%.

95

SPLASH DISPERSAL OF PHYTOPHTHORA CACTORUM FROM INFECTED STRAWBERRY FRUIT CAUSED BY FOLIAGE DRIP. K. M. Reynolds, L. V. Madden, D. L. Reichard, and M. A. Ellis, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster 44691.

To simulate canopy drip, low velocity water drops were used to study splash dispersal of P. cactorum from infected strawberry fruit. Drops $\leq$ 1 mm diameter did not produce splash droplets when released from heights < 40 cm. Drops of 1, 2, and 3 mm diameter produced splash droplets when released from heights $\geq$ 40, 20, and 10 cm, respectively. Maximum and mean splash distance, mean droplet diameter, and number of splash droplets per impact all increased with increasing kinetic energy of the impacting drop. Mean droplet diameter decreased with splash distance for droplets with and without sporangia, but mean diameter was generally greater for droplets carrying sporangia. Multiple-exposure photographs of drop impacts suggested that residence time of splash liquid on the fruit surface determined both incidence of sporangium-bearing splash droplets, and mean number of sporangia per droplet.

96

A MODEL FOR PREDICTING THE SPORULATION OF *PLASMOPARA VITICOLA* BASED ON TEMPERATURE AND DURATION OF HIGH RELATIVE HUMIDITY. N. Lalancette, L. V. Madden, and M. A. Ellis. Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691

The amount of sporulation of *Plasmopara viticola* on Catawba grape leaves was observed under various temperatures and durations of high relative humidity. Potted vines with 7-day-old lesions were placed in a dark, humid (>95% RH) growth chamber at 6 pm. Plants were removed for observation at 6, 7.5, 9, 10.5, and 12 hr; temperatures examined were 10, 15, 20 25, and 30 C. A maximum sporulation of 3.3×10^5 sporangia/cm^2 of lesion area occurred after 12 hr at 20 C. Little or no sporulation occurred at 10 and 30 C. A predictive model for sporulation was developed by fitting the Richards model to the data. The model described 90% of the variation in sporulation when the asymptote and rate parameters were expressed as functions of temperature.

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EFFECT OF PRUNING PRACTICES ON PHOMOPSIS CANE AND LEAF SPOT OF GRAPE (Vitis labrusca). J.W. Pscheidt and R.C. Pearson. Dept. of Pl. Path., NYSAES, Cornell Univ., Geneva, N.Y., 14456.

The occurrence of Phomopsis cane and leaf spot (Phomopsis viticola) and use of mechanical pruning (hedging) practices have been increasing in western New York vineyards for several years. Our goal was to determine if pruning practices had an effect on disease severity. Hand pruned and hedged vines in vineyards unsprayed prior to bloom were examined for disease. The surface area of internodes 2-5 infected by Phomopsis averaged 6.9 and 5.7% on hedged and hand pruned vines, respectively. However, only vines that had been hedged for more than 2 years had significantly higher disease severity. Disease severity was significantly reduced when vines, that had been hedged for 6 years, were hand pruned (5.3 v.s. 8.0%). Increased disease severity in hedged vines could be due to several factors such as higher inoculum levels or increased shoot susceptibility.

99

USE OF AN ORBITAL THEMATIC MAPPER FOR DETECTION OF FOREST DECLINE IN THE SOUTHERN APPALACHIAN MOUNTAINS. R. I. Bruck, and S. Khorram. Dept. Plant Pathology and Computer Graphics Center, NC State University, Box 7616, Raleigh, NC 27695.

Studies have been undertaken to assess the utility of employing the NASA LANDSAT IV Thematic Mapper (TM) for the detection and quantification of mortality and decline symptoms in spruce-fir forests of the southern Appalachian mountains. Permananent field survey plots were installed in high altitude boreal-montane ecosystems on Mt. Rogers (VA), Mt. Mitchell, Grandfather Mt., Roan Mt., and Clingman's Dome (all NC) beginning in 1983. Regression and principal component analyses revealed that TM band 5 data (indicating water stress), plot basal area, percent mortality, and percent defoliation were correlated ($p=.01$) with TM digital number values (measures of spectral reflectance). Significant relationships ($p=.01$) between eigenvectors for digital number values and percentages of slightly declining trees suggests the possibility of detecting presymptomatic stress.

100

GRADIENTS OF DISEASE INCIDENCE, SEVERITY, AND DEFOLIATION IN PEANUT, CAUSED BY CERCOSPORIDIUM PERSONATUM. S. C. Alderman F. W. Nutter, Jr., and J. L. Labrinos. Dept. Plant Pathology, University of Georgia, Athens, GA 30602.

Peanut plants ('Florunner') supporting 50 sporulating lesions of Cercosporidium personatum were placed in the center of four 30-m-square blocks of Florunner peanuts in July, 1986. Overhead irrigation was applied nightly for 2 weeks to provide conditions favorable for infection. Incidence (leaflets with lesions), severity (lesions per leaflet), and % leaflet defoliation were determined on 4 stems at 15 cm intervals from the source plant in 4 directions (N, S, E, W). Data were collected weekly for 4 weeks, beginning 3 wk after introduction of the inoculum source. Incidence, severity and defoliation data (y) with respect to distance (x) were fitted to log(y)-log(x), ln(y)-linear(x), logit(y)-log(x), and logit(y)-linear(x) models. Gradients were best described by the logit-linear model, based on examination of r^2 and residuals. Velocities of spread for incidence, severity, and defoliation were 0.049, 0.039, and 0.036 m/day, respectively.

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EFFECT OF LOW TEMPERATURE ON PUCCINIA RECONDITA POPULATIONS CARRYING VIRULENCE TO LR24 IN WHEAT. D. Marshall, Texas Agricultural Experiment Station, Texas A&M University Research and Extension Center, Dallas, 75252

Virulence to LR24 has been found in P. recondita races UN2, UN5, UN13 and UN17 in Texas collections. Over two years, these four races have been found in equal frequencies during the months of November through February in commercial wheat fields. However, from March through June the frequency of UN5 greatly increases over the other three races. Seedlings of 'Thatcher' and the near-isogenic 'LR24-Thatcher' were inoculated and incubated at various temperature combinations ranging from 5 to 25 C. Genotypes with LR24 virulence in races UN2, UN13 and UN17 exhibited reduced sporulation, chlorosis and necrosis on LR24-Thatcher at 20 and 25 C. The same genotypes produced compatible reactions at all lower temperatures. The UN5 race with LR24 virulence exhibited a compatible reaction on LR24-Thatcher at all temperatures tested. All four P. recondita genotypes produced compatible reactions on Thatcher regardless of temperature.

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Microclimate of Grapevine Canopies Associated with Leaf Removal and Control of Botrytis Bunch Rot. J.T. English, C.S. Thomas, J.J. Marois, and W.D. Gubler, Department of Plant Pathology, University of California, Davis 95616.

Removal of leaves around clusters has been shown to reduce Botrytis bunch rot of grapes. Microclimates of leafed and nonleafed canopies were characterized and compared at three locations in California. Temperature, relative humidity, wind speed, and leaf wetness were recorded hourly in each canopy throughout the growing season. Vapor pressure was calculated from temperature and relative humidity. Within leafed and nonleafed canopies, climatic factors varied over time and location. As determined by multivariate analysis, wind speed, vapor pressure, temperature, and leaf wetness, considered together, accounted for 18 to 66% of microclimatic differences between leafed and nonleafed canopies over the entire season. These factors generally accounted for more than 60% of differences between canopies at each location when evaluated at 10-day intervals over the season.

103

USE OF INCIDENCE-SEVERITY RELATIONSHIPS TO DETERMINE THE ACTION THRESHOLD FOR COMMON MAIZE RUST ON SWEET CORN. H. R. Dillard and R. C. Seem, Dept. of Plant Pathology, N.Y.S. Agr. Expt. Station, Cornell Univ., Geneva, NY 14456.

Individual plants from three commercial fields of processing sweet corn (cv. Jubilee) in western New York were evaluated for common maize rust (Puccinia sorghi). Incidence and severity were assessed as percent leaves infected per plant and plant mean of pustule count or Horsfall-Barratt rating per leaf, respectively. The relationship between incidence (0-100%) and percent leaf area diseased (0-1%) was linear (R^2,adj.=0.99). Pustule counts of 5.5 to 6.8 per leaf corresponded to 1% leaf area diseased or 80% leaves infected. We propose use of these relationships to simplify the assessment of a 6-pustules-per-leaf action threshold for initiation of fungicide sprays to reduce the rate of epidemic development.

104

DISPERSAL AND SURVIVAL OF CLEISTOTHECIA OF UNCINULA NECATOR. David M. Gadoury and Roger C. Pearson, Department of Plant Pathology, N.Y.S. Agr. Expt. Station, Cornell University, Geneva 14456.

Mature cleistothecia of Uncinula necator were removed from mildew colonies by rain, but immature ascocarps remained attached to mildew colonies by anchorage hyphae. Dispersal of mature ascocarps during rain began in late July in severely diseased vineyards and continued until leaves senesced or were killed by frost. Large numbers of ascocarps were deposited in bark crevices where they remained throughout winter. The percentage of viable ascocarps in collections from leaves, canes, berry clusters, soil, and bark averaged 65, 63, 93, 78, and 96%, respectively, at leaf fall, and 3, 8, 3, 0, and 54%, respectively, the following spring. This differential survivorship may reflect the selective removal of mature ascocarps from leaves, canes, and berry clusters; dispersal of mature ascocarps to bark; and the death of ascocarps in soil.

105

USE OF LATE LEAFSPOT DISEASE GRADIENTS TO EVALUATE DISEASE ASSESSMENT SCHEMES FOR ACCURACY, PRECISION, RESOLUTION AND SPEED. F. W. Nutter, Jr. and S. C. Alderman. Dept. Plant Pathology, University of Georgia, Athens 30602.

Disease gradients provide a unique opportunity to compare assessment systems for use within a single pathosystem since disease severity decreases in a continuum with increasing distance from an inoculum source. This provides a wide range of disease levels simultaneously to make comparisons for accuracy, precision, resolution, and speed. Gradients of late leafspot in peanut, caused by Cercosporidium personatum, were established in Plains, GA, and assessed twice using the following methods: Incidence, defoliation, severity (lesion area/total leaf area remaining), ICRISAT 9 pt. scale, canopy layer method, height to defoliation/-height of stem, dry weight of stems and peanut canopy reflectance at 800 nm using a multispectral radiometer. Incidence, defoliation and the radiometer assessment systems provided the most accurate and precise estimates of leafspot severity and also had the highest correlation with yield.

106

THE USE OF MITOCHONDRIAL DNA (mtDNA) RESTRICTION FRAGMENT LENGTH POLYMORPHISMS AS A TAXONOMIC AID FOR THE GENUS PYTHIUM. F. N. Martin and H. C. Kistler, Plant Pathology Dept., University of Florida, Gainesville, 32611.

Speciation in the genus Pythium is often difficult due to large numbers of described species, similarity in morphologies, and variation in taxonomic characteristics. Because of the distribution and importance of this genus, alternative methods for species identification are needed. Analysis of restriction digests of mtDNA from 21 different Pythium spp. revealed significant and consistant differences in the banding patterns of each species. Restriction fragment length polymorphisms were observed at a high frequency for interspecific comparisons and at a low frequency for intraspecific comparisons. When 10 isolates of P. oligandrum collected from diverse locations in the U.S.A., South Africa, and Czechoslovakia were compared, mtDNA banding similarity of 85 to 100% was observed. Less than 60% similarity was observed with comparisons to other species (including morphologically similar species). All species examined had unique banding patterns.

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SURVIVAL AND GROWTH OF SECONDARY SPORIDIA OF Tilletia indica AT VARIOUS RELATIVE HUMIDITIES AND TEMPERATURES. J. L. Smilanick, L. R. Secrest, K. Weise, and J. A. Hoffmann. Crops Research Laboratory, USDA-ARS, Logan, Utah 84322-6300. Present address of first author: Horticultural Crops Research Laboratory, USDA-ARS, Fresno, CA 93727.

The survival of secondary sporidia of Tilletia indica at different relative humidities was determined by suspending sporidia on coverslips in chambers in which the relative humidity was controlled with glycerine and water. Secondary sporidia survived for 2.0, 3.5, 8.0, 10.5, and 12 hours at 25, 50, 70, 85, and 95% relative humidity, respectively, at 25 C. The effect of temperature on germ tube elongation on potato dextrose agar was also determined. The rates of germ tube elongation were 0.33, 0.42, 1.86, 4.04, 5.40, 0.94, and 0.0 µm/hr at 5, 10, 15, 20, 25, 30, and 35 C, respectively. The findings of the in vitro studies reported here are consistent with epidemiological observations of Karnal bunt in India and Mexico.

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SCLEROTIA FORMATION OF A SNOW MOLD CAUSING LOW TEMPERATURE BASIDIOMYCETE. J. H. McBeath and J. Plaskowitz. Agricultural and Forestry Experiment Station, University of Alaska, Fairbanks, AK 99775-0080 and Systematic Botany, Mycology & Nematology Laboratory, USDA Beltsville Agricultural Research Center, MD 20705.

Sclerotial low temperature basidiomycete (SLTB) is a snow mold commonly found in Alaska. Observations made of it since 1981 indicated that sclerotia-forming is characteristic of SLTB. In 1986, light and scanning electron microscopy of SLTB sclerotia at different stages of growth and development were conducted. Swelling of terminal and intercalary cells in the mycelia was the first sign of the sclerotia-forming process. As the process progressed, globose- and pyriform-shaped cells increased in number and became tightly interwined. Cell differentiation to the rind or medulla was not observed. Freeze fracture of sclerotial cells showed dense granules and oil globules in the cytoplasts. Sclerotia formation is probably essential to the propagation and survival of SLTB.

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CYANOGENESIS OF A SCLEROTIAL LOW TEMPERATURE BASIDIOMYCETE. J.H. McBeath and M. Adelman. Agricultural and Forestry Experiment Station, University of Alaska, Fairbanks, AK 99775-0080.

Coprinus psychromorbidus, a snow mold disease causing low temperature basidiomycete (LTB), produces HCN but no sclerotia. Sclerotial low temperature basidiomycete (SLTB), a snow mold common to Alaska, forms sclerotia consistently. In 1986, experiments were conducted to study the cyanogenesis of SLTB. SLTB and Fusarium nivale (a non-cyanogenic control) were cultured on a liquid medium (containing glucose, pepton, yeast extract, mineral salts) with 10% fresh wheat straw at 10 C for 6 weeks. After adding 2% KOH, fungal mass-wheat straw (well colonized by fungus) and culture filtrates were distilled. The distillates (5 ml) were then added to sodium picrate (10 ml). Had HCN been present, the color would have changed from yellow to red. No HCN was detected either in samples of SLTB and F. nivale, or in the controls (medium and KOH). HCN controls produced positive results. Absence of cyanogenesis further strengthened suspicion that SLTB and LTB differ taxonomically.

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SEROLOGICAL RELATIONSHIPS AMONG ACREMONIUM SPECIES. R. A. Shelby[1], A. E. BROWN[2], and E. M. CLARK[1]. [1]Dept. of Plant Pathology, and [2]Dept. of Botany and Microbiology, Ala. Agric. Expt. Sta., Auburn University, AL 36849.

Antigens of 7 endophytic, pathogenic, or saprophytic Acremonium species isolated from nine grass hosts were characterized by crossed immunoelectrophoresis (CIE) using antiserum developed against a single isolate of A. coenophialum from tall fescue. Endophytic isolates from grasses reacted with anti-A. coenophialum antiserum to varying degrees. As many as five antigen moieties were detected when the homologous antigen was tested. Other A. coenophialum isolates were less reactive to this antiserum. The endophytic fungi, A. starrii, A. huerfanum, and A. chisosum, had 1 to 4 common antigens, while none were detected in A. loliae, or the saprophytic species, A. strictum. One isolate of A. typhinum, had a single reactive antigen. These data suggest a common phylogeny among most endophytic Acremonium species, as well as A. typhinum, but that some biochemical variation between isolates of A. coenophialum is present.

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FUNGI ON PLANTS AND PLANT PRODUCTS IN THE UNITED STATES-A SOURCEBOOK FOR PLANT PATHOGENIC FUNGI. **G.F. Bills, G.P. Chamuris, D.F. Farr, & A.Y. Rossman. Systematic Botany, Mycology, and Nematology Laboratory, Agricultural Research Service, Beltsville, MD 20705.**

In the past, the "Index of Plant Diseases in the United States" (Agr. Handb. No. 165) has been the most comprehensive reference documenting fungi in this country. The old Index is outdated because it was based on information gathered primarily before the 1940's. In addition, the Index only provided a host-oriented coverage of fungal pathogens and lacked a unified treatment of the fungi. Scientist in this laboratory are compiling a new book, "Fungi on Plants and Plant Products in the United States." This book has been designed to: 1) provide host distribution data for plant pathogenic fungi; 2) aid in the identification of fungi on plants and plant products; and 3) serve as a source of accepted names and synonyms of these fungi. The construction of computer databases used to generate the book will be described. Summary statistics of the literature surveyed, numbers of host plants and fungal pathogens included, and taxonomic and economic trends based on the organisms covered will be presented.

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EFFECT OF D(+)-XYLOSE ON THE RESPONSE OF MYCELIAL GROWTH, SPORULATION AND POLYPHENOXIDASE OF BIPOLARIS MAYDIS RACE T TO GUAIACOL. Y-S Shin and M. O. Garraway, Department of Plant Pathology, Ohio Agr. Res. Dev. Centr. and The Ohio State University, Columbus, OH 43210.

Mycelial growth and sporulation of Bipolaris maydis race T (BMT) was inhibited when grown for 7 days on synthetic agar media (pH 6.0) containing glucose (10 g/l) and a guaiacol (400 mg/l) amendment. When the glucose in the medium was substituted by xylose the inhibitory effect of guaiacol was more pronounced. This effect of guaiacol was seen on both non-buffered media and media containing 0.05 M of 4 Morpholine Ethane Sulfonic acid (MES) buffer. Fungal polyphenoloxidase activity (ΔOD/hr/gm dry wt) with glucose and xylose was 36 ± 10 and 6 ± 2, respectively, without guaiacol, and 23 ± 8 and 3 ± 1 with a guaiacol amendment, indicating that the activity of this enzyme from thalli grown on xylose was several fold less than that from thalli grown on glucose. Decreased polyphenoloxidase contributes to the increased guaiacol inhibition of growth and sporulation of BMT seen when glucose in the media is replaced by xylose.

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REGULATION OF CERCOSPORIN PRODUCTION BY GROWTH MEDIA COMPONENTS. A. E. Jenns, M. E. Daub, and R. G. Upchurch. Department of Plant Pathology, N. C. State University, Raleigh, NC 27695-7616.

Cercosporin production by Cercospora asparagi, C. beticola, C. nicotianae, C. zea-maydis and C. kikuchii was affected by the media on which the fungi were grown. Cercosporin content was determined over time by soaking mycelial plugs, cut from cultures on agar, in 5N KOH and measuring absorbance at 480 nm. Results of this partial extraction were positively correlated ($R=0.99$, $P<0.01$) with the total toxin concentration in acetone extracts of ground mycelium. Both the medium most conducive to cercosporin production and the time of maximum accumulation differed among species and among isolates of the same species. In general, toxin production was highest on potato dextrose agar or a medium containing malt extract, peptone and glucose. Two media reported to enhance sporulation suppressed toxin production by all isolates. Measurement of cercosporin production on a range of media over time is necessary to assess the toxin-producing ability of a Cercospora isolate.

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OOSPORE PRODUCTION BY PHYTOPHTHORA SYRINGAE AT LOW TEMPERATURES IN VARIOUS MEDIA AND IN ALMOND ORCHARDS. M. A. Doster and R. M. Bostock, Department of Plant Pathology, University of California, Davis, CA 95616.

Isolates of Phytophthora syringae pathogenic on almond trees produced oospores in media after 4 weeks at 5 and 9 C but most did not produce oospores at > 12 C. Similarly, oospores were only observed at 12 C and below in inoculated almond leaves. Oospore production greatly increased when corn oil, linseed oil, or wheat germ oil was added to various base media. The addition of β-sitosterol resulted in a slight but significant increase in oospores. In almond orchards P. syringae was isolated from 18 to 26% of recently fallen almond leaves on the orchard floor and abundant oospores were observed in these leaves. Oospores were also produced by an almond isolate of P. syringae in almond leaf medium (75 g fresh leaves/L) and in leaf media of apricot, cherry, french prune, peach and plum but not in apple leaf medium.

115. Withdrawn

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AN ULTRASTRUCTURAL STUDY OF PEANUT RUST. J. TAYLOR, C. W. Mims, and E. S. Luttrell, Department of Plant Pathology, University of Georgia, Athens, GA. 30602.

Transmission electron microscopy was used to examine the host-parasite relationship between the rust fungus Puccinia arachidis and its host Arachis hypogaea as well as the formation of urediniospores by the fungus. Hyphae of P. arachidis ramified within intercellular spaces of infected leaves and gave rise to haustorial mother cells that formed typical D-haustoria. Each haustorium consisted of a slender neck region and an expanded haustorial body. An electron-dense neck band was characteristically present and an extrahaustorial membrane separated the haustorium from the host cytoplasm. Hyphae typically aggregated within the leaf adjacent to either epidermis and proliferated to form uredinia. A layer of sporogenous cells within each uredinium gave rise to stalked, binucleate urediniospores. A peridium initially covered the uredinium but was ruptured along with the host epidermis as spores developed.

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ULTRASTRUCTURE OF THE HOST-PARASITE RELATIONSHIP IN LATE LEAF SPOT DISEASE OF PEANUT. C. W. MIMS, E. S. Luttrell and S. C. Alderman. Dept. of Plant Pathology, Univ. of Georgia, Athens, Ga. 30602.

Transmission electron microscopy was used to examine the host-parasite relationship between the fungus Cercosporidium personatum and leaf cells of its host, Arachis hypogaea. In developing lesions hyphae were most numerous in intercellular spaces of the spongy mesophyll but were also found between the cuticle and the cells of the lower epidermis. Branched, irregularly septate, and multinucleate haustoria were observed in living host cells. Haustorial branches were separated from the host cytoplasm by an extra-haustorial membrane. At the site of host cell wall penetration, haustorial diam. was the same or slightly less than that of an intercellular hypha. No collar of host cell wall material was observed at the penetration site. Host cells containing haustoria eventually died although haustoria within the cells remained intact and appeared healthy.

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CHLAMYDOSPORE PRODUCTION BY PHYTOPHTHORA CACTORUM. T. W. Darmono and J. L Parke, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Morphological descriptions of Phytophthora cactorum include reference to chlamydospores; however, no effective procedure for producing chlamydospores has been reported. Abundant spherical structures 28-56 µm in diameter matching the descriptions of P. cactorum chlamydospores were formed by growing the fungus in 20% clarified V-8 juice broth at room temperature for 3 days under continuous light, replacing the medium with sterile distilled water, and then placing the cultures at 4 C for an additional 7 days. Compared to oospores, chlamydospores were larger, had thinner walls, and were easily distinguished by staining with tetrazolium bromide. Chlamydospores were non-papillate, persistent, and were terminal or rarely intercalary. They survived freezing at -23 C for more than 24 hr and germinated readily at room temperature. Of 11 isolates examined, all produced chlamydospores in vitro.

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EFFECTS OF A STEROL BIOSYNTHESIS INHIBITOR ON HYPHAL TIP CELLS OF THREE BASIDIOMYCETOUS FUNGI. R. W. Roberson and M. S. Fuller, Department of Botany, University of Georgia, Athens, GA 30602.

Transmission electron microscopy was used to study the effects of the triazole fungicide cyproconazole on hyphal tip cells of Rhizoctonia solani, Sclerotium rolfsii, and Fomes annosus. Cyproconazole is known to inhibit C14-demethylation in sterol biosynthesis resulting in the accumulation of C14-methylated sterols and a decrease in the functional sterol ergosterol. The sterol biosynthesis inhibiting sensitive step is a cytochrome P_{450} mediated reaction. In this study the effects of the fungicide were tested at EC_{50} concentrations. Samples of fungicide treated and nontreated material were prepared for study using freeze-substitution fixation and flat embedding techniques. Nontreated tip cells had well organized Spitzenkörper regions composed of masses of apical vesicles. In treated samples the number of apical vesicles within the Spitzenkörper regions were greatly reduced.

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THE USE OF HILL PLOTS FOR THE EVALUATION OF BROWN STEM ROT RESISTANCE AND YIELD IN SOYBEANS. Alemu Mengistu, H. Tachibana, A. H. Epstein, and K. G. Bidne. Department of Plant Pathology, Iowa State University, Ames IA 50011-1020.

Differences in brown stem rot (BSR) severity between resistant and susceptible soybean cultivars were obtained in hill plots. Relative BSR differences were similar whether cultivars were grown on highly or lightly infested land. Yields of resistant cultivars were not affected by the level of infestation, but yields were different for susceptible cultivars. Significantly higher yields under low levels of infestation and significantly lower yields under high levels of infestation were obtained for susceptible cultivars. Based on mean yields, cultivars with above average resistance to BSR were identified under high but not under low infestation levels. Effective evaluation for BSR resistance is possible in hill plots in highly infested soils.

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OCCURRENCE AND PATHOGENICITY OF FUSARIUM SOLANI ON SOYBEAN SEEDLINGS. F. Killebrew, K. Roy, G. Lawrence, and K. McLean, Dept. of Plant Pathology and Weed Science, MS State University, MS State, MS 39762

Soybean (Glycine max (L.) Merr.) seedlings collected from 36 different locations in MS were evaluated for symptoms and incidence of Fusarium solani (Mart.) Sacc. on root and hypocotyl tissues. Incidence of the fungus on seedlings was negatively correlated with stand and positively correlated with root and hypocotyl disease indices. Isolates of F. solani caused dark brown to black cortical lesions on primary and secondary roots of soybean seedlings inoculated in the greenhouse. Though differing in virulence, all were pathogenic as measured by disease severity, root volume, and stand. Pathogenicity of F. solani was verified in 1985 and 1986 field tests. Final stand, seedling height, and yield were significantly reduced. In another field test in 1985, F. solani was more pathogenic on seedlings when poor quality seeds were inoculated than when higher quality seeds were inoculated.

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COMPARISON OF METHODS FOR EVALUATION OF POTATO GENOTYPES FOR RESISTANCE TO VERTICILLIUM WILT. S.K. Mohan, J.R. Davis, D.L. Corsini, L.H. Sorensen, and J.J. Pavek. Univ. of Idaho and ARS-USDA, Aberdeen, ID 83210.

Nineteen potato genotypes were evaluated in the greenhouse and field for resistance to wilt caused by Verticillium dahliae and for sensitivity to the toxin produced by the pathogen in vitro. There was a highly significant correlation (r=0.90; P=<0.001) between the rate of wilt progress in the greenhouse and in the field. Similarly, stem colonization by V. dahliae in the field was highly correlated (r=0.82; P=<0.001) with wilt incidence. However, the sensitivity of a number of genotypes to V. dahliae toxin did not correspond with their wilt reaction. Some wilt-susceptible genotypes were not sensitive, while some wilt-resistant genotypes were sensitive to the toxin. These results indicate that greenhouse evaluation for wilt and measurement of the degree of stem colonization by V. dahliae in the field provide the most reliable means for determining Verticillium wilt resistance of potato genotypes.

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VARIABILITY AMONG SINGLE CONIDIAL ISOLATES OF MACROPHOMINA PHASEOLINA. R.T. Chitima-Matsiga and T.D. Wyllie, Dept. of Plant Path., Univ. of Missouri, Columbia, MO 65211.

Macrophomina phaseolina, the causal agent of charcoal rot of numerous agronomic crops, is extremely variable. In order to determine the nature of this variability & to establish its origin, pycnidial formation was stimulated by exposure to ultra-violet light. Four hundred single pycnidiospores were isolated. Comparisons were made between conidia derived from the same pycnidium & between pycnidia of the same isolates as well as between isolates. Differences in growth rate under different cultural conditions, pathogenicity, virulence & host range were determined. The data suggest that differences occur in morphology, growth rate & pathogenicity among isolates of M. phaseolina, & also to some extent between individual conidia originating from a single pycnidium. The differences in growth of single conidial cultures ranged from 45 mm to 50 mm & from 57 mm to 66 mm at temps of 22C & 32C, respectively. Pathogenicity ranged from 20% disease incidence in cultures derived from one pycnidium to 90% in another. Morphologically, the alfalfa isolate had the most appressed growth whereas the pine isolate had an aerial type of growth. This diversity enables the organism to adjust to a multitude of environments & hosts.

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RESISTANCE TO ALTERNARIA BRASSICAE AND PHYTOALEXIN-ELICITATION IN RAPESEED AND SOME OTHER CRUCIFERS. K.L. Conn, J.P. Tewari and J.S. Dahiya, Dept. of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5.

All cultivars of rapeseed are susceptible to Alternaria brassicae, which causes the blackspot disease of rapeseed. Many vegetable, oleiferous and wild crucifers were screened for resistance to A. brassicae. An accession of Eruca sativa showed hypersensitive response to A. brassicae. Accessions of Camelina sativa and Capsella bursa-pastoris were classed as being the most resistant. On these plants, the germination of A. brassicae conidia was reduced and penetration of the leaves usually did not take place, resulting in almost no infection. Preliminary research has indicated the elicitation of low levels of phytoalexins in rapeseed, and high levels in C. sativa. This may explain their differential disease susceptibility. Phytoalexin production in the other crucifers is being investigated. This appears to be the first report on elicitation of phytoalexins in crucifers upon being challenged by a fungal pathogen.

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EFFECTS OF IN VITRO METALAXYL TREATMENTS ON VIRULENCE OF PHYTOPHTHORA MEGASPERMA F. SP. GLYCINEA. Jana S. Holt and Jack D. Paxton. Department of Plant Pathology, University of Illinois, C-519 Turner Hall, 1102 S. Goodwin, Urbana, IL 61801

Variation in virulence and aggressiveness was observed after successive transfers of Phytophthora megasperma f. sp. glycinea on media amended with 0.01 ppm metalaxyl. Following hypocotyl wound inoculation of 8-day-old soybean plants, metalaxyl treated race 11 was able to cause disease on Harosoy 63 (normally resistant to race 11), and both Harosoy and Harosoy 63 plants grown from seeds treated with metalaxyl. Colonies derived from single zoospores of treated race 11 were uniform in causing disease in Harosoy 63. The rate of disease development was greater with metalaxyl treated cultures than their parents. Metalaxyl treated fungi retained sensitivity to metalaxyl in vitro.

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PRELIMINARY INVESTIGATION ON A CYLINDROSPORIUM LEAF SPOT ON OAK. G. P. Munkvold, University of Illinois, Urbana-Champaign 60801.

In September 1985, a leaf spot was observed on northern red oak (Quercus rubra) in Urbana and Lisle, IL. Symptoms consisted of numerous 2-4 mm circular necrotic lesions on the lower leaves, and early defoliation. Lesions are dark brown at first and later become tan, with dark margins, surrounded by a yellowed or water-soaked halo. Cylindrosporium quercus was consistently observed on and isolated from lesions. Of the media tested (V-8 juice agar, cornmeal agar, prune agar, and potato dextrose agar), PDA was best for growth. Growth and spore germination occurred at temperatures from 18 to 27°C. Growth was best at 27°C and germination was highest at 24 to 27°C. Fifty-six oak seedlings were leaf-inoculated with conidia by atomization, brush inoculation, and placement of pre-germinated conidia on the leaves via cellophane disks. Typical lesions formed on one northern red oak. Cylindrosporium quercus was reisolated from the lesions.

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INFLUENCE OF TANNIN-RELATED COMPOUNDS FROM PEANUT SEED COATS AND COTYLEDONS ON ASPERGILLUS PARASITICUS GROWTH AND AFLATOXIN PRODUCTION. A. H. Azaizeh and R. E. Pettit, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station 77843.

Twenty three peanut genotypes were evaluated in a humidity chamber for infection and subsequently for aflatoxin production. Tannin-related compounds were extracted with methanol from seed coats and cotyledons. Tannin extracts from peanut parts were evaluated in liquid medium (Yeast extract) for their effect on A. parasiticus growth and aflatoxin production. Aflatoxin production was determined by using high pressure liquid chromatography. Infection and aflatoxin production differed among genotypes. Florunner, PI 337409, SN 55-437, and Texas 7 were the most resistant genotypes with low infection and low aflatoxin production. Levels of tannin compounds in seed coats and cotyledons differed among genotypes, with higher levels in seed coats. Tannins extracted from seed coats and cotyledons of peanut kernels revealed that some extracts significantly decreased the growth of A. parasiticus and slowed or partially inhibited aflatoxin production.

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LATENT COLONIZATION BY COLLETOTRICHUM SPP.- IMPLICATIONS FOR MYCOHERBICIDES. R.F. Cerkauskas. Agriculture Canada, Vineland Research Station, Vineland Station, Ontario LOR 2EO.

Isolates of Colletotrichum coccodes (CC) from tomato, C. orbiculare (CO) from cucumber, C. gloeosporioides f.sp. aeschynomene (CGA-commercial mycoherbicide product-Collego), and C. gloeosporioides (CG) from Stylosanthes spp. were inoculated (5×10^5 spores/ml) separately onto 4 soybean and tomato cultivars, and onto various weed species in growth room studies. Paraquat was used to detect latent colonization of plant stems by the fungi(Phytopath. 70:1036-1038). Field tests for latent colonization by CC on 6 tomato cultivars also were conducted during the growing season. Paraquat-treated tomato and soybean stem pieces had significantly greater($P \simeq 0.01$) stem colonization by CC and CGA respectively than nontreated pieces. Soybean is a host for CGA but fungal colonization is not evident until plant material is treated with paraquat. Testing for the host range of fungi considered as potential mycoherbicides should include the paraquat technique for latent colonization.

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VEGETATIVE COMPATIBILITY AMONG RACES OF Fusarium oxysporum f. sp. cubense. R.C. Ploetz, TREC, Univ. Florida, Homestead, Florida 33031.

A collection of race 1, race 2, race 4, and isolates of unknown race of Fusarium oxysporum f. sp. cubense (incitant of Panama disease of bananas) from Australia, Florida (USA), Honduras, South Africa, and Taiwan were characterized for vegetative compatibility. Vegetative compatibility was assessed by observing complementation between nit mutants selected using Puhalla's technique. Four vegetative compatibility groups (vcgs) among race 1 isolates, two among race 2, and three among race 4 isolates were tentatively identified; no vcg contained isolates from more than one race. In general, isolates within a vcg were from the same geographic region.

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EFFECTS OF VERTICILLIUM DAHLIAE ON PHOTOSYNTHESIS AND TRANSPIRATION OF POTATO. R. L. Bowden and D. I. Rouse, Department of Plant Pathology, University of Wisconsin, Madison, 53706.

Axenically propagated plantlets of potato cv. Russet Burbank were grown in a growth chamber ($550 \mu E$ PAR m^{-2} sec^{-1}; 25C day (16h), 15C night (8h)) in a medium containing a mixture of eight isolates of Verticillium dahliae (220 microsclerotia/ml of medium). Net photosynthesis and transpiration of individual leaves of different ages were measured every 4 days with a Li-Cor 6200 system. Reductions in photosynthesis and transpiration of inoculated plants were first detected 38 days after planting (DAP), usually prior to visible leaf symptoms. During early symptom expression, variation was large within and between inoculated plants. Later, photosynthesis and transpiration were more uniformly depressed. Relative to controls, leaves of inoculated plants averaged 41% lower photosynthesis, 61% lower transpiration, 70% lower stomatal conductance, 99% higher leaf water-use efficiency, 5% higher leaf temperature, and 18% lower internal CO_2 concentration by 62 DAP.

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SEPARATE AND COMBINED EFFECTS OF HEAT AND MOISTURE ON SYMPTOM INDUCTION IN EUPHORBIA LATHYRIS INFECTED WITH MACROPHOMINA PHASEOLINA. P. T. Rotkis and S. M. Alcorn, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Two-week-old E. lathyris seedlings were inoculated with M. phaseolina at two root sites. After 2 weeks at 25 C, asymptomatic infected seedlings and healthy controls were placed in growth chambers at 25 C and 34 C and watered to induce water potentials of approximately -20 bars and -40 bars. Thermocouple psychrometers were used to measure water and osmotic potentials. Although no aerial symptoms attributed to M. phaseolina were observed in any treatment, the fungus could be recovered from all inoculation sites. Root colonization extended beyond inoculation sites in all treatments except 25 C and -20 bars. The reduction in available soil moisture at 25 C appears to have a limiting effect on the extent of root

colonization of seemingly symptomless plants by M. phaseolina that is not observed at 34 C.

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INVOLVEMENT OF PHYTOPHTHORA IN VANILLA ROOT ROT. Peter H. Tsao and Leon Mu. Univ. of California, Riverside 92521, and CIRAD, B.P.494, Papeete, French Polynesia.

Phytophthora blight of vanilla affects all above-ground tissues and is a serious disease in all vanilla growing regions of the world. Because previous attempts failed to isolate Phytophthora from roots, vanilla root rot has been attributed to Fusarium spp. In 1985/86, we conducted a survey in French Polynesia involving vanilla samples (bean, leaf, stem, collar, root, and soil) from 59 sites on 5 islands (Tahiti, Moorea, Tahaa, Raiatea, and Huahine). By plating tissues on selective $P_{10}VP$ and PVPH media, Phytophthora was isolated from 36 of 82 samples, including 20 of 40 root or collar samples from all 5 islands. Species recovered from roots were P. palmivora (15 isolates), P. parasitica (4 isolates), and P. capsici (='P. palmivora' MF4) (1 isolate). Pathogenicity of selected isolates on roots of excised vanilla cuttings was demonstrated by fulfilling Koch's Postulates. We conclude that Phytophthora spp. are important causal agents of vanilla root rot.

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IDENTITIES OF PHYTOPHTHORA ISOLATES CAUSING VANILLA BLIGHT AND ROOT ROT IN FRENCH POLYNESIA. Leon Mu and Peter H. Tsao. CIRAD, B.P.494, Papeete, French Polynesia, and Univ. of California, Riverside 92521.

Phytophthora isolates recovered from top and root samples of vanilla in French Polynesia belonged to 3 species. They were: 25 isolates of P. palmivora (A1 & A2 mating types) from islands of Tahiti, Moorea, and Raiatea; 10 P. parasitica (A1 & A2) from Tahiti, Raiatea, Tahaa, and Huahine; and 1 P. capsici (='P. palmivora' MF4) (A1) from Moorea. Identification was based on following main characters: P. palmivora: sporangia papillate and caducous, pedicels 2-3 µm, L/B ratio 1.6-1.9, chlamydospores present, antheridia amphigynous; P. parasitica: sporangia papillate and noncaducous, L/B 1.2-1.3, chlamydospores present, antheridia amphigynous, colonies patchy with uneven margins; P. capsici: sporangia papillate and caducous, pedicels 149 µm, L/B 2.2, antheridia amphigynous. Phytophthora from vanilla has hitherto been called P. jatrophae, a nomen nudum. Its use should be discontinued.

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IMPORTANCE OF BINUCLEATE RHIZOCTONIA-LIKE FUNGI AS PATHOGENS OF CENTROSEMA SPP. IN COLOMBIA. G. Olaya H. and J. M. Lenné, Tropical Pastures Program, CIAT, Cali, Colombia.

Foliar blight caused by Rhizoctonia spp. is the most widespread and serious disease of the tropical pasture legume genus Centrosema. From 1985 to 1987, more than 200 isolates were collected across a wide range of locations and soil types in the savannas and at several locations in the humid tropical forests of Colombia mostly from diseased C. acutifolium, C. brasilianum and C. pubescens. Approximately 50% of isolates were binucleate Rhizoctonia-like fungi (BNR), the rest being R. solani and, less commonly, R. zeae. In seedling inoculation trials, comparing 5 isolates each of the BNR and R. solani on 7 accessions of C. brasilianum, mean severity ranged from 2.27 to 2.70 and from 2.39 to 2.89, respectively, on a 0-5 scale. BNR and R. solani are regarded as equally important causal agents of foliar blight of Centrosema spp. in Colombia.

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GENOME ORGANIZATION OF BARLEY YELLOW DWARF VIRUS. W.A. Miller, P.M. Waterhouse, W.L. Gerlach, and K. Helms. CSIRO Division of Plant Industry, Box 1600, Canberra, A.C.T., 2601, Australia.

Barley yellow dwarf virus (BYDV) is the most widespread virus of cereals worldwide. We have determined the complete nucleotide sequence of the PAV serotype. Open reading frames which include the RNA-dependent RNA polymerase and coat protein genes have been identified. The polymerase shows striking sequence homology with that of carnation mottle virus. The coat protein gene is located near the middle of the viral genome, and it shows structural similarity to the coat protein of southern bean mosaic virus. The possible role of viral gene products in aphid transmission will be discussed. In addition, a satellite RNA has been found associated with the RPV serotype. It is similar to the satellites of the nepo- and sobemoviruses (virusoids). Effects on symptoms and the specificity of the association are under investigation. This is the first identification of a satellite RNA of a luteovirus.

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SINGLE GENE OF CaMV INDUCES DISEASE. K. B. Goldberg, M. J. Young*, J. E. Schoelz, J. M. Kiernan, and R. J. Shepherd. Departments of Plant Pathology, University of Kentucky, Lexington, KY 40546, and University of California*, Davis, CA 95616.

Expression of gene VI of cauliflower mosaic virus (CaMV) in transformed tobacco plants induces disease symptoms similar to those of natural virus infections. Gene VI and its homologous 19S promoter of either strain CM1841 or D4 were cloned into a Ti plasmid vector (pGA472) which was used for transformation of Burley 21 tobacco leaf discs. Regenerated plants showed mottling symptoms somewhat similar to those obtained with natural virus infections. A few transformed plants showed severe generalized chlorosis. Northern blot analysis confirmed the presence of gene VI RNA when probed with a CaMV region VI nick-translated DNA. Western blots with antiserum to P62, the gene product of region VI, were positive indicating that expression of gene VI was associated with symptom expression.

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HOST ADAPTION BY FIGWORT MOSAIC VIRUS. S. Gowda, R. D. Richins, and R. J. Shepherd. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Nucleotide changes in the genome of figwort mosaic virus (FMV) were observed during the adaption of the virus to a new host. When DNA of an infectious clone of FMV isolated from figwort (Scrophularia californica) was inoculated to and maintained in a solanaceous host (Datura innoxia) for a two year period, restriction endonuclease mapping of isolated viral DNA showed that several new restriction sites were clustered in the region VI portion of the genome. DNA sequencing of region VI of the original and adapted strains revealed that extensive changes had occurred in the inferred amino acid sequence of the region VI gene product. One segment of the protein of the adapted strain over 100 amino acids in length showed only a 37% homology at the amino acid level with the original strain. In addition, a 75 base pair deletion had occurred near the 3' end of the gene.

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CHEMICAL EVIDENCE THAT TOBACCO RINGSPOT VIRUS (TRSV) IS A T=1 ICOSAHEDRON. R.N. Skopp, L.C. Lane, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE, 68583-0722.

The major capsid protein of TRSV is 56K. TRSV also contains 13K and 30K polypeptides. It has been proposed that TRSV is a T=4 icosahedron with the 13K polypeptide as the asymmetric unit and that the 56K protein is a tetramer. We have found that the 13K polypeptide is not in association-dissociation equilibrium with the 56K protein, and that the 56K protein contains a single formic acid labile site (presumably asp-pro). Tetramers, pentamers, or hexamers must dissociate to form T=4 icosahedra. These aggregates should contain respectively 4, 5 and 6 identical formic acid labile sites. Our evidence shows that TRSV is not a T=4 icosahedron. TRSV is most likely a T=1 icosahedron, structurally similar to comoviruses and picornaviruses and with the 56K polypeptide as the asymmetric unit. The role of the smaller polypeptides is unclear, but they could arise from ambiguity in polyprotein cleavage.

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Further studies on the identification and mapping of cloned potato leafroll virus cDNAs. 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.

A potato leafroll virus (PLRV) cDNA plasmid (pUC9) library (400 transformants) was screened with cDNA probes selected from an initial set of overlapping PLRV cDNA

clones. These probes represented the left and rightmost ends of previously cloned and mapped PLRV cDNAs (Phytopathology 76:1090). New cDNA sequences were identified and extended in both directions. Three clones, pPLRV4-173, -228, and -323, containing cDNA inserts of 3.4, 2.4, and 1.2 kilobase pairs (kbp), respectively, were physically mapped with restriction endonucleases. Physical mapping data and Southern-blot hybridization analysis indicated that cDNAs 173, 228, and 323 formed a composite map of 5.9 kbp. Assuming a mol. wt. of 2 x 10^6 daltons (ca. 6.2 kb) for PLRV RNA, this map represents about 95% of the PLRV RNA genome. Clones were verified to be PLRV-specific based on positive hybridization to PLRV RNA and total cellular RNA of PLRV-infected Physalis floridana plants in a dot-blot hybridization assay. The 5' to 3' orientation of PLRV cDNA 228 was determined by the use of M13 strand-specific hybridization probes which indicated that the internal Pst I site of this cDNA was 3'-orientated relative to PLRV RNA. The latter, in conjunction with the composite cDNA map, established the 5' to 3' order of the PLRV cDNAs as 5' - cDNA 173 - cDNA 323 - cDNA 228 - 3'.

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CLONED COMPLEMENTARY DNAS FOR BEET WESTERN YELLOW VIRUS (BWYV) AND A BWYV-ASSOCIATED RNA. **Bryce W. Falk and Lana Anderson. Department of Plant Pathology, University of California, Davis, CA 95616.**

Complementary DNA clones (cDNAs) were made to the viral nucleoprotein RNAs of the ST9 severe isolate of beet western yellows virus (BWYV). So far, 12 cDNA clones of sizes 400-800 bp have been analyzed. Individual ^{32}p labelled cDNAs hybridized with either the 1.9 x 10^6 genomic RNA, or with the 9.0 x 10^5 "extra" RNA. None of the cDNAs reacted with both ST9 RNAs. The cDNAs which reacted with the BWYV genomic RNA also reacted with sap extracts from plants infected with 5 other BWYV isolates. The cDNAs which reacted with the BWYV genomic RNA also reacted with extracts from plants infected with 5 other BWYV isolates. The cDNAs which reacted with the "extra" RNA did not react with these extracts, but only with extracts from ST9-infected plants. These latter clones have proven useful for identifying ST9-infected plants which do not react with severe symptoms.

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A GENETIC ANALYSIS OF THE HOST RANGE OF THE CAULIFLOWER MOSAIC VIRUS STRAIN W260. J. E. Schoelz and R. J. Shepherd. University of Kentucky, Lexington KY 40546.

Previous studies with cauliflower mosaic virus (CaMV) strains D4, CM1841, and Cabbage-B have shown that a host range determinant of CaMV is encoded within the first half of gene VI, a gene which codes for the 62 kd inclusion body protein. In order to further study the host specificity of CaMV, a fourth CaMV strain, W260, was chosen that has a host range that is intermediate between D4 and CM1841. W260 systemically infects Nicotiana bigelovii and induces a hypersensitive response in Datura stramonium and Nicotiana edwardsonii. Recombinant viruses made between W260 and both D4 and CM1841 indicated that gene VI of W260 was responsible for induction of the hypersensitive response in D. stramonium and N. edwardsonii. Systemic spread of W260 in N. bigelovii was dependent on at least two loci, one of which corresponded to gene VI and a second which mapped within genes I-V.

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Barley Yellow Dwarf Virus-RPV-IL RNA has a Protein Covalently Linked to its 5' Terminus. J.F. Murphy[1], J.M. Clark Jr.[2], and C.J. D'Arcy[1]. Departments of Plant Pathology[1] and Biochemistry[2], University of Illinois at Urbana-Champaign, IL. 61801, USA.

We have characterized the 5' terminus of the RNA of an Illinois isolate of barley yellow dwarf virus-RPV (BYDV). We first assayed whether the 5' terminus of BYDV RNA was blocked, i.e. contained any derivatization other than phosphate(s). BYDV RNA, satellite tobacco necrosis virus RNA (STNV RNA; positive control) and tobacco mosaic virus RNA (TMV RNA; negative control) were treated with calf intestinal phosphatase, then with T4 polynucleotide kinase with [γ-^{32}P] ATP. The 5' terminus of STNV RNA was labeled with ^{32}P, while BYDV RNA and TMV RNA were not labeled by this procedure. We then assayed whether BYDV RNA contained a protein covalently linked to its 5' terminus (VPg) by treating BYDV RNA, cowpea mosaic virus RNA (CPMV RNA; positive control) and TMV RNA (negative control) with ^{125}I using the ^{125}I-Bolton-Hunter reagent. Acid precipitation assays and gel analyses detected ^{125}I-CPMV RNA VPg (MW = 5,000) and an apparent ^{125}I-BYDV RNA VPg (MW = 17,000) as well as their coat proteins.

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

Six of 370 tomato (Lycopersicon esculentum, Mill.) somaclones regenerated from a fully ToMV-susceptible line (GCRI-26) were found to be resistant to viral infection after inoculation with ToMV, as detected by ELISA and back-inoculation to Nicotiana glutinosa. The resistance is stably inherited as shown by screening four generations of self-pollinated progeny. Screening of reciprocal crosses and their progeny indicated a nuclear gene is involved as well as a resistance factor which is maternally inherited. Somaclones appear normal in morphology, pollen viability and chromosome number. The somaclonal resistance appears to be specific for ToMV and is not temperature sensitive, resembling the type of resistance given by the resistance gene, Tm-1. Further studies on possible resistance mechanisms are in progress.

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COMPARISONS OF PROTEINS AND SEROLOGICAL REACTIONS OF THREE ISOLATES OF MAIZE MOSAIC VIRUS WITH THOSE OF THE IRANIAN (SHIRAZ) MAIZE MOSAIC VIRUS. R. G. Gomez-Luengo and D. T. Gordon, The Ohio State Univ., OARDC, Wooster, OH 44691.

Isolates of maize mosaic virus (MMV) from Costa Rica (MMV-CR), Florida (MMV-FL),and Hawaii (MMV-HI) were compared with the Iranian (Shiraz) maize mosaic virus (IMMV). Analysis of viral proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed identical migration patterns for proteins from MMV-CR, MMV-FL and MMV-HI, whereas corresponding proteins of IMMV migrated faster, except for the G protein which migrated at the same rate. All G proteins were positive for carbohydrate. Remaining proteins were identified as putative L, N, NS, M_1 and M_2 proteins. Western blots stained with antibodies to MMV-HI showed identical protein band staining patterns for MMV-CR, MMV-FL and MMV-HI, whereas IMMV proteins failed to stain. MMV-CR, MMV-FL and MMV-HI appear closely related, whereas IMMV is either a very distantly related strain of MMV or a different maize-infecting rhabdovirus.

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MOLECULAR CLONING OF SOWTHISTLE YELLOW VEIN VIRUS RNA. **D. C. Stenger, T. J. Morris, and A. O. Jackson. Department of Plant Pathology, University of California, Berkeley, CA 94720.**

Genomic RNA of sowthistle yellow vein virus (SYVV), a plant rhabdovirus, was extracted from virions and estimated to be similar in size (13 kilobases) to Sonchus yellow net virus RNA. DNA complimentary to SYVV RNA was synthesized by reverse transcription of viral RNA using DNase-digested calf thymus DNA random primers and cloned into E. coli plasmid pUC9. Recombinant plasmids containing SYVV sequences were selected by colony hybridization and screened for insert size by gel electrophoresis. The use of recombinant plasmids as probes to analyze virus-specific RNAs in infected host extracts will be discussed.

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INFECTION OF ISOLATED MAIZE PROTOPLASTS BY THE MAIZE MOSAIC VIRUS AND REPLICATION OF THE VIRUS. R. G. Gomez-Luengo, E. D. Ammar, and D. T. Gordon. The Ohio State Univ., OARDC, Wooster, OH 44691.

Mesophyll protoplasts were isolated from Aristogold Bantam Evergreen sweet corn by digestion of tissue with cellulase and pectinase followed by low speed centrifugation, under sterile conditions. Protoplasts were inoculated with the Hawaiian isolate of maize mosaic virus (MMV-HI) by polyethylene glycol (PEG) mediated fusion. Numerous inoculated protoplasts fluoresced after labelling with a monoclonal antibody to G protein of MMV-HI and tagging with a fluorescein isothiocyanate-labelled antibody. Rhabdoviruslike particles (RVLP) were observed attached to protoplast membranes, and uneveloped RVLP and putative nucleocapsid strands were observed in the cytoplasm. ELISA of viral antigen concentration at 0 to 48 hr post inoculation demonstrated viral antigen at 0 hr followed by a decrease at 12 hr and then an increase at 24 hr with doubling of antigen titer at 48 hr.

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APPARENT SELECTION OF SEROLOGICALLY DISTINCT TYPES OF BARLEY YELLOW DWARF VIRUS FROM A SINGLE ISOLATE. R.M. Lister and C.H. Lei, Botany and Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Serological tests of subcultures of Rochow's Cornell MAV isolate ("C-type") of barley yellow dwarf virus, passaged in Clintland 64 oats for 6 years at Purdue by sequential mass transfer of Sitobion avenae, revealed a change in reactivity with two monoclonal (mc) antibodies, mc MAV-1 and mc MAV-3. Whereas the earlier C-type subcultures reacted with both mc antibodies, the current Purdue subcultures ("P-type") react only with mc MAV-1. Both C-type and P-type isolates react indistinguishably with a polyclonal rabbit antiserum to the Cornell MAV. The change is presumed due to selection from a mixture of MAV-like viruses, for similar changes were observed among subcultures of a newly - acquired culture of the Cornell MAV isolate, transferred using single S. avenae. Tests of MAV-like isolates from elsewhere revealed that some are also of the P-type.

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cDNA CLONING AND GENOME ORGANIZATION OF RED CLOVER NECROTIC MOSAIC VIRUS. Z. Xiong, and S. A. Lommel, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Full-length cDNA clones of the bipartite genomed red clover necrotic mosaic virus (RCNMV) were constructed. Direct RNA sequencing of RNA-1 and RNA-2 in conjunction with DNA sequencing is currently being used to determine if RNA-1 and RNA-2 clones are complete. RNA-1 programs polypeptides of 90, 50, 39 (capsid protein), and 34 kd and RNA-2 directs the synthesis of a single, 36 kd protein in an *in vitro* translation system. The monocistronic nature of RNA-2 was confirmed by identification of an open reading frame deduced from the sequencing data. In addition, a RNA-2 transcript synthesized from the RNA-2 clone directed the synthesis of only the 36 kd polypeptide. The organization of the RNA-1 genes encoding the observed polypeptides was determined by hybrid-arrested *in vitro* translation with complementary M13 ssDNA-RCNMV subclones and translation of transcripts from cDNA clones representing different portions of RCNMV RNA-1.

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ROLE OF PLANTS AS RESERVOIRS FOR AN APHID-INFECTING VIRUS. F. E. Gildow and C. D'Arcy, Departments of Plant Pathology, The Pennsylvania State University, University Park, PA, and University of Illinois, Urbana, IL.

The isometric Rhopalosiphum padi virus (RhPV) was transmitted from chronically infected aphid clones to virus-free clones of R. padi and Schizaphis graminum when reared together 1 wk on 7-day old barley or oats. Healthy aphids were consistently infected when fed 24 hr on washed detached leaves from plants used to rear infected colonies. When healthy aphids were fed 24 hr on 20% sucrose previously fed on by RhPV-infected aphids, 43 of 64 aphids acquired RhPV. When virus-free aphids were fed 1 wk on seedlings simultaneously with, but physically separated from infected aphids, RhPV was acquired by 36 of 80 healthy aphids, apparently through the plant vascular system. Evidence for RhPV replication in 4 barley cultivars was negative by ELISA. Two ds-RNA's (7.5 and 1.7 x 10^6 mw) associated with RhPV replication in aphids were not detected in plants used to rear infected R. padi. Results suggest that barley and oats function as nonhost reservoirs for RhPV.

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DEVELOPMENT OF AN IMMUNOSORBENT ASSAY FOR SEED BORNE ERWINIA STEWARTII. G. L. Lamka, D. C. McGee, J. H. Hill, and E. J. Braun, Dept. of Plant Pathology, Iowa State University, Ames, IA. 50011.

Polyclonal antibodies were produced by immunizing New Zealand white rabbits with either live Erwinia stewartii or a lithium chloride extract from the outer cell wall of the bacterium. The antibodies recognized 16 different isolates of Erwinia stewartii, including 3 avirulent strains in a double sandwich ELISA. No cross reactivity was found when 13 bacterial strains from 6 genera and 10 unidentified bacterial isolates from maize seed were tested. An epiphytotic was produced in the field by leaf inoculation of A632 corn plants at tasseling. Harvested seed was dried and stored for 5 months. Seed samples then were ground, suspended in buffer, and tested for infection by double sandwich ELISA. Infection was readily detected in seeds from inoculated plants, but not in those from uninoculated plants.

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RECOVERY OF CERCOSPORA KIKUCHII AND OTHER SEEDBORNE FUNGI FROM SOYBEAN SEEDS FROM PLANTS INOCULATED WITH FOUR ISOLATES OF C. KIKUCHII. M. A. Pathan, J. B. Sinclair, and M. Khan, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801-4709.

Recovery of C. kikuchii (Ck), Phomopsis spp. (Ph), Fusarium spp. (Fs) and Alternaria spp. (Al) from soybean seed lots of Bedford, Bragg, Dare, Davis, Forrest, Hood 75, Lee, Mack, Pickett 71 and Tracy was recorded from field plots inoculated with one of four isolates of Ck (IN-C4, IL-ATCC, PR, and ATCC-36864). Three samples of 100 seeds each from each cultivar was randomly selected, surface sterilized, placed on moist cellulose pads (Kimpac), and incubated in 12-hr light for 5 days at 25C. The mean percentage of seeds showing purple stain was greatest over all cvs. inoc. with isolate PR, least with IL-ATCC, intermediate with ATCC-36864 and IN-C4. Recovery of Ck was greatest from seeds of all cvs. inoc. with isolate PR and ATCC-36864, least with IL-ATCC, intermediate with IN-C4. Recovery of Ck over all isolates was greatest for Tracy, least for Bedford; for Fs and Ph, greatest for Bedford and least for Tracy; and for Al no differences were recorded.

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DETECTION OF CLAVIBACTER MICHIGANENSE PV. INSIDIOSUM IN ALFALFA SEED BY ISOLATION AND ELISA. D. C. Erwin and R. A. Khan, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

We confirmed the report (Phytopathology 46: 407-409) that C. m. insidiosum (Cmi) was seed-borne by isolation of Cmi from seed harvested from artificially-inoculated alfalfa plants at Five Points, CA and at El Centro, CA. Cmi was isolated on yeast dextrose $CaCO_3$ agar amended with polymyxin B sulfate (20 µg/ml), potassium tellurite (10 µg/ml), benomyl (12 µg/ml) and chlorothalonil (0.66 µg/ml) (YDCPTBC) after enrichment by germinating seeds in buffered saline or broth media for 2-3 days in shake culture. Seeds with Cmi varied from 0 to 15%. Plants with extensive vascular discoloration yielded seed-borne Cmi more frequently than plants with slight infection. When 20 Cmi cultures, isolated from seeds, were sprayed on freshly cut cotyledonary leaves of cv. CUF101 (susceptible), 20% were highly virulent, 35% moderately virulent, and 45% did not induce symptoms. Identification of Cmi by ELISA required only one day compared with 5-10 days by dilution on YDCPTBC.

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LEVELS OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA IN SOYBEAN SEED LOTS. D. V. Phillips and D. L. Pinnow, Georgia Experiment Station and Georgia Department of Agriculture, Experiment, GA 30212.

The level of southern strains of Diaporthe phaseolorum var. caulivora (SDPC) in soybean seed was determined in randomly selected seed lots intended to be planted or sold as seed in Georgia. Three hundred nonsurface-sterilized seed from each lot were plated on a selective medium and the number of SDPC colonies was counted after 6-7 days. The fungus was isolated from 5 of 50 seed lots harvested in 1984, from 12 of 47 seed lots in 1985, and from 5 of 50 seed lots in 1986. The highest percentage of infested seed within a seed lot was 1%, with a mean of 0.47% over the three years. Thirty-two percent of the infested seed lots and 12% of the others had germination rates below 50% and would probably not have been used for seed. Approximately 12% of the Georgia soybean acreage is being planted with seed containing a low level (0.5%) of SDPC.

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CONSEQUENCES OF SOYBEAN SEED INFESTATION BY DIAPORTHE PHASEOLORUM VAR. CAULIVORA. D. V. Phillips, and P. L. Raymer, Georgia Experiment Station, Experiment, GA 30212.

A soybean seed lot containing 4% seed naturally infested with a southern isolate of Diaporthe phaseolorum var. caulivora (SDPC) was used to determine surface contamination, emergence, and effects of storage on infestation level.

There was no significant difference in recovery of SDPC from surface-sterilized and non-sterilized seed, indicating that infestation was internal. The percentage of infested seed did not change significantly during storage under 3 different conditions. Isolation from seedlings emerging from sand in the greenhouse indicated that only about 12% of the infested seeds emerged. Plants which grew from infested seed had no symptoms before flowering. Because of low levels of infested seed, poor emergence of infested seed, and no effective secondary dissemination of the pathogen, there appears to be very little chance of a stem canker epidemic resulting directly from planting seed infested with SDPC.

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TRANSMISSION OF PHAEOISARIOPSIS GRISEOLA IN BEAN SEED. A.W. Saettler and F.J. Correa, ARS/U.S. Department of Agriculture, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312 .

Severe outbreaks of angular leaf spot (ALS), caused by Phaeoisariopsis griseola, were noted in Michigan seed fields of 'Montcalm' red kidney beans in 1982 and 1983. Seed samples obtained from fields were assayed by incubating them at 100% RH for 4 days on a wire mesh screen; presence of P. griseola spores indicated pathogen-contaminated seed. Nine of 20 samples (45%) in 1982 and 6 of 59 samples (10%) in 1983 carried P. griseola; contamination was mainly localized on the seed coat surface. Greenhouse studies revealed that the ALS pathogen could spread from infected seedlings (arising from contaminated seed) to healthy seedlings in the same pot. Field tests with Charlevoix, Montcalm, and Laker bean cultivars showed that when contaminated seed was planted, significant levels of ALS developed in field plots. The results were consistent and reproducible and indicate that ALS is seed-transmitted in beans.

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FREEZE DAMAGE TO SOYBEAN SEEDS AND RELATIONSHIPS TO GERMINATION AND FUNGAL COLONIZATION. J. A. Osorio and D. C. McGee, Dept. of Plant Pathology, Iowa State University, Ames IA 50011-1020.

Separate sets of greenhouse-grown soybean plants, inoculated at growth stage R/5 with isolates of Fusarium graminearum, Alternaria alternata, Phomopsis longicolla, or not inoculated, were subjected to freezing at -2.5 C or -4.5 C for 4 hours at R/7, or were left unfrozen. Field plots similarly inoculated were subjected to a natural frost. Both greenhouse and field freezing significantly increased colonization of seeds by F. graminearum and A. alternata. Conversely, P. longicolla seed infection was significantly lowered by freezing treatments. P. longicolla presence greatly reduced germination in non-freezing treatments, while F. graminearum and A. alternata caused only slight reductions. Freezing per se reduced seed germination, but it was reduced further by inoculation with all three fungi. Damage by freezing and by fungal invasion was confirmed by staining the seeds with 2,3,5-triphenyl tetrazolium chloride.

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AN AGAR PLATING ASSAY FOR DETECTION OF PSEUDOMONAS SYRINGAE PV. SYRINGAE AND P. SYRINGAE PV. PHASEOLICOLA IN BEAN SEED. S.K. Mohan and N.W. Schaad. Univ. of Idaho, Dept. of Plant, Soil & Ent. Sci., Moscow, ID 83843.

An assay for detection of Pseudomonas syringae pv. syringae (Pss) and P. syringae pv. phaseolicola (Psp) in bean seed is described. One kg sample is soaked in 3 L of sterile saline with 0.01% Tween-20 at 5 C for 20 hr. Aliquots (0.1 ml) of 10-fold diluted, undiluted and 10-fold concentrated (by centrifugation) extracts are plated on KBC (Phytopathology 75:1351) and MSP (sucrose-peptone-agar with cephalexin, vancomycin and bromthymol blue). After 72 hr incubation at 23 C, Pss appears on KBC as flat, circular, translucent colonies whereas Psp is inhibited. On MSP, both Pss and Psp appear as convex, globose, glistening, light yellow colonies. Over 80% of seed-associated saprophytic bacteria are inhibited on these media, compared to King's medium B. As few as 2.1 x 10^4 cfu of Pss and ca. 10^5 cfu of Psp contained in 3 L seed extract are detectable on KBC and MSP, respectively.

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INCIDENCE OF SOYBEAN SEED MICROORGANISMS ASSOCIATED WITH STINK BUG FEEDING DAMAGE. J.S. Russin, M.B. Layton, D.B. Orr, and D.J. Boethel. Dept. of Entomology, Louisiana State University, Baton Rouge, LA 70803

A two-year field study was conducted to determine effects of stink bug feeding damage on incidence of seedborne microorganisms. Microorganisms were identified after incubating surface-disinfested seeds on potato dextrose agar for 7 days. In both 1985 (cv 'Forrest') and 1986 (cv 'Centennial'), stink bugs reached levels of 4-fold economic threshold. These populations correlated positively with incidence of seedborne Fusarium spp., Phomopsis spp., and bacteria; they correlated negatively with Cercospora sp., and did not correlate with Colletotrichum sp. In 1986, populations reached 1.6-fold economic threshold. These lower populations were associated with increased incidence of Fusarium spp., decreased incidence of Cercospora sp., and unchanged incidence of Phomopsis spp., Colletotrichum sp., Alternaria spp., and bacteria.

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EFFECTS OF A CERCOSPORA LEAF DISEASE ON SEED PRODUCTION OF SUBTERRANEAN CLOVER. R. G. Pratt, USDA, ARS, Forage Research Unit, Mississippi State, MS 39762.

Relationships of infection by a host-specialized form of Cercospora zebrina to seed production and seed quality of subterranean clover were evaluated over three years at Mississippi State. Plots of cultivar Woogenellup, surrounded by wide borders of winter wheat, were inoculated in late winter. Burrs were collected from uniform sampling areas of plots after senescense of plants in spring and seed were threshed and evaluated. Infection by Cercospora was associated with significant reductions in numbers of burrs and in the amount, weight, and size of seed in one or more years. No significant differences in seed germination or percentages of hard seed were observed in standard germination tests. Flowering and disease development were compared during one season; peak flowering occurred approximately 2 weeks before rapid disease spread. Results suggest that Cercospora principally affects seed production in this cultivar indirectly by reducing plant vigor and longevity.

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BIOLOGICAL CONTROL OF FUSARIUM WILT OF CUCUMBER WITH NONPATHOGENIC ISOLATES OF FUSARIUM OXYSPORUM AND STRAINS OF PSEUDOMONAS PUTIDA. T. C. Paulitz and Ralph Baker, Colorado State University, Fort Collins, 80523

The addition of chlamydospore inoculum of nonpathogenic isolates of Fusarium oxysporum (F.o.) to a raw soil infested with Fusarium oxysporum f. sp. cucumerinum (F.o.c.) significantly reduced the infection rate of cucumber (Cucumis sativus L. 'Marketer Long'). Some isolates were not effective in disease suppression. These ineffective isolates of F.o., when combined with an ineffective strain of Pseudomonas putida (A12), induced significant disease suppression in F.o.c.-infested soils of pH 6.7 and 8.1, but not 5.5. Population densities of P. putida (strains A12 and N1R) increased significantly in the rhizosphere of cucumber in the presence of an ineffective nonpathogenic isolate of F.o., in a soil of pH 8.1.

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CHARACTERIZATION OF BACTERIA INVOLVED IN SUPPRESSION OF PYTHIUM DAMPING-OFF. Oliver C. H. Kwok, Harry A. J. Hoitink and Weidong Chen, Dept. of Plant Pathology, Ohio State University and Ohio Agricultural Res. and Dev. Center, Wooster, OH 44691

Heterotrophic bacteria were isolated from suppressive bark compost container media and cucumber roots by dilution plating on full strength and 100-fold diluted nutrient agar, and ten-fold diluted trypticase soy agar. Bacteria isolated on nutrient-rich and nutrient-poor media are considered as copiotrophs and oligotrophs, respectively. Populations of oligotrophs in edaphic container medium were 100-fold higher than those of copiotrophs. Oligotrophs isolated were mostly aerobic, gram-negative rods, and were generally smaller than copiotrophs. Strains of many bacterial genera were identified that induced suppression of Pythium damping-off in a conducive container medium. We conclude that a wide diversity of oligotrophic as well as copiotrophic bacteria can induce suppression of Pythium damping-off. Oligotrophs have been overlooked as beneficial microorganisms in soil.

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PROTECTION OF COTTON SEEDLINGS AGAINST RHIZOCTONIA SOLANI BY BACTERIAL SEED TREATMENT. M. L. Courtney and E. B. Nelson, Department of Plant Pathology, University of Arkansas, Fayetteville 72701.

Bacteria were isolated from the hypocotyls of cotton seedlings grown in field soil naturally infested with Rhizoctonia solani and from mycelium of R. solani buried for 24 hrs in a soil suppressive to Rhizoctonia. Cotton seeds were treated with 139 isolates and grown for 2 wks in infested soil in a growth chamber at 21 C. Seven isolates significantly increased stands relative to the carrier control and were not significantly different from the fungicide control (PCNB), although none provided better control than PCNB. These seven isolates were included in field tests. At 57 days, one isolate identified as Arthrobacter globiformis provided seedling stands (29.1%) which were not significantly different from stands resulting from PCNB treated seeds (45.9%).

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BIOLOGICAL CONTROL OF PYTHIUM SEED ROT AND PRE-EMERGENCE DAMPING-OFF OF COTTON WITH ENTEROBACTER CLOACAE AND ERWINIA HERBICOLA APPLIED AS SEED TREATMENTS. E. B. Nelson, Dept. of Plant Pathology, University of Arkansas, Fayetteville 72701.

Thirteen strains of Enterobacter cloacae and Erwinia herbicola were evaluated as biological seed treatments on cotton (Acala SJ-2). All strains reduced levels of Pythium seed rot and pre-emergence damping-off in naturally infested soil. Although 3 of 4 strains were as effective as metalaxyl when tested at 25 C, all strains of both bacteria were less effective at 15 C. At 35 C little disease development was observed among all treatments. The control of Pythium seed rot and pre-emergence damping-off by E. cloacae and E. herbicola strains was correlated with the suppression of seed colonization by Pythium spp. during the first 24 hr of seed germination. Bacterial strains suppressed seed colonization by Pythium spp. at 15, 25, and 35 C.

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THE EFFECT OF CARBON AND NITROGEN SOURCES, pH, AND TEMPERATURE ON THE EXPRESSION OF GENE(S) INVOLVED IN ANTIFUNGAL COMPOUND BIOSYNTHESIS BY A STRAIN OF PSEUDOMONAS FLUORESCENS. W. Howie and T. Suslow. Advanced Genetic Sciences, 6701 San Pablo Ave., Oakland, CA 94608

The influence of carbon/nitrogen sources, pH, and temperature on antifungal gene expression was studied by using a B-galactosidase gene fusion in the afuE region of strain Hv37aR2 (Hv37aR2::Tn3HoHo1-142 denoted as strain WH108). Cotton seeds were soaked in suspensions with strain WH108 with or without various carbon/nitrogen sources and planted into moistened sand (7%) adjusted from pH 3 to 8 or not adjusted but incubated at temperatures from 16 to 32 C. After 24 hr bacterial cells were washed from the seed and B-galactosidase activity and seed colonization was determined. Several simple sugars, amino acids, and dicarboxylic acids increased gene expression from 1 to 9 fold. Highest gene expression was recorded from pH 6 to 8 and at 20 C. Substrates which enhanced gene expression also increased the in vivo suppression of Pythium ultimum by parental strain Hv37aR2 from 5 to 20 % in a natural soil at day four.

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MICROBIAL ACTIVITY AS AN INDICATOR OF SUPPRESSION OF PYTHIUM DAMPING-OFF. W. Chen, H. A. J. Hoitink, and O. H. Touvinen, Depts. of Plant Pathology and Microbiology, The Ohio State Univ., OARDC, Wooster 44691.

Container media amended with composts produced at high temperature are conducive to Pythium damping-off, but they become suppressive in 3 to 4 days as the biological vacuum is colonized by mesophilic microorganisms. Changes in total microbial activity, total biomass and microbial populations were followed during the time span that the container medium changed from conducive to suppressive. Microbial activity and biomass were based on rates of hydrolysis of fluorescein diacetate and extractible phospholipid phosphate, respectively. Disease suppressiveness was highly correlated with microbial activity (R^2a=0.840), but not with total biomass (R^2a=0.245). After the container medium had become suppressive, microbial populations stabilized or declined, indicating that microbiostasis prevailed in the suppressive system.

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EFFECTS OF ORGANIC MATTER DECOMPOSITION LEVEL AND CELLULOSE AMENDMENTS ON THE INOCULUM POTENTIAL OF R. SOLANI. Y. R. Chung, H. A. J. Hoitink, and W. A. Dick, Depts. of Plant Pathology and Agronomy, The Ohio State Univ., OARDC, Wooster 44691.

Container media amended with fresh hardwood tree bark (FB, ~45% cellulose, w/w) were conducive to Rhizoctonia damping-off of radish. Those amended with hardwood tree bark compost (BC) were suppressive. Significantly higher cellulase activity was present in the FB medium colonized by R. solani (R) than in the BC medium. The population of R increased in the FB but not in the BC medium. Addition of low levels of cellulose (5%, w/w) to the BC medium decreased damping-off. High levels of cellulose (20%, w/w) established high cellulase levels in the R-infested BC media, increased the population of R and negated suppression. Even in the presence of a suppressive microflora, the high cellulose amendment increased conduciveness. We conclude that high cellulose levels increase the inoculum potential of R resulting in increased damping-off severity.

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INFLUENCE OF LIGHT/DARK CYCLES ON DEVELOPMENT OF CROWN GALLS. B. W. Kennedy and R. L. Denny, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Seedling sunflower (Helianthus annuus L. 'Challenger') and tomato (Lycopersicon esculentum L. 'Homestead') were grown in controlled environments with light-dark cycles of 12-12 hrs at 25 C. At full light, intensity was 400 μE.m-2.s-1; toward end of each cycle there was a gradual light-dark and dark-light transition over 45 min. Multiple wounds were made immediately below first leaf attachments when plants were 5-10 cm tall. Plants were then subjected to a series of altered light-dark regimes, or free run in continuous light or dark, for 48 hrs before inoculation with Agrobacterium tumefaciens. Plants subjected to 48 hr of continuous darkness after injury and before inoculation consistently produced galls 2-4 times larger than those produced on plants continuing on 12-12 light-dark schedules. Notable differences also occurred in crown gall development due to timing during the diurnal light-dark cycle when plants were injured and (or) inoculated.

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PHASE SHIFTING OF LEAF MOVEMENTS IN SUNFLOWER AFFECTED BY BACTERIAL TOXEMIA. B. W. Kennedy and R. L. Denny, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Seedling sunflower (Helianthus annuus L. 'Challenger') were grown in controlled environments with light-dark cycles of 12-12 hrs at 25 C. At full light, intensity was 400 μE.m-2.s-1; toward the end of each cycle there was a gradual light-dark and dark-light transition over 45 min. Six days after planting, plants were inoculated via stem puncture with an insect pin dipped in 24-36-hr-old, agar-grown culture of Pseudomonas syringae pv. tagetis and leaf angles were measured for 28-36 hr at 4 hr intervals beginning on day 9. Amplitude of leaf movement was significantly greater in diseased plants compared to controls and acrophase (greatest angle of leaf elevation from the horizontal) was significantly shifted to a different portion of the circadian diurnal cycle. We conclude that the bacterium can alter at least one entrained rhythmic function in its host.

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PSEUDOMONAD EPIPHYTE RESPONSE TO COPPER-BASED BACTERICIDES ON DRY BEANS. K. A. Garrett and H. F. Schwartz. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, Colorado 80523.

Field samples of dry beans were evaluated in 1986 for the presence of copper resistance in epiphytic populations of Pseudomonas syringae pv. syringae (PSS) and P. syringae pv. phaseolicola (PSP) after treatment with four sprays of cupric hydroxide. Bacterial populations from five sites were enumerated on King's B medium, and 450 isolates were screened for copper resistance on nutrient glucose agar amended with 10 levels of cupric hydroxide (Kocide 606). PSS isolates exhibited greater copper resistance ($P < 0.001$) than PSP isolates. Significant differences in resistance occurred among both pathovars relative to number of sprays, although increased resistance did not always correspond to increased number of sprays.

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A NOVEL SOURCE OF TRANSFERABLE RESISTANCE TO TUBER SOFT ROT CAUSED BY ERWINIA SPP.: SOMATIC HYBRIDS BETWEEN SOLANUM TUBEROSUM AND SOLANUM BREVIDENS. S. Austin, J.P. Helgeson, E. Lojkowska, and A. Kelman. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Hexaploid somatic hybrids were produced by protoplast fusion between S.brevidens, a diploid non-tuber bearing species, and a tetraploid S.tuberosum (line PI 203900, a late blight differential line). Tubers from hybrids were screened for resistance to soft rot erwinias using an infectivity titration assay. Tubers of PI 203900 as well as cvs. Katahdin and Russet Burbank were susceptible to soft rot; all somatic hybrid clones produced highly resistant tubers. Furthermore, pentaploid sexual hybrids derived from crosses of hexaploid somatic hybrids with cv. Katahdin also showed high levels of resistance. High resistance was also present in tubers from the subsequent backcross progeny of a pentaploid with cv. Katahdin. Results indicate sexual transmission of valuable germplasm derived from wild Solanum species via protoplast fusion procedure.

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TEMPERATURE AND MOISTURE EFFECTS ON BACTERIAL SPOT OF PEACH CAUSED BY XANTHOMONAS CAMPESTRIS PV. PRUNI. D. Petra Shepard and E. I. Zehr, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Severe epiphytotics of bacterial spot occur under warm, humid conditions. Leaf infection often results in defoliation. Leaf wetness duration as related to severity of bacterial spot was examined at 24 and 30 C in dew chambers. All plants were exposed to 48 hr continuous leaf wetness at 27 C prior to inoculation to induce water-congestion. Plants were spray-inoculated (10^7 cfu/ml), incubated for 0, 6, 18, 24, and 48 hr under moist conditions and held at 24 or 30 C in dry growth chambers. At 24 C a 24- or 48-hr wetting period was required for significant infection. Symptoms appeared 10-14 days later. Shorter wetness periods resulted in 1% or less leaf area infected. At 30 C light infection occurred after 0 and 6 hr. Incidence and severity were greater at 30 C than 24 C for all wetness periods. After wetting for 48 hr at 30 C, severe symptoms appeared after 3 days.

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SURVIVAL AND DISPERSAL OF AN ANTIBIOTIC RESISTANT STRAIN OF PSEUDOMONAS SYRINGAE IN A MAPLE NURSERY. D. K. Malvick and L. W. Moore, Dept. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331.

Maple twigs (Acer rubrum) and perennial rye grass (Lolium perenne) were sprayed with a pathogenic Pseudomonas syringae strain resistant to rifampicin and nalidixic acid. Inoculated twigs were sampled biweekly from July 1985 to September 1986; epiphytic populations of the marked strain ranged from nondetectable to 10^4 cfu/g. Results show that P. syringae can overwinter on maple twigs and may serve as a source of inoculum in the spring. Aerial dispersal was also investigated. The marked strain was detected on medium in inverted petri plates and on maple leaves positioned 12 to 100 cm above inoculated grass. The number of cells that dispersed vertically and were detected was low even in the presence of wind, irrigation water and rain. Data suggest that small quantitities of P. syringae can disperse from grass to trees. The identity of the marked strain was confirmed with DNA profile analysis.

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A MODIFIED BEAN POD BIOASSAY TO DETECT PSEUDOMONAS SYRINGAE PV. SYRINGAE (Pss) STRAINS CAPABLE OF CAUSING BACTERIAL BROWN SPOT (BBS) OF SNAPBEANS. G. Y. Cheng, D. E. Legard, and J. E. Hunter, Dept. of Plant Pathology, NYS Agr. Expt. Station, Cornell University, Geneva, NY 14456.

Detached snapbean pods of 'Bush Blue Lake 274' were inoculated by dipping an insect pin in a Pss colony and inserting the pin laterally under the epidermis. Pod age, size and angle of inoculation were critical. Inoculated pods were kept in a moist chamber (at 22-25 C). Stock cultures of 11 Pss strains isolated from BBS lesions in WI, ND, CO and 689 of 696 strains from fresh BBS lesions in NY and WI induced Pss green water-soaked sunken lesions (GWS) without browning in 5 days. None of 44 strains of Pss from 15 other hosts, nor 69 strains of 17 other PS pathovars induced BBS type lesions. Avirulent strains produced brown or water-soaked lesions which turned brown in 1-3 days. Of 337 Pss strains recovered from weeds, 23 found on 3 weeds near BBS-infected beans induced GWS lesions identical to BBS type Pss.

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MOLECULAR DIVERSITY OF A PATHOGENICITY-SPECIFIC GENE WITHIN POPULATIONS AND PATHOVARS OF *Pseudomonas syringae*. D. K. Willis and S. S. Hirano, USDA/ARS and Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706.

The *pgsA* locus of *Pseudomonas syringae* pv.*syringae* isolate B728a (brown spot disease of bean, *Phaseolus vulgaris*) affects pathogenicity specific functions, i.e. mutation at this locus eliminates the pathogenic response but does not affect epiphytic growth or HR induction on heterologous host plants. The 6.8 kb *Eco*RI fragment which contains this gene is conserved with respect to size and homology among several taxonomically diverse *P. syringae* pathovars including *P.s. phaseolicola*, *P.s. glycinea*, and *P. syringae* isolate BR2 (bean wildfire blight). However, the *pgsA* fragment shows significant polymorphism among pathogenic *P.s. syringae* strains isolated from field grown bean plants in Wisconsin. It is our goal to determine if this physical diversity is correlated with *pgsA* gene function.

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PATHOGENIC VARIATION IN SOYBEANS OF XANTHOMONAS CAMPESTRIS PV. GLYCINES. I. Hwang, S. M. Lim, Department of Plant Pathology, University of Illinois, IL 61801.

The pathogenicity of twenty isolates of X. campestris pv. glycines collected from the United States, Brazil, and Africa was determined, based on reactions of 25 soybean cultivars differing in their genotypes. Reaction type, symptomless, necrotic spots or chlorotic spots, varied depending on the soybean genotype and the bacterial isolate. Cultivars carrying the resistant gene rpx remained symptomless after inoculation with each of the 20 isolates. Cultivar "Peking" was susceptible (chlorotic spots) to all isolates. Reactions of the other cultivars varied with the isolate. A set of differential cultivars was developed for the identification of the pathogenic specificity in X. campestris pv. glycines.

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DIFFERENTIATION OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI INTO PATHOGENIC RACES BASED ON THE TEPARY BEAN REACTIONS. Mildred Zapata and Anne K. Vidaver. Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE 68583.

Little is known about host specialization in X. campestris pv. phaseoli (Xcp). A narrow host range with variations in virulence on cultivars of Phaseolus vulgaris is known, as is high levels of resistance in P. acutifolius, a natural host of Xcp. However, the use of tepary as a differential for the pathogen is unknown. Five lines of P. acutifolius (cultivated teparies) obtained from Puerto Rico (P.R.) were inoculated on pods and cotyledons to differentiate strains of Xcp. Four pathogenic races, three from P.R. and one from the Dominican Republic were determined by qualitative (hypersensitivity and compatibility reaction) and quantitative analysis. Correlations of the reaction on the pods and cotyledons were observed at approx. 25°C. This is the first report of host specialization in Xcp into pathogenic races by using phenotypic reactions in P. acutifolius; which suggests specific resistance within the species. The identification of Xcp races on P. acutifolius differentials is important for the identification of resistance genes in the host and virulence genes in the pathogen and for the study of pathogen distribution in specific localities.

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Association of vascular occlusion and water stress with Pierce's disease of the grapevine. P. H. Goodwin, J. E. DeVay and C. P. Meredith*, Dept. of Plant Pathology, *Dept. of Viticulture and Enology, University of Calif., Davis, CA 95616.

Leaves with marginal chlorosis and necrosis (MCN) from Vitis vinifera cv. 'Chardonnay' infected with Pierce's disease bacteria were compared to leaves from healthy grapevines. Vascular occlusion in the node and petiole of leaves with MCN was indicated by a xylem flow resistance of 1.49 MPa $cm^{-1}ml^{-1}hr$ in contrast to 6.43 x 10^{-3} MPa $cm^{-1}ml^{-1}hr$ for comparable healthy tissue. Stomatal closure was also associated with MCN. Stomatal resistance of leaves with MCN was not significantly different from that of healthy leaves during the early morning; however, the stomatal resistance of diseased leaves increased rapidly throughout the day and was 8 times greater than that of healthy leaves by early evening. Diseased leaves contained 6 times more proline and 73% more cis-abscisic acid than healthy leaves. These results indicate that vascular occlusion in the node and petiole reduces the uptake of water into diseased leaves resulting in water stress and possibly MCN.

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PLANT DENSITY EFFECTS ON THE INCIDENCE OF AERIAL STEM SOFT ROT OF POTATOES. M.R. Cappaert and M.L. Powelson. Department of Botany and Plant Path. Oregon State Univ., Corvallis, 97331.

Field plots were established at two different sites at each of two locations in Oregon to study the effect of plant density on incidence of aerial stem soft rot of potatoes. Plant populations ranged from 52 to 13 x 10^3 plants per hectare. Leaf area index and disease incidence were measured bimonthly beginning at row closure. For all plant densities, largest leaf area index values preceded the highest incidence of disease by two weeks. AUDPC and leaf area index values were significantly greater (p 0.05) in the high compared to the low plant density plots at both locations. Mean leaf area index values at one location were 8.75 and 2.96 in plots with the highest and lowest plant densities, and mean AUDPC values were 13.23 and 4.73, respectively. At the second location, mean leaf area index values were 4.19 and 2.12 for high and low plant densities with corresponding AUDPC values of 6.45 and 0.79, respectively.

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A SCREENING TECHNIQUE FOR ANGULAR LEAF SPOT (PSEUDOMONAS SYRINGAE PV. TABACI) RESISTANCE OF TOBACCO. M.J. Wannamaker and R.C. Rufty, Dept. of Crop Science, North Carolina State University, Raleigh, N.C. 27695

A greenhouse technique was developed to screen tobacco genotypes for angular leaf spot resistance. The effects of inoculation method, bacterial concentration, and mist were evaluated. An artist's air brush proved to be the most effective method of administering inoculum. A bacterial suspension containing 3.0-9.0 x 10^7 cfu/ml produced symptoms typical of those observed in the field. A mist period of 0-18 hr prior to inoculation had no influence on disease development on the 4 cultivars tested. Post-inoculation mist periods of 24, 36, and 48 hr greatly increased the amount of disease. A quantitative disease assessment scale permitted classification of genotypes for disease reaction. The similarity between symptoms produced in the greenhouse and field symptoms indicates that screening for angular leaf spot resistance should be feasible under greenhouse conditions.

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ISOLATION OF PINK STRAINS OF CURTOBACTERIUM FLACCUMFACIENS PV. FLACCUMFACIENS PATHOGENIC TO SOYBEAN. J. M. Dunleavy, U.S. Dept. Agriculture, ARS, Dept. Plant Pathology, Iowa State University, Ames IA 50011.

Curtobacterium flaccumfaciens pv. flaccumfaciens is a yellow-pigmented bacterium that causes bacterial tan spot of soybean. Four isolates of a pink-pigmented bacterium were obtained from infected soybean leaves collected in Iowa and plated on tryptic ase soy agar (TSA). All isolates produced tan spot symptoms when inoculated to Clark soybeans, and the same strains were reisolated from infected leaves. When the yellow strain was compared with the pink strains, all strains were gram-positive, motile rods; obligately aerobic, not acid fast, catalase-positive, and were chemoorganotrophs. When the yellow and pink isolates were transferred from TSA to nutrient dextrose agar, all colonies were yellow. All bacterial isolates gave a positive test for carotenoid pigments, and pink isolates were identified as C. flaccumfaciens pv. flaccumfaciens.

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ORIGIN AND RELATEDNESS OF RACES WITHIN FUSARIUM OXYSPORUM F. SP. LYCOPERSICI AND NONPATHOGENIC STRAINS OF F. OXYSPORUM. 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. Included in this group are the vascular wilt pathogens which attack over 70 plant species of agronomic and horticultural importance. Fusarium oxysporum f. sp. lycopersici (FOL) causes Fusarium Wilt of tomato. Three races of FOL exist (races 1, 2, and 3). Races 1 and 2 have a worldwided distribution whereas race 3 has only been confirmed in Australia and Florida. Inasmuch as there is no resistance to race 3, this race has the potential to cause severe crop losses. Thus, knowledge of the evolution of races within FOL would be invaluable. Data from pathogenicity tests, vegetative compatibility studies, and isoenzyme analyses have been utilized in this study. Some conclusions concerning the origin and relatedness of races within FOL and nonpathogenic strains will be presented.

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ISOZYME ANALYSIS REVEALS GEOGRAPHICAL DIFFERENTIATION OF *Rhynchosporium secalis* POPULATIONS. S. B. Goodwin, R. W. Allard, and R. K. Webster[1], Department of Genetics and Department of Plant Pathology[1], University of California, Davis, CA 95616.

The frequencies of different electromorphs at each of eight putative enzyme loci were determined for populations of *Rhynchosporium secalis* from three regions: California (N = 278 isolates), Idaho (N = 111), and Oregon (N = 156). Polymorphisms were found for five loci (phosphoglucose isomerase, phosphoglucomutase, leucine aminopeptidase, catalase, and β-glucosidase); the remaining three loci (aconitase, glucose-6-phosphate dehydrogenase, and hexokinase) were monomorphic. Strong geographical differentiation was found between populations; each region was characterized by a unique electromorph in high frequency (> 0.5). The multilocus phenotype of each isolate was determined by the specific combination of electromorphs at all loci. Of the 72 phenotypes possible, only fifteen to eighteen were found within each region. Furthermore, only three or four phenotypes were predominant in a region, accounting for more than 60% of all isolates. All other phenotypes occurred in low frequency (< 0.08). The pathogenicities of isolates from Idaho and Oregon are currently being determined to further elucidate the population biology of this organism.

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Recovery of spontaneous selenate resistant mutants from *Fusarium oxysporum* and *Fusarium moniliforme*. J.C. Correll and J. F. Leslie, Dept. Plant Path., Kansas State Univ., Manhattan.

Selenate is a toxic analog of sulfate that greatly restricts the growth of these fungi. Spontaneous selenate-resistant mutants were recovered from several strains of *F. oxysporum* and *F. moniliforme* cultured on a minimal medium (MM) without sulfate amended with taurine (0.1 g/l) and Na selenate (1.0 g/l). Fast-growing selenate-resistant sectors were transferred to MM with either Mg sulfate (0.5 g/l) or taurine (1.0 g/l). The selenate-resistant sectors had wild-type morphology on MM + taurine. Some mutants (*sel* mutants) were unable to use sulfate as a sulfur source and grew as thin expansive colonies on MM + sulfate. Two different *sel* mutants, recovered from a strain of *F. oxysporum*, were able to complement one another by forming a heterokaryon when co-cultured on MM + sulfate. All of the *sel* mutants were able to complement *nit* mutants derived from a vegetatively-compatible strain. *Sel* mutants may be useful in tests to identify vegetative compatibility groups in natural populations of these fungi.

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Observations on the heritability of heterokaryon "self-incompatibility" in *Gibberella fujikuroi* (*Fusarium moniliforme*). J. C. Correll, J. F. Leslie & C.J.R. Klittich, Dept. of Plant Path., Kansas State Univ., Manhattan, Kansas 66506.

Nitrate nonutilizing (*nit*) mutants were induced in over 50 wild-collected isolates of *F. moniliforme* recovered from corn and sorghum. In most of these isolates, phenotypically distinct *nit* mutants derived from the same parent were able to complement one another by forming a heterokaryon; these

isolates were designated "self-compatible" (SC). The *nit* mutants recovered from two isolates, however, were unable to form a prototrophic heterokaryon with a phenotypically distinct *nit* mutant derived from the same parent; these isolates were designated "self-incompatible" (SI). When an SI strain was crossed with an SC strain, SC and SI progeny were recovered in a 1:1 ratio, indicating a single nuclear gene controlling this phenotype was segregating. Light microscopic examination of SC isolates revealed frequent anastomosis between hyphal side branches; neither hyphal interaction nor anastomosis was observed in the SI isolates.

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PHENOTYPIC BEHAVIOR OF PROGENY FROM THE CROSS BETWEEN CHLORAMPHENICOL-RESISTANT A^1 AND STREPTOMYCIN-RESISTANT A^2 MATING TYPES OF PHYTOPHTHORA PARASITICA. P. J. Ann and W. H. Ko, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI 96720

When chloramphenicol-resistant A^1 mating type of P. parasitica, was mated directly with streptomycin-resistant A^2 mating type, all 432 single-oospore cultures from three crosses were resistant to either chloramphenicol or streptomycin but not to both, indicating the absence of genetic exchange in the progeny. The ratios of A^1:A^2:A^1A^2 were 67:59:2 among the chloramphenicol-resistant cultures and 178:119:7 among the streptomycin-resistant cultures. The appearance of A^2 type resistant to chloramphenicol and A^1 type resistant to streptomycin is apparently due to the mating type change during oospore formation because oospores produced by A^1 type through hormonal stimulation gave rise to A^2 type cultures in addition to A^1 type cultures and vice versa. Chemically induced oospores produced by A^1 type also gave rise to A^1A^2 type cultures.

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VEGETATIVE-COMPATIBILITY GROUPS OF FUSARIUM OXYSPORUM F.SP. VASINFECTUM. Talma Katan and J. Katan, Dept. Pl. Pathol. Volcani Center, Bet Dagan 50250, and Dept. Pl. Pathol. and Microbiol., Faculty of Agriculture, Rehovot 76100, Israel.

Fusarium populations in root tissue and the rhizosphere of diseased cotton (Gossypium barbadense cv. Pima S-5), growing in soils naturally infested with F. oxysporum f. sp. vasinfectum race 3, were analyzed by pathogenicity and the vegetative-compatibility grouping (VCG) methods. About 700 Fusarium isolates, obtained from four sites at two separate geographic regions in Israel, were tested for pathogenicity and their VCG was determined using pathogenic nit testers. All nit mutants of pathogenic isolates gave heterokaryons with the testers, indicating one VCG. None of the nonpathogenic isolates was compatible with the testers. Percentage of pathogenic isolates in tissues was 97 and in the rhizospheres ranged from 3-91. In contrast, pathogenic isolates in tissue and rhizosphere of plants of the resistant, Pima-type cv. F-**2**7 comprised 1-3%.

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CUTINOLYTIC ENZYMES FROM COLLETOTRICHUM LAGENARIUM. A. M. Bonnen, and R. Hammerschmidt. Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The role of cutinolytic enzymes (cutinases) in penetration was studied in the Colletotrichum lagenarium cucurbit system. C. lagenarium, a direct penetrating fungus, was grown in liquid culture with cutin as the sole carbon source. Cutinolytically active enzymes were isolated from the culture filtrate utilizing p-nitrophenylbutyrate assay and a more specific tritiated-cutin assay. Initial characterization shows one cutinase to be acidic with an approximate molecular weight of 60,000. Another cutinase exhibits properties of a basic protein and is currently being characterized. EMS-generated mutants of C. lagenarium and enzyme inhibitors are being used to determine the role each of these enzymes might have in penetration.

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IN VITRO PROPAGATION OF SPINELESS RED SPANISH PINEAPPLE. L.J. Liu, Evelyn Rosa-Márquez and Enid Lizardi, Agricultural Experiment Station, Univ. of Puerto Rico, Río Piedras, P.R. 00928.

The sharp spines on the leaves of the Red Spanish pineapple are a serious problem at harvest and a potential limiting factor for commercial production. More than 1,000 lateral buds (0.4-0.8 mm) and meristem tips from crowns of the spineless pineapple were dissected and surface sterilized in Clorox 10% and Tween 80 (2 drops/100 ml) for 15 minutes. The explants were cultured in various media for shoot differentiation. Murashige and Skoog (MS) medium without hormones, MS and AZ media with low concentrations of hormones (MS + 0.1 mg/l 2,4-D and AZ + 0.1 mg/l NAA) were the best combinations for shoot differenciation. In addition, 18 liquid culture media consisting of modified MS with low concentrations of NAA, IBA, BA, Kinetin, IAA, 2,4-D and coconut water were used to culture the plantlets derived from lateral shoots of pineapple on a high speed shaker. Shoot differentiation and proliferation were achieved in 4-8 weeks. The culture medium (MS + 0.1 mg/l 2,4-D + 0.5 mg/l BA) produced the best results.

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CALLUS INDUCTION AND REGENERATION FROM RED SPANISH PINEAPPLE. Evelyn Rosa-Márquez, Lii-Jang Liu and Enid Lizardi, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, Puerto Rico 00928.

Benzyl adenine (BA), Naphthalene acetic acid (NAA), 2,4-Dichlorophenoxy acetic acid (2,4-D) and 6-Furfuryl amino purine (Kinetin) at various concentrations and combinations were added to the basal medium of Murashige and Skoog (MS) for callus induction and regeneration. Callus formation was observed when BA hormone was added to the medium and incubated in the dark. The BA hormone played an important role in callus formation in the dark. The culture medium (MS + 4 mg/l NAA) yielded the best results for plant regeneration. Calli of the Red Spanish pineapple were regenerated into green plantlets 2-3 weeks after incubating them in diffuse light (2-3 footcandle), at 26 C.

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NUCLEOTIDE SEQUENCE OF COPPER RESISTANCE GENES FROM PSEUDOMONAS SYRINGAE PV. TOMATO. M. A. Mellano and D. A. Cooksey. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

A 4.5 kb DNA fragment that conferred copper resistance in Pseudomonas syringae was previously cloned from an indigenous plasmid of P. syringae pv. tomato. Deletion analysis and Tn5 mutagenesis indicated that at least 4.1 kb of the cloned sequence was involved in copper resistance. Nucleotide sequence analysis indicated the presence of five open reading frames (ORFs); four ORFs ranged from 850-1000 bp, and the fifth was

approximately 375 bp. All of the ORFs were closely grouped and in the same orientation. Tn5 insertions which inactivated copper resistance mapped within the five ORFs.

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SCREENING FOR SWEET POTATO STORAGE ROOT REACTION TO ERWINIA CHRYSANTHEMI. Clark, C. A., and Pace, C. S., Dept. Plant Pathology & Crop Physiology, LAES, Louisiana State University Agricultural Center, Baton Rouge, 70803-1720.

Reaction of different sweet potato clones and cultivars was determined by stabbing plastic pipette tips containing 50 µl of varying concentrations of inoculum of Erwinia chrysanthemi into whole storage roots. Lesion dimesions were determined after 4-6 days at room temperature. Significant differences were found among cultivars. The cultivars 'Porto Rico' and 'Centennial' were rated as resistant, the cultivars 'Jewel' and 'Travis' as intermediate and the clones L81-10 and L82-508 as susceptible. Susceptible selections developed much larger lesions with active decay at the margins while resistant selections had smaller lesions with a dry, dark margin. Time course analysis revealed that lesion enlargement ceased at 2-3, 4-6 and >10 days after inoculation for 'Centennial', 'Jewel' and L81-10, respectively. Storage root reaction differed from reaction of vines to inoculation with E. chrysanthemi for several selections.

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BACTERIAL SOFT ROT OF BROCCOLI IN TENNESSEE. C. H. Canaday, C. A. Mullins, J. E. Wyatt, D. L. Coffey, J. A. Mullins, and T. Hall, Depts. of Entomology & Plant Pathology, Plant & Soil Science, and Agricultural Engineering, Univ. of Tennessee, Knoxville, TN 37901 and Dept. of Plant & Soil Science, Tennessee Technological Univ., Cookeville, TN 38505.

A bacterial soft rot has hampered commercial production of broccoli in Tennessee. The disease occurs on broccoli heads following extended periods of rainy or damp weather. Both Pseudomonas marginalis and Erwinia carotovora have been isolated from infected heads. Conventional sprays of maneb, copper, maneb + copper, mancozeb, benomyl, chlorothalonil, anilazine, or sulfur failed to adequately control this disease. Sprays of dilute sulfuric acid, sodium hypochlorite, calcium propionate, bacterial antagonists, or a water surfactant were also ineffective in reducing disease incidence or severity. Moderate levels of disease tolerance have tentatively been identified in broccoli cultivars 'Shogun', 'Green Defender', and 'Corvet'.

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CONTROL OF CUCUMBER POWDERY MILDEW (SPHAEROTHECA FULIGINEA) BY STEPHANOASCUS SPP. W. R. Jarvis, L. A. Shaw, and J. A. Traquair, Agriculture Canada, Harrow, Ontario, Canada NOR 1GO

Two newly-described species of Stephanoascus with Sporothrix anamorphs were isolated from Erysiphe cichoracearum on clover and from the epiphytic microflora of corn. When applied as a suspension of conidia to colonies of Sphaerotheca fuliginea on leaves of greenhouse cucumber (Cucumis sativa), both Stephanoascus spp. caused rapid collapse of conidiophores and conidia of the powdery mildew. Antagonism is most active at high relative humidities but since death of the powdery mildew occurs in a few hours, the risk of inviting water-dependant cucumber pathogen is not great. These fungi are considered to have good potential as biological controls.

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FIELD EVALUATION OF CELERY GERMPLASM FOR RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2. **K. F. Ireland, W. H. Elmer, and M. L. Lacy. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824.**

Eight-week old celery transplants were planted in fields naturally infested with *F. oxysporum* f. sp. *apii* race 2 in Muskegon MI (1985) and Decatur, MI (1986), and rated for disease severity 12 weeks later. Disease ratings were based on the degree of vascular discoloration. Tall Utah 52-70 HK was rated as moderately resistant in 1985 and moderately susceptible in 1986. Deacon was moderately resistant both years. Pilgrim and Companion (MSU cultivars released in 1986) were highly resistant and moderately resistant, respectively, both years. Breeding line MSU 74-70 was highly resistant both years with uniform field appearance. Tall Utah 52-70 HK, Deacon, and Companion had comparable trimmed fresh weights each year. Pilgrim had a significantly higher yield than all other cultivars in 1986 but not 1985. MSU 74-70 produced a slightly, but not significantly higher yield than Tall Utah 52-70 HK in 1986.

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CHEMICAL SEED PIECE TREATMENTS FOR THE CONTROL OF RHIZOCTONIA BLACK SCURF AND OTHER STORAGE DISEASES OF POTATO. T. A. Zitter and D. E. Halseth, Departments of Plant Pathology and Vegetable Crops, Cornell Univ., Ithaca, NY 14853.

Rizolex (tolcolfos-methyl) 5D and 50W formulations and SN 84364 50W applied as dusts (2 seasons) or as in-furrow sprays (1 season) performed significantly better than standard treatments of captan 7.5 D, maneb 8D or Tops 2.5 D or an untreated control. Higher final stands, plant heights, total and marketable yields and lower Rhizoctonia black scurf ratings were noted both seasons when relying upon natural soilborne R. solani inoculum. Tuber discoloration was apparent on progeny tubers after the 1986 test, and following 3 mo storage Helminthosporium solani (silver scurf) and Colletotrichum coccodes (black dot) were readily isolated. Two treatments (CGA 449 and SN 84364) significantly reduced silver scurf. None of the treatments reduced black dot.

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INTERACTIONS BETWEEN FUNGICIDE AND PINK ROOT TREATMENTS ON ONION BULB YIELD. Marvin E. Miller, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596.

Interactions between fungicide and pink root treatments on bulb yield were determined for cultivars Texas Grano 1015Y and Texas Grano 1105Y (TG 1105) in field trials. Pink root treatments were inoculation and no inoculation with Pyrenochaeta terrestris; purple blotch fungicide treatments were weekly applications of mancozeb at 3.36 kg (ai)/ha, iprodione at 1.68 kg (ai)/ha, and no fungicide. Pink root and fungicide treatments each had significant ($p = 0.01$) effects on yield; however, there were no significant interactions between the two factors. Within TG 1105 plants infected with P. terrestris, there was a highly significant correlation ($R^2 = 0.66$, $p = 0.008$) between severity levels of pink root and severity levels of purple blotch.

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EFFECT OF IRRIGATION METHOD ON THE DEVELOPMENT OF BACTERIAL SPOT EPIDEMICS IN TOMATO. M.A. Moss, Ken Pohronezny, James Schenk and Wilbur Dankers, Univ. of Fla., Tropical Research and Education Center, 18905 S.W. 280 St., Homestead, FL 33031

Epidemics of bacterial spot of tomato caused by Xanthomonas campestris pv. vesicatoria were evaluated for nine weeks under drip, overhead sprinkler, and overhead gun irrigation systems. Weekly disease assessments were made beginning four weeks after seeding and ending one week prior to harvest. Analysis of disease progress indicated that the rate of bacterial spot development was slower under drip irrigation. Rates of disease development between overhead sprinkler and overhead gun were similar. The rate-reducing effect of drip irrigation was probably due to the absence of a dispersal mechanism for the pathogen and/or the lack of free moisture on leaf and fruit surfaces. The current lack of effective chemical control for bacterial spot suggests that cultural practices such as drip irrigation may aid in reducing crop losses attributed to this serious disease.

202. Withdrawn

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EFFECT OF SOLARIZATION ON VERTICILLIUM WILT AND FUSARIUM WILT OF TOMATO. J. P. Jones and A. J. Overman, IFAS, University of Florida Gulf Coast Research and Education Center, 5007 60th Street East, Bradenton, FL 34203.

EauGallie fine sand was solarized by covering the soil with four mil clear polyethylene mulch from July 10 to September 10, 1985. Soil temperatures were 3 to 6 C higher in the four solarized areas compared to the 27 to 29.5 C temperature maintained in the upper 30 cm layer of soil in the four control areas of Sesbania macrocarpa cover crop. Solarization significantly reduced the incidence of Verticillium wilt (V. albo-atrum race 2) of tomato, cv. Sunny, in the fall crop (2.3 vs. 62.3%) and in the succeeding spring crop (16.7 vs. 53.0%). The incidence of Fusarium wilt (F. oxysporum f. sp. lycopersici race 3) was not significantly reduced by solarization during either crop season.

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A NEW LEAF DISEASE OF ONION IN NEW YORK CAUSED BY STEMPHYLIUM VESICARIUM. Nina Shishkoff and James W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, N.Y., 14853.

A new disease of onion (Allium cepa L.) in New York was first noticed in Orange County in 1985. Affected onion leaves had oval water-soaked lesions that coalesced to girdle the leaves. Pseudothecia formed in dead tissue. The fungus isolated from lesions was identified as Stemphylium vesicarium (Wallr.) Simm. Lesions developed on wounded and unwounded leaves after inoculation by spraying 1.4-1.7 x 10^5 conidia/ml and incubating in a dew chamber at 20°C. The number of lesions per leaf increased with the number of hours of exposure to free moisture in a growth chamber at 20°C, with appreciable damage occurring only after 24 hours of exposure. These studies indicate that S. vesicarium may be a potentially serious pathogen of onion during wet periods in New York, as it is in Texas.

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OCCURRENCE OF PHYTOPHTHORA CAPSICI ON COTTON, CHILI, AND PUMPKIN IN SOUTHEASTERN ARIZONA. P. A. Mauk and R. B. Hine, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

An unusual epidemic caused by Phytophthora capsici occurred in furrow irrigated cotton, chili, and pumpkin in the high elevation (1200 m) agricultural area of southeastern Arizona during the fall of 1986. The epidemic was triggered by high humidity, high rainfall and irrigation. Cotton boll rot, caused by P. capsici, had not previously been described from Arizona. Losses in the variety Coker 101 ranged from 5-10% in different fields. Pod decay in chili, which also had not been previously observed in Arizona, resulted in extensive losses throughout the valley. Decay of pumpkin fruits ranged from 50% to 100% in different fields. Compatibility type A-1 of P. capsici was found on cotton and chili and A-2 on pumpkin.

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EVALUATION OF MULCHES AND ROW COVERS TO DELAY WATERMELON MOSAIC VIRUS I IN YELLOW SQUASH. K. E. Conway[1], B. D. McCraw[2] and J. L. Sherwood[1], Department of Plant Pathology[1] and Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078-0285.

Combinations of mulches and row covers were evaluated during fall 1986 at Stillwater and Lane, OK. Treatments consisted of aluminum reflective (AF) or white plastic mulches, Vispore® or Reemay® row covers, and controls. Virus-infected plants were included at Stillwater to provide a known disease source. Squash cultivars Lemondrop and Multipik were planted in each treatment. At Stillwater, yield from AF mulch alone and both mulches in combination with row covers was significantly greater than other treatments. Row covers delayed virus onset from 2 to 6 days, with greatest delay in combination with mulch treatments. At Lane, where no virus was detected, yield from all combinations of mulches and row covers was significantly greater than no mulch-row cover treatments. Removal of row covers after initial flowering reduced yield.

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EFFECT OF MACROPHOMINA PHASEOLINA AND NEMATODES ON CANTALOUPE. B. D. Bruton* and C. M. Heald**, *USDA-ARS, Lane, OK 74555, **USDA-ARS, Weslaco, TX 78596.

The effect of Macrophomina phaseolina (MP) on cantaloupe in the presence of Meloidogyne incognita (MI) or Rotylenchulus reniformis (RR) was studied in the greenhouse. Nematodes were the primary cause of plant injury, expressed as top dry and root dry weight, with no apparent interaction with MP. Top dry weight between MP and control treatment was not significantly different. Reduction in top dry weight was greatest in MI and MP + MI treatments. With one exception, RR injury was less than MI singularly or in combination with MP. Root dry weight was less than control in all treatments with the exception of those involving MI which increased root weight due to excessive galling. In one test, symptom expression (charcoal rot) increased due to the presence of either nematode.

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THE EFFECT OF COPPER BACTERICIDES ON XANTHOMONAS CAMPESTRIS PV. VESICATORIA (XCV) POPULATIONS ON ASYMPTOMATIC TOMATO LEAVES. J. B. Jones, S. S. Woltz, J. P. Jones, R. D. Gitaitis, and K. M. Portier, IFAS, University of Florida Gulf Coast Research and Education Center, 5007 60th Street East, Bradenton, FL 34203 and University of Georgia, Tifton, GA 31794.

Tomato plants cv 'Sunny' were grown on plastic mulched raised beds in the spring and fall of 1986. In the spring, plants were either treated or not treated with $Cu(OH)_2$ + mancozeb at 2.3 kg and 1.3 kg/ha. Leaflets were sampled weekly for XCV. XCV populations on apparently non-diseased leaflets in treated plots were significantly reduced compared to the non-treated plots. In the fall, $Cu(OH)_2$ alone was added as a third treatment. $Cu(OH)_2$ alone and plus mancozeb significantly reduced populations compared to the control. The bactericide treatments did not differ significantly. In greenhouse studies, surface populations of a Cu resistant strain of XCV were reduced significantly by $Cu(OH)_2$ as compared to the control. These results support the concept that copper resistance is actually copper tolerance.

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DNA-DNA HYBRIDIZATION OF FOUR MELOIDOGYNE SPECIES. E. Pableo, and A. C. Triantaphyllou, Departments of Plant Pathology and Genetics, North Carolina State University, Raleigh, N. C.,27695.

Homology in the genomes of root-knot nematodes was measured by the binding of hybridized DNA to hydroxyapatite. DNA from M. incognita and M. arenaria was separated into repetitive and non-repetitive fractions, labeled, and reassociated with total DNA from twelve populations of Meloidogyne spp. At optimum conditions of reassociation (0.12 M sodium phosphate, 60°C), one half of the populations showed higher DNA homology with the non-repetitive sequences, and the other half with the repetitive fraction. This may indicate that the rate of divergence of the repetitive fraction may be similar to that of the non-repetitive sequences, and may imply that the repetitive sequences are important in the biological functions of these organisms. Results indicated that M. incognita, M. javanica, and M. arenaria are closely related with 80 to 100% DNA homology to the probes. M. hapla showed wide divergence with 24.5 to 33.5% DNA homology to the probes.

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SOYBEANS RESISTANT TO SOYBEAN CYST NEMATODE RACE 5. L. D. Young and E. E. Hartwig, USDA-ARS, 605 Airways Blvd., Jackson, TN 38301 and P. O. Box 196, Stoneville, MS 38776.

Commercial soybean cultivars resistant to soybean cyst nematode (SCN) populations similar to race 5 are not available. Breeding lines resistant to race 5 (SCN population TN-79) were developed through greenhouse evaluations. Lines were tested at Wheatley, AR (WA) and Tiptonville, TN (TT), where SCN populations (> 1300 cysts/L soil) reproduced well on race 3- and 4-resistant cultivars. Lines were evaluated for yield in absence of SCN at Stoneville, MS (SM). Mean final cyst densities for 'Essex' and 'Bedford' were 474 and 1036/L soil at WA and TT, respectively. Four lines had final cyst densities less than 20% of susceptible cultivar Essex and race 3- and 4-resistant Bedford. Two of the lines (D82-2397A and J82-190) yielded significantly greater than Bedford and Essex at TT and Essex at WA, yielded greater than Bedford at WA, and equaled Bedford at SM. D82-2397A has accumulated genes from several sources and is resistant to SCN races 3 and 4.

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INTERACTION BETWEEN MELOIDOGYNE HAPLA AND PYTHIUM MYRIOTYLUM POPULATIONS IN PEANUT SEEDLING DISEASE. H. H. Fagbenle and A. B. Filonow, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Interactions between Pythium myriotylum and Meloidogyne hapla in peanut seedling disease were investigated in greenhouse factorial experiments. Plants were inoculated with P. myriotylum at plant, 1,2, or 3 weeks after nematode inoculation (0, 1,500, and 3000 eggs per pot). Interaction decreased fresh shoot weight, and increased disease index and recovery of P. myriotylum from peanut roots. Three inoculum densities of M. hapla (0, 2000, and 4000 eggs per pot) and four inoculum densities of P. myriotylum (0, 0.4, 1.2 and 10.9 propagules per 100 g soil) were tested in all possible combinations. Nematode-fungus interaction increased disease index in the presence of 2000 and 4000 M. hapla eggs. Interaction decreased shoot and root weights, increased recovery of fungus from roots, and increased recovery of fungus from soil in the presence of 4000 M. hapla eggs.

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SCANNING ELECTRON MICROSCOPY OF CONCOMITANT INFECTIONS OF MELOIDOGYNE HAPLA AND RHIZOCTONIA SOLANI ON PEANUT. H. H. Fagbenle and P. M. Inskeep, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

The scanning electron microscope (SEM) was used to study the spatial relationship of concomitant infections of Meloidogyne hapla and Rhizoctonia solani on Pronto peanuts. Two-day-old peanut seedlings were inoculated with either R. solani (0.8 propagules/100 g soil), M. hapla (4,000 eggs/cup), or R. solani + M. hapla. Eleven weeks after inoculation, root and hypocotyl samples were prepared and observed with the SEM. The epidermis of root galls often split, leaving a rough surface of crushed cortical cells. Hyphae and mycelial "mats" of R. solani were abundant on gall and hypocotyl surfaces. Dense infection cushions occurred on the hypocotyls of plants inoculated with both organisms. Fungal hyphae penetrated nematode egg masses which had broken through the outer surface of the gall. Mycelial masses were found within some nematode cuticles and some nematode cavities.

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MITOCHONDRIAL DNA DIVERSITY IN POPULATIONS OF SOYBEAN CYST NEMATODE, HETERODERA GLYCINES. A.D. Radice, T.O. Powers, L.J. Sandall, and R.D. Riggs. Departments of Plant Pathology, University of Arkansas, Fayetteville, AR 72701 and University of Nebraska, Lincoln, NE 68583-0722.

Restriction patterns of Mitochondrial DNA from 12 geographically diverse populations of H. glycines were examined. Fourteen restriction enzymes yielded 45 different fragments. Surprisingly few restriction fragment length polymorphisms exist in North American populations or in populations from China and Columbia. A single identical "wild type" mitochondrial genome was identified in all populations, and was the only mitochondrial genome in most of the North American populations. Variant mitochondrial genomes present in the Colombian, Chinese and Virginia populations differed from "wild type" by the expansion of a localized region of the genome, and not loss or addition of restriction sites. Conversely, closely related Heterodera species display considerable divergence from the mitochondrial genome of H. glycines. This reduced mtDNA variability in H. glycines supports the view that North American populations have passed through a recent bottleneck, possibly through introduction by man, in connection with soybean production.

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INFLUENCE OF SOYBEAN CULTIVAR ROTATION SEQUENCES ON RACE DEVELOPMENT OF HETERODERA GLYCINES, RACE 3. G. W. Lawrence, J. M. McGuire, Dept. of Plant Pathology & Weed Sci., MS State University, MS State, MS 39762.

Race status of a field population of soybean cyst nematode (SCN) (Heterodera glycines Ichinohe) previously characterized as race 3 was measured after 3 years of continuous SCN-susceptible, race 3-resistant, race 3 and 4-resistant and sequences of susceptible and resistant soybeans [Glycine max (L.) Merr.]. Reproduction indices (Ri) on SCN race differentials Pickett 71, Peking, PI 90763 and PI88788 were measured as % of reproduction on Lee. Populations from 6 rotation sequences were not identifiable as race 3 because of Ri on Pickett 71 of 17-53%, but reactions on the other differentials were atypical for race 4. Five populations were identified as race 3; however, 4 of these populations had Ri of 2-21% on Bedford (resistant to races 3 and 4). The SCN population from continuous race 3-resistant soybean had Ri of 36,7,9, and 8% on Pickett 71, Peking, PI90763 and PI88788, respectively. The population from continuous susceptible soybeans did not reproduce on the differentials.

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EFFECT OF SOIL SALINITY ON THE CITRUS NEMATODE. R. M. Davis, C. J. Farrald, and D. Swietlik, Texas A & I University, Citrus Center, Weslaco, Texas 78596.

The ability of the citrus nematode, Tylenchulus semipenetrans, to reproduce on sour orange roots subjected to salinity stress was studied in the greenhouse. More nematodes were recovered from plants irrigated with $MgSO_4$ (6 dS/m) than from plants irrigated with an equivalent amount of $CaCl_2$, Na_2SO_4, a combination of the 3 salts, or no salt. The nematode significantly reduced seedling growth only in the $MgSO_4$ and salt combination treatments. In a clay soil the nematode reduced growth of phosphorus-deficient plants irrigated with saline (3 dS/m) water but not in plants irrigated with a saline, complete nutrient solution. No growth reduction by the nematode occurred in a sandy soil regardless of salinity or phosphorus status. Apparently, the citrus nematode can reduce plant growth under certain conditions of plant stress.

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RESISTANCE OF CORN HYBRIDS AND INBREDS TO MELOIDOGYNE SPP. G. L. Windham and W. P. Williams, USDA, ARS, Crop Science Research Laboratory, Mississippi State, MS 39762.

Sixty-four corn hybrids were evaluated for resistance to Meloidogyne incognita (MI), M. arenaria (MA), and M. javanica (MJ) in greenhouse experiments. Forty-nine inbreds were also evaluated for resistance to MI and MA. Seeds were planted in Todd Planter Flats containing a sterilized sand-soil mixture (1:1). Seven-to ten-day-old seedlings were inoculated by pipetting a water suspension containing 3,000 eggs into each cell. After 60 days, eggs were extracted from each root system using NaOCl and Oostenbrink's R factor (RF) (final egg number/initial number of eggs) was determined. Northrup King 508 (RF = 0.8) and Pioneer Brand 3147 (RF = 0.8) maintained MA and MJ below the initial population level, respectively. All hybrids were excellent hosts for MI with RF values ranging from 20.7 by Sunbelt 1860 to 49.5 by Pioneer Brand XC941. Inbreds Mp307 (RF = 1.8) and Mp313 (RF = 0.2) were poor hosts for MI and MA, respectively.

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VARIATION OF PATHOGENICITY OF MELOIDOGYNE INCOGNITA ON WHITE CLOVER. G. L. Windham and G. A. Pederson, USDA, ARS, Forage Research Unit, Mississipi State, MS 39762.

The pathogenic variation of nine Meloidogyne incognita populations on five white clover cultivars and germplasms was determined in greenhouse experiments. The clover cultivars/germplasms evaluated were 'Regal', 'Osceola', Drought tolerant synthetic #6, 'Louisiana S-1', and SC-1. Seedlings were transplanted to 'Super Cell Cone-tainers' and inoculated with 1500 eggs/cell three weeks after transplanting. After 60 days, the amount of root galling, and shoot and root dry weights were determined. A tolerance index (shoot weight of infected plant/shoot weight of uninfected plant X 100) was calculated. Root systems of all cultivars/germplasms were extensively galled by all nematode populations. Differences (P = 0.05) in pathogenicity of the nematode populations were reflected by the effect of the nematodes on shoot and root weights. SC-1 was the only entry that exhibited tolerance to M. incognita.

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FLUORESCENT PSEUDOMONAS SPP. POPULATIONS IN SOIL AND RHIZOSPHERE OF CITRUS PLANTED IN SITES AMENDED WITH VARIOUS AMENDMENTS. A. B. Gould*, R. M. Sonoda* and S. Nemec**. *University of Florida, IFAS, AREC, Ft. Pierce, FL 33454, **U. S. Department of Agriculture, ARS, Orlando, FL 32803.

Populations (colony-forming units/g soil) of fluorescent pseudomonads (fp) in the rhizosphere and soil of citrus in two groves, one on a flatwoods soil and one on a ridge soil in Florida, differed only slightly among deep-tilled treatments containing phosphogypsum, phosphoclay, peat, humate and lime. Fp populations (cfu/g) were highest in the first sampling (3 mo after planting) than in the later two samplings. Late in the first year after planting, fp populations in the no-till, no amendment treatment were higher than in all deep-tilled treatments at both locations. Fp numbers were greater in the rhizosphere than nonrhizosphere soil (10^{-6} to 10^{-9}, and 10^{-3}

to 10^{-6} cfu/g, resp.) in all treatments at each sampling. Species commonly isolated were Pseudomonas putida, P. fluorescens and P. aeruginosa.

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POPULATION DYNAMICS OF *STREPTOMYCES SCABIES* AND OTHER ACTINOMYCETES IN SOIL CROPPED TO POTATOES. A. P. Keinath and R. Loria, Department of Plant Pathology, Cornell University, Long Island Horticultural Research Laboratory, Riverhead, NY 11901.

Streptomyces scabies, an actinomycete which causes potato scab, produces melanin, spiral spore chains, and gray aerial mycelia. Populations of actinomycetes were sampled in field plots of scab-susceptible (Chippewa) and -resistant (Superior) potato cultivars and in fallow plots. The population densities of total and melanin- producing actinomycetes and gray, melanin-producing actinomycetes with spiral spore chains were monitored during the growing season in rhizosphere (RS) and nonrhizosphere soil and on the tuber surfaces. In 1985 and 1986 most actinomycete populations increased logarithmically over time, and approximately 10% of the total actinomycetes produced melanin. In 1986, population densities in RS were 7-30 times greater than those in fallow soil. More total and spiral spore-chain actinomycetes were found on the surfaces of Chippewa tubers than on those of Superior. The population density of these spiral actinomycetes was correlated positively with scab severity for both cultivars, suggesting a relationship between population density and disease.

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IMMOBILIZATION OF MN PREDISPOSES WHEAT TO TAKE-ALL, D. M. Huber, Botany & Plant Pathology Department, Purdue University, W. Lafayette, IN 47907.

Although a deficiency of many nutrients will predispose wheat to take-all root, crown, and foot rot, a sufficiency of ammoniacal-N and Mn have recently been closely associated with reduced disease. To evaluate the independant effects of these two nutrients, peat (as a "sink" for Mn) was applied with and without Mn or siderophonre-producing bacteria as seed-dressings to winter wheat prior to planting in field soils naturally infested with Gaeumannomyces graminis. Severe take-all occurring on plants from sterilized peat seed treatment was correlated with reduced uptake on Mn. The addition of Mn, and stabilization of N in the ammoniacal form with nitrapyrin which increases the availability of Mn, nullified the peat induced predisposition to take-all. Siderophore producing bacteria partially off-set the peat induced predisposition to take-all by apparently increasing the availability of Mn for plant uptake.

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EFFECT OF ONION SEED BACTERIZATION ON THE GERMINATION OF SCLEROTIA OF SCLEROTIUM CEPIVORUM IN MUCK SOIL. M.S. Reddy and J.E. Rahe. Department of Biological Sciences, Simon Fraser University. Burnaby, British Columbia, Canada V5A 1S6.

The effects of diallyldisulfide and of onion seedlings grown from sterilized non-bacterized and bacterized seeds on the germination of sclerotia of Sclerotium cepivorum were compared. Diallyldisulfide and bacterized onion seedlings stimulated germination of sclerotia significantly relative to both non-bacterized seedlings and control (no seedlings) in muck soil; non-bacterized seedlings were not significantly different from control. Significant differences in the abilities of the five bacterial isolates being compared to enhance the stimulatory effect of onion seedlings on germination of sclerotia were observed. Effects on sclerotial mycosphere fungi and bacteria varied among the treatments. Significant inverse correlations between sclerotial germination and sclerotial mycosphere bacterial and fungal populations were detected.

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MICROBIAL PHENOMENA RELATED TO INCREASED GROWTH RESPONSE IN SOLARIZED SOILS. A. Gamliel and J. Katan. Dpt. of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Increased growth response (IGR), i.e. improved growth in the absence of known pathogens, was recorded in 14 out of 16 solarized soils. Dry wt of bioassayed tomato plants increased in the solarized (S) soils by 20-180%. Some reduction in the total populations of bacteria was observed but populations of fluorescent pseudomonads were much increased (20-1000 fold) in rhizosphere and root tissue of tomato plants in S soils. Solarization greatly reduced the total populations of fungi, Pythium spp. and fungi causing plant stunting and slightly affected populations of actinomycetes. Solarization also improved growth of cotton plants in monoculture system. The effect on microbial populations was similar to that found with IGR.

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Influence of temperature, wheat chaff and metalaxyl on infection of wheat embryos by Pythium spp. David M. Ingram and R.J. Cook. Washington State University and USDA-ARS Pullman, Wa. 99164.

Infection of wheat seed embryos occurred within 40 degree-days (0 C base) after sowing in steam-treated soils reinfested, respectively, with oospores of four Pythium spp. At all temperatures (5-25 C), the incidence of embryo infection at 40 degree-days was greatest (64-94%) in soil infested with P. ultimum var. sporangiiferum. Pythium torulosum was the next most frequent embryo-colonist (38-68%), followed by P. heterothallicum (8-52%) and P. irregulare (6-36%). Metalaxyl prevented embryo infection by P. ultimum var. sporangiiferum and P. irregulare at 5-20 C, but at 25 C, 10-12% of the treated seed embryos were infected by these species. With fresh (natural) wheat chaff added to the soil (1% w/w), the frequency of embryo infections was increased 21-58% for P. ultimum var. sporangiiferum and 7-32% for P. irregulare, and 10-12% of metalaxyl-treated seeds were embryo-infected by P. irregulare at both 20 and 25 C.

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DISEASE DEVELOPMENT OF THIELAVIOPSIS BASICOLA ON PIMA COTTON AS INFLUENCED BY INOCULUM DENSITY AND TEMPERATURE. P. A. Mauk and R. B. Hine, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

The influence of inoculum density and temperature on disease development was evaluated for Thielaviopsis basicola (= Chalara elegans) on Pima cotton (Gossypium barbadense). A naturally infested soil containing 600 ppg of air-dried soil was diluted with autoclaved field soil to obtain inoculum densities ranging from 0 to 600 ppg. Seeds were sown in infested soils in growth chambers maintained at 20 and 28 C. Ten days after germination plants were evaluated for cortical root decay, plant height, and number of true leaves. The extent of cortical decay was related to inoculum density but was not related to the temperature of incubation. However, dramatic reductions in plant height and number of true leaves occurred only at the high inoculum levels and lowest incubation temperatures.

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RHIZOCTONIA SOLANI AND RHIZOCTONIA-LIKE BINUCLEATES ASSOCIATED WITH SUGAR BEET PLANTS IN THE RED RIVER VALLEY. C. E. Windels and D. J. Nabben, Northwest Expt. Stat., Univ. of Minn., Crookston, MN 56716.

Rhizoctonia was isolated from dying or lesioned sugar beet (Beta vulgaris L.) seedlings and from diseased crowns and roots of older plants (>6 wk old). Isolates of R. solani were identified by anastomosis group (AG). Of 197 cultures from seedlings collected from soils tested in the greenhouse and in two field seasons, 2% were AG-1; 8.6%, AG-2-2; 0.5%, AG-3; 48.7%, AG-4; 28.9%, AG-5; and 3.6% were multinucleate isolates that did not anastomose with any tester isolates (AG-1 through AG-8 and AG-B2). The remaining 7.6% isolates were binucleate. Of 95 cultures from older plants, 91.6% were AG-2-2 and 7.4% were AG-4; 1% were binucleate. The number of AGs isolated per field by plant assay ranged from one to three. Overall, there was a greater diversity of AGs from diseased sugar beet seedlings than from older plants, with AG-4 and AG-5 predominating on seedlings and AG-2-2 on older plants.

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GERMINATION INHIBITION AND FUNGISTATIC ACTIVITY OF WATER-SOLUBLE LEACHATES FROM SUGARBEET SEED. J. J. Gallian and S. K. Kober, Dept. of Plant, Soil and Entomological Sciences, University of Idaho, 1330 Filer Ave. E., Twin Falls, ID 83301.

Germination rate of sugarbeet seed in blotter tests for the first 7 days was inversely related to the concentration of leachate used as the moisture source. Leachate was obtained by shaking 2000 sugarbeet seed in 132 ml of water for 24 hr. Rhizoctonia solani isolated from sugarbeet roots was incubated at 30 C for 4 days in liquid media to which the filter sterilized leachate had been added at 0, 25, 50 and 75% concentrations. Wet and dry weights of mycelium were higher in the 25% treatment and lower in the 50 and 75% treatments

than in the control. The increase of fungal growth in the 25% treatment suggests a concentration threshold for fungistatic activity. Removal of germination inhibitors to speed germination and improve crop establishment may have a detrimental effect by also removing a natural protection against seedling pathogens.

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Control of Cylindrocladium black rot through cultural practices that modify the soil physical environment. J. R. Sidebottom and M. K. Beute, Plant Pathology Department, North Carolina State University, Raleigh, NC, 27695-7616

Development of Cylindrocladium black rot (CBR) in peanuts caused by Cylindrocladium crotalariae is slowed when soil temperatures exceed 25C and stops if the temperature exceeds 30C. Cultural practices modifing the soil physical environment were evaluated in 1985 and 1986 for their effect on the development of CBR in susceptible (Florigiant) and moderately resistant (NC 18416) cultivars. Cultural practices included planting date (early, mid and late May), bed preparation (bedded, flat) and row orientation (north-south, east-west). In both years, CBR incidence was less for NC 18416 than Florigiant and for the last planting date than the first. In 1985, disease incidence in Florigiant was less in bedded rows; in 1986 there was no difference due to bed preparation. In 1986, CBR development was less in north-south than east-west rows. Soil temperature in the row at 10 cm depth was greater than 28C for more hours in east-west rows and in bedded rows.

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ETIOLOGY AND IMPACT OF DISSIMILATORY SULFATE REDUCTION IN HIGHLY MAINTAINED TURFGRASS SOILS. W. Lee Berndt and J.M Vargas, Jr. Department of Botany and Plant Pathology Michigan State University East Lansing, MI 48824.

Dissimilatory sulfate reduction in the soil profile has been shown to be associated with pathogenic conditions in some highly maintained turfgrasses. The relationship is hypothesized to result from supplemental application of elemental sulfur and sulfur containing compounds. In affected soils, turfgrass vigor quickly declines; death may soon follow. Toxic metabolites such as sulfide, produced by reducing conditions, may initiate the decline. These same metabolites, when reacting with metal cations, produce a black sulfide precipitate layer which furthur encourages reducing conditions by chemically scavenging oxygen and hindering drainage. The objectives of this preliminary research are to define the inputs leading to such reducing conditions and turf decline, and to develop cultural strategies for managing these inputs.

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EFFECT OF TILLAGE ON PROPORTION OF FLUORESCENT PSEUDOMONADS EXHIBITING IN VITRO ANTAGONISM TO PYTHIUM GRAMINICOLA. D. P. Miller, I. W. Deep, and P. E. Lipps, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

In a study of the effects of tillage on continuous corn production in a heavy clay soil, fluorescent pseudomonads were isolated from soil and tested for in vitro antagonism against Pythium graminicola (Subr.). Soil samples were collected on 5 dates from mid June through early September, 5-10 cm from the stalk and 2-5 cm below the surface. There were no detectable effects of tillage on the population levels of fluorescent pseudomonads. However, of 3130 isolates tested, the proportion of these bacteria that exhibited antagonism against the test fungus was consistently higher in tilled treatments (avg. = 40% antagonists) than in non-tilled treatments (avg. = 9% antagonists). P. fluorescens, especially biotype D (P. chlororaphis), comprised the majority of isolates identified, including both antagonistic and non-antagonistic isolates. Different tillage practices alter the organic nutrient status of the sampling zone, and this may influence the proportion of antagonistic pseudomonads.

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QUANTIFICATION OF MICROSCLEROTIAL GERMINATION OF MACROPHOMINA PHASEOLINA IN THE RHIZOSPHERE OF SOYBEAN. D.J. Collins and T.D. Wyllie, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

A nylon mesh technique was used to quantify microsclerotial germination of Macrophomina phaseolina in the rhizosphere of soybean. Approximately 2 X 1 cm pieces of nylon mesh with attached microsclerotia were buried in the following soils: rhizosphere (nylon placed beneath a two day old Williams 79 soybean seedling), nonrhizosphere, and sterile soil. After four days incubation at 28^{0} C, the nylon mesh was retrieved and examined microscopically. Microsclerotial germination was determined by counting the number of hyphal intercepts on the grids of the nylon mesh. Preliminary results show germination of microsclerotia to be greatest in the rhizosphere and sterile soils with 25 and 24 hyphae per mesh, respectively; whereas, the nonrhizosphere soil showed the least microsclerotial germination with 14 hyphae per mesh. This method may be useful in determining the effect of various environmental factors on pathogen activity.

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EFFECT OF ATRAZINE ON UNGERMINATED CONIDIA OF COCHLIOBOLUS SATIVUS IN SOIL. T. Isakeit and J. L. Lockwood, Dept. Botany and Plant Pathology, MI State Univ., East Lansing, MI 48824-1312

The effect of atrazine on conidia of Cochliobolus sativus exposed to fungistatic conditions in soil was determined. Conidia borne on Nuclepore membranes on the surface of Boyer sandy loam (-1 kPa matric potential) containing 24 ppm atrazine were examined for germination on soil and viability when placed on carrot agar. Germination of isolates 504, R002, and 2442 was 3%, 13%, and 24% on atrazine-treated soil after two weeks, and their viability was 96%, 49%, and 24%, respectively. These isolates did not germinate on untreated soil and their viability was 99%, 96%, and 87%, respectively. Thus, viability after incubation on soil treated with atrazine was inversely correlated with rate of germination on soil. Atrazine did not increase germination of three other isolates on soil, nor did it affect their viability. In conclusion, atrazine increased conidial germination of some (but not other) isolates of C. sativus in soil. These conidia rapidly lost viability.

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DENDROCLIMATIC EXAMINATION OF WHITE OAK ALONG A SOIL-MOISTURE GRADIENT IN THE PIEDMONT OF SOUTH CAROLINA. J. C. Jacobi and F. H. Tainter. Department of Forestry, Clemson University, Clemson, SC 29634-1003.

The purpose of this research was to determine the role of drought as an inciting factor in the decline of white oak. Radial growth data were collected from 16 undisturbed hardwood stands in which white oak was a major component of the overstory. The stands were classified into 4 community types ranging from xeric upper flats to mesic lower slope positions. Trees on all sites exhibited below normal radial growth during years of extreme drought such as 1911, 1924, 1936, 1954 and 1980. The period of time following a severe drought before radial growth recovered to predrought levels was longest for trees on xeric sites. Thus, one might expect a greater incidence of oak decline and mortality on the xeric sites which are more prone to drought stress due to their low-soil moisture holding capacity.

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NOCTURNAL DISPERSAL IN CONIFER PLANTATIONS: IMPLICATIONS FOR THE AEROBIOLOGY OF CRONARTIUM SPP. K. E. Moore, Department of Atmospheric Science, State University of New York at Albany, Albany, New York 12222.

Dispersal and deposition processes for nocturnally-dispersed basidiospores of Cronartium spp. are poorly understood. Van Arsdel (Phytopath. 57: 1221-1229) suggested a variety of nocturnal circulations that would account for some observed distributions of disease. In the present study some characteristics of nocturnal, near-surface flow in young conifer plantations were measured using two tracers: neutrally buoyant soap bubbles, and sulfur hexafluoride gas. Even on very slight slopes, drainage flow is an important feature. However, sporadic upslope flow does occur in the form of waves or wave-like oscillations; the breaking of such waves may increase deposition velocity several fold.

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EARLY STIMULATION OF ETHYLENE PRODUCTION IN HOSTS CHALLENGED BY ENDOTHIA PARASITICA OR ITS CULTURE FILTRATES. F.V. Hebard and L. Shain, Dept of Plant Path, Univ of Kentucky, Lexington 40546.

Ethylene production was stimulated near Endothia cankers on oak and chestnut. In the field, stimulation occurred within 5 days of inoculation, prior to lignification. Stimulation occurred within 2 days when bark plugs were placed on agar-mycelium disks or culture filtrates. With mycelium, bark plugs from American chestnut commonly were stimulated more than plugs from Chinese chestnut or scarlet oak; likewise, virulent E. parasitica commonly stimulated more than hypovirulent strains. With filtrates, stimulation occurred for young (ca 7 days) cultures on all media; for old (10-20 days) cultures, stimulation occurred with filtrates from bark extract broth but not potato dextrose

broth or a defined medium. Oxalate, a suggested toxin from E. parasitica, did not stimulate ethylene production. These results suggest that American chestnut is more sensitive than resistant hosts to metabolites of virulent strains, and that oxalate is not one of those metabolites.

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BIOLOGICAL AND CHEMICAL CONTROL OF OAK WILT IN TEXAS LIVE OAK. T. H. Filer, Jr., USDA - Forest Service, Southern Hardwoods Laboratory, Stoneville, MS 38776.

Chemicals and bacterial isolates were screened in the greenhouse and laboratory in 1983 for control of oak wilt. In 1984, systemic and non-systemic chemicals were field tested. Propiconazol treated trees remained healthy while untreated trees became infected with Ceratocystis fagacearum. In 1985, randomized plots with 5 replications and 5 trees per replication were established bimonthly beginning in March. In each replication, 25 trees were treated with Pseudomonas chicorii or propiconazol and 25 trees were selected as checks. In 1986, 375 trees which were treated in 1985 were re-treated and inoculated with C. fagacearum. There were significantly fewer dead trees in the propiconazol-treated plots than in the check plots in March 1987. Propiconazol treated trees were not significantly different from trees which were inoculated with bacteria.

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FIELD CONVERSION OF CHESTNUT BLIGHT CANKERS INITIATED AFTER APPLICATION OF HYPOVIRULENT CONIDIA. K.L. Scibilia, F.V. Hebard, and L. Shain. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Conidia from two hypovirulent (H) strains of Endothia parasitica (Ep780 and Ep905) were sprayed on 56 American chestnut trees. Mycelium from Ep155, the isogenic virulent (V) strain of Ep780 and Ep905, was used to initiate a total of 192 cankers 3 weeks before spraying and 1-6 weeks after spraying. Isolations made 8 weeks after spraying with conidia were judged hypovirulent by culture morphology and their ability to convert Ep155 in vitro. Conidia of the aggressive strain Ep780 converted significantly ($p < .001$) more cankers [58] to H than did those of the highly debilitated strain Ep905 [15]. A similar number of cankers were converted 1-3 and 4-6 weeks after spraying, but there was a tendency ($p < .16$) for a decrease in conversion with time. There also was a tendency ($p < .11$) for more conversion on trees sprayed with a conidial suspension amended with a pinolene-based sticker/extender.

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INFLUENCE OF DISEASE MANAGEMENT PRACTICES ON PRODUCTION OF WHITE SPRUCE SEEDLINGS IN A FOREST TREE NURSERY. S.A. Enebak[1], M.A. Palmer[2], R.A. Blanchette[1], and R.W. Stack[3]. Univ. of Minn., Dept. of Plant Path.[1], USDA Forest Service[2], St. Paul, MN, 55108 and North Dakota State Univ.[3], Fargo, 58105

In a forest tree nursery in northern Wisconsin, damping-off of 1st-year white spruce was least in plots treated with dazomet (D), and intermediate in plots treated with thiram seed coat (T), captan soil drench (C), silica sand (S), and both C plus T. The greatest amount of damping-off occurred in nontreated plots. Incidence of stunted, mycorrhizal-deficient seedlings, was greatest in D plots, intermediate in plots treated with C, T, or C plus T, and lowest in plots treated with S and in nontreated plots. First-season yield of healthy seedlings was greatest in plots treated with S (421 seedlings/m^2), intermediate in plots treated with T (331/m^2), D (330/m^2), or in nontreated plots (304/m^2). Yield was lowest in plots treated with C (278/m^2) and C plus T (272/m^2). The size and percent mycorrhizal rootlets of healthy seedlings were not significantly different ($P = 0.05$) among all treatments.

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AIR POLLUTION AND THE DECLINE OF A SPRUCE-FIR ECOSYSTEM IN THE SOUTHERN APPALACHIAN MOUNTAINS. Robert I. Bruck, Dept. Plant Pathology, NC State University, Box 7616, Raleigh, NC 27695.

Studies have been initiated to relate data collected from the EPA-Mt. Mitchell Mountain Cloud Chemistry Project and from permananent field plots established to quantify dieback and decline of red spruce and Fraser fir. In 1986, cloud impaction via frontal and/or orographic cloud processes occurred 8 of 10 days. Cloud acidity ranged from pH 2.4 to 5.6 (mean 3.1). Nitrogen compounds and sulfur compounds accounted for equal proportions of hydrogen ions. The incidence and duration of ozone concentrations >85 ppb were often >25% of all hours during the growing season (June - Sep.). Pollutant deposition appears to be stratified by elevation (greater at higher elevations) and aspect (greater on west aspects). These observations closely correlate to the incidence of damage to this spruce-fir ecosystem where stands above 1800 m exhibit >60% mortality, stands 1600-1800 m >25% mortality, and stands below 1600 m <15% mortality. Although pollutant deposition/concentration and decline are correlated, no cause and effect data are yet available.

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A SYSTEM FOR EPIDEMIOLOGICAL ANALYSIS OF OAK WILT. D. N. Appel, R. C. Maggio, E. L. Nelson, Texas Agricultural Experiment Station, College Station, TX., and M. J. Jeger, Tropical Development and Research Institute, London, England.

A computer based system was developed to analyze local, tree-to-tree spread of Ceratocystis fagacearum. After acquiring yearly, sequential color-infrared aerial photographs of expanding oak wilt foci, detailed photointerpretation is done to produce a base map with individual trees and clumps of trees classified as healthy, diseased, or dead. When digitized on a microcomputer geographic information system, the coordinate and disease-class data are analyzed over time and illustrated with a software routine termed IOWAP (Interactive Oak Wilt Analysis Package). Data are computed on an Amdahl V/8 and a Tektronix 4170/4107 graphics workstation. IOWAP generates linear distances and directions of fungal spread among trees connected by common root systems and root grafts. Also, this system facilitates study of differential infection and mortality rates that appear to be common among oak wilt foci in Texas. These analyses are expected to aid in understanding the dynamics of localized oak wilt epidemics.

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SPREAD OF ARMILLARIA ROOT ROT OF LODGEPOLE PINE IN WEST-CENTRAL ALBERTA. H.W. Klein-Gebbinck, P.V. Blenis and Y. Hiratsuka, Department of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

Eighty 12-year-old lodgepole pine trees (Pinus contorta var. latifolia Engelm), were excavated in a single stand in west-central Alberta. Rhizomorphs, rather than root contact with stumps or debris, appeared to be responsible for most infections. Rhizomorphs were found on or very near 91% of the infected trees. Of 19 roots to which rhizomorphs were attached, 18 were successfully colonized. Lateral root infection typically did not result in tree mortality, because subsequent colonization was usually distal to the point of infection. The majority of killed trees had resinosis on the tap root or root collar, thus suggesting that the lethal infection had taken place there. Spatial analysis indicated, that diseased trees often occurred in clumps less than 0.9 m in diameter.

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A NEW CANKER DISEASE OF JUNIPERUS SCOPULORUM IN KANSAS WINDBREAKS. N. Tisserat and A. Nus, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A previously undescribed canker disease of Rocky Mountain juniper was widely distributed in central Kansas windbreaks; more than 20% of the trees in some windbreaks were affected. Diseased trees first exhibited dieback of one or more branches, followed by death of the top 1/3 to 1/2 of the tree crown. Large, flattened, resin-soaked cankers appeared on branches and the main stems. Trees with multiple, coalescing stem cankers were killed. A fungus, tentatively identified as Diplodia sp., was consistently isolated from and observed fruiting in the cankers. Single-spore isolates of the fungus produced both microconidia (2.5 X 5.0 µm) and macroconidia (11.8 X 25.6 µm) in pycnidia on PDA. One- to two-year-old Rocky Mountain juniper and eastern redcedar trees were inoculated by inserting mycelium and spores of the fungus into fresh stem wounds. All trees developed sunken cankers within 1 mo in the greenhouse, and most were killed within 3 mo.

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INTERACTIONS AMONG THE THREE VARIANTS OF VERTICICLADIELLA WAGENERI AND THE THREE HOST TYPES. F. W. Cobb, Jr., T. T. Lawson and T. L. Popenuck. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Isolates of the hard pine, Douglas-fir and pinyon variants were used to inoculate seedlings of all three hosts under greenhouse conditions. Twenty seedlings per host were inoculated with four isolates of each variant. The hard pine variant infected an av. of 15 ponderosa pine, 10 Douglas-firs and 6 pinyons but killed an av. of 13, 1 and 3 seedlings, respectively. The Douglas-fir variant infected an av. of 7 ponderosa pines, 13

Douglas-firs and 2 pinyons but killed an av. of only 3, 4, and 1 seedlings, respectively. The pinyon variant infected an av. of 13 ponderosa pine, 15 Douglas-firs and 12 pinyons and killed an av. of 11, 6, and 9 seedlings, respectively. Differences in infection and mortality among isolates and among hosts were highly significant (P= 0.01) as was the mortality interaction. The interaction for infection was significant at 0.05. These results and isozyme studies suggest a third fungus variety.

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HISTOPATHOLOGY OF PLOIODERMA HEDGCOCKII ON LONGLEAF PINE NEEDLES. F.F. Jewell, Sr., School of Forestry, Louisiana Tech University, Ruston, LA 71272.

Longleaf pine(Pinus palustris Mill.) needles with typical symptoms of Ploioderma hedgcockii were sampled, prepared for, and examined by light microscopy. Non-symptomatic needle tissue sampled was typical for longleaf. Symptomatic tissue abnormalities were limited mostly to the mesophyll in symptom areas. Partial or complete cell collapse and limited cell dissolution in the mesophyll was the dominant host reaction with hyphae(textura globulosa) abundant between collapsed cells. Pathogen or host reaction rarely extended into or past the endodermis. Gradual separation of non- and affected tissues and limited hyphal presence at outer edges of symptom areas was common. Hysterotheca formed between epidermis and hypodermis. The latter often disrupted by hyphae. A black pseudoparenchymatous layer with hyphae passing between epidermal cells to needle surface covered the hysterotheca, which were often subtended by filamentous, branched, and large(5-7) hyphae(textura intricata).

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PATHOGENICITY STUDIES WITH SEPTORIA MUSIVA. J. M. Krupinsky, USDA, ARS Northern Great Plains Research Lab, P. O. Box 459, Mandan, ND 58554

Isolates of Septoria musiva were obtained from diseased leaves of Populus spp. which were collected from nursery propagation beds and experimental plantations in central North Dakota, and from windbreaks and native stands in Minnesota, North Dakota, and South Dakota. Isolates of S. musiva were compared by inoculating rooted cuttings in the glasshouse to detect differences in pathogenicity on five Populus clones (Northwest, Robusta, Norway, Siouxland, and Walker). One million spores/ml and an incubation time of 48 hr were used to promote good symptom development. The percent necrosis, number of lesions, and lesion size were estimated visually three weeks after inoculation. Symptoms caused by different isolates could be distinguished from one another in tests conducted in 1985, 1986, and 1987.

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E.G. Kuhlman. Relationship of early symptoms of fusiform rust infection to later presence of galls. U.S. Forest Service, Southeastern Forest Experiment Station, Athens, GA.

Open pollinated, 4-wk-old progeny from 160 loblolly pine trees were inoculated by the CBS system with basidiospores of Cronartium quercuum f. sp. fusiforme. The susceptible control family had significantly more galls 9 months after inoculation than 118 of the 159 families tested. Symptoms of infection were observed at 1.5, 3, 6, and 9 months after inoculation. Symptoms observed at 1.5 mo after inoculation were not as good predictors of the presence of galls at 9 mos as were symptoms at 3 mos. The most accurate predictor at 3 mos was the presence of stem swelling, which had more than a 90% probability of being a gall at 9 mos. For all other symptoms, the relative susceptibility of the family affected the frequency that seedlings with any given symptom at 3 mos developed galls at 9 mos. Seedlings from susceptible families that had no stem symptoms (only needle spots or no symptoms at all) at 3 mos were 2-3 times more likely to have galls at 9 mos than were similar seedlings from reistant or intermediate families.

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SEQUENCES OF TWO ADDITIONAL PECTATE LYASE GENES FROM ERWINIA CHRYSANTHEMI EC16. S. E. Gold, S. J. Tamaki, S. Manulis, M. Robeson and N. T. Keen. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Pectate lyases produced by soft-rotting Erwinia species are important in disease development. The pel A and pel C genes were sequenced and compared with the previously sequenced pel E and pel B. The EC16 pel genes occur in two clusters, one containing pel A and pel E and the second pel C and pel B. Within each cluster transcription appears to occur from independent promoters but from the same sense strand. The pel C and pel B genes encode highly homologous proteins sharing 84% amino acid homology and similar pI values of 9.0 and 8.8, respectively. The pel A and pel E genes encode more diverged proteins, with 61% amino acid homology and pI values of 4.6 and 9.8, respectively. Two conserved regions occur in the four pel gene products, separated from each other by about 65 amino acids. These regions may play a role in catalysis or calcium binding.

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MOLECULAR EPIDEMIOLOGY OF XANTHOMONAS CAMPESTRIS PV. PHASEOLI AND X. C. PHASEOLI VAR. FUSCANS. R. L. Gilbertson, S. A. Leong, D. J. Hagedorn, and D. P. Maxwell, Department of Plant Pathology, University of Wisconsin-Madison.

Molecular hybridization techniques were used to differentiate Xanthomonas campestris pv. phaseoli (Xcp) from xanthomonads isolated from bean debris that were avirulent on beans, and other X. campestris pvs. Avirulent isolates were distinguished from Xcp by absence of a plasmid found in Xcp, by presence of different restriction enzyme digestion patterns of total genomic DNA, and by different restriction fragment length polymorphisms (RFLPs) detected by hybridization with random Xcp genomic DNA probes. Xcp, X. c. phaseoli var. fuscans (Xcpf), and other X. campestris pvs. had different total genomic DNA restriction patterns, and some Xcp genomic DNA probes demonstrated different RFLPs. The Xcp plasmid was used as a DNA probe to differentiate Xcp from avirulent isolates. Xcpf had a different plasmid than Xcp, but both plasmids shared regions of homology. Genetic conservation of plasmid DNA in Xcp and Xcpf might allow for development of a pathovar phaseoli-specific DNA probe.

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FUSIONS TO A PROMOTERLESS ICE NUCLEATION GENE: A SENSITIVE TOOL FOR MEASURING BACTERIAL GENE EXPRESSION IN VITRO AND IN PLANTA. P. B. Lindgren, R. Frederick, N. J. Panopoulos, S. E. Lindow, and B. J. Staskawicz. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

We have constructed transposable elements capable of generating transcriptional fusions with a promoterless ice nucleation gene (inaZ). The utility of such fusions in the study of bacterial gene expression in culture and in planta was established by quantitative measurements of ice nucleation activity of virB::inaZ fusions in A. tumefaciens and hrp::inaZ fusions in Pseudomonas syringae phaseolicola. A log-linear relationship between the ice nucleation activity and the amount of ice protein detected by Western blotting with an anti-ice protein antibody was established (r=0.94). The ice nucleation reporter gene system provides a convenient inexpensive and very sensitive method for measuring bacterial gene activity in planta with very low inoculum levels normally encountered in natural situations.

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SEQUENCE ANALYSIS OF AN AVIRULENCE GENE FROM PSEUDOMONAS SYRINGAE PATHOVAR TOMATO. D. Kobayashi, S. Tamaki and N. T. Keen, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Three classes of avirulence genes were previously cloned from Pseudomonas syringae pv. tomato which, when introduced into P. syringae pv. glycinea, elicited the hypersensitive response on soybean. One of the original clones, designated as avrD, was subcloned as a 5.6 Kb HindIII fragment. Sequencing of this fragment disclosed the presence of four open reading frames (ORF) on the same sense strand, each of approximately 1 Kb in size. Three of the ORFs were closely clustered and occurred about 1 KB 3' from the end of the first ORF. Transposon insertions which mutated the avirulence phenotype of the original cosmid clone all mapped to the first ORF. However, subcloning experiments have suggested that one or more of the other three ORFs in the 5.6 Kb HindIII fragment may be required for the avirulence phenotype.

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CHARACTERIZATION OF HR$^-$ TN5 MUTANTS OF PSEUDOMONAS SYRINGAE PV. SYRINGAE UNABLE TO INDUCE RESISTANCE IN CUCUMBER.

J. A. Smith, L. W. Fulbright and R. Hammerschmidt. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The hypersensitive response (HR) caused by Pseudomonas syringae pv. syringae (wheat isolate) on cucumber results in the systemic induction of peroxidase activity and resistance more rapidly than the cucumber pathogen Pseudomonas syringae pv. lachrymans. TN5 mutants of P. syringae pv. syringae were

generated by conjugal transfer of the suicide plasmid vector pSUP1011. Two HR⁻ mutants were obtained after screening approximately 2000 potential mutants for HR inducing ability in cucumber leaves. Loss of the ability to induce a hypersensitive response resulted in the loss of ability to cause disease on wheat or induce systemic resistance in cucumber. In both mutants TN5 had inserted into a high molecular weight Eco R1 fragment of genomic DNA.

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CONSTRUCTION AND ANALYSIS OF AN ERWINIA CHRYSANTHEMI MUTANT CONTAINING DELETIONS IN THE PEL GENES ENCODING ALL OF THE MAJOR PECTATE LYASE ISOZYMES. J.L. Ried and A. Collmer. Dept. of Botany, Univ. of Maryland, College Park, MD 20742.

Strain EC16 of the soft-rot pathogen, E. chrysanthemi, produces four extracellular isozymes of pectate lyase (PL). The pelB and pelC genes, encoding PLb and PLc, were deleted from the chromosome by marker exchange-eviction mutagenesis. An nptI sacB sacR cartridge, encoding kanamycin resistance and sucrose sensitivity, was inserted in a cloned E. chrysanthemi DNA fragment in place of the pelB and pelC genes. The marked deletion was introduced into the chromosome by exchange recombination and then evicted by a second recombinational exchange with an unmarked deletion derivative. The pelA and pelE genes were subsequently deleted by similar methods, resulting in a mutant deficient in PLa, PLb, PLc, and PLe and producing less than 0.1% of the extracellular PL activity of the wild type. However, the mutant was able to utilize polygalacturonic acid as a sole carbon source and caused maceration of potato tuber, carrot, and pepper tissues.

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POLYSACCHARIDES IN INTERCELLULAR WASH FLUIDS FROM RESISTANT AND SUSCEPTIBLE COTTON COTYLEDONS INOCULATED WITH *XANTHOMONAS CAMPESTRIS PV. MALVACEARUM*. M. Pierce, M. Essenberg and A. Mort. Oklahoma State Univ., Dept. of Biochemistry, Okla. Agricultural Expt. Station, Stillwater, OK 74078.

Analysis of polysaccharides washed out with water from intercellular spaces of infected cotyledons provides information on two fronts: 1) determination of whether exopolysaccharide (EPS) production by the bacteria is affected differently in a susceptible versus a resistant host and 2) determination of any alterations in the host's cell wall polysaccharides that are easily extracted. The congenic cotton lines WbM 4.0 (bacterial-blight susceptible) and WbM 0.0 (highly resistant) were used. During growth *in plant*, the amount of EPS per bacterial cell was similar in both lines. However, the levels of arabinose- and galactose-containing polymers increased and those of galacturonic acid-containing ones decreased early during the incompatible interaction, but not during the compatible interaction. These differences observed in the resistant line would be expected from the digestion of the galacturonic acid-rich regions of the host's pectin. Differential induction of pectinases may explain the results.

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PHYSIOLOGICAL RESPONSES OF SOYBEAN TO BROWN STEM ROT. P. M. Higley, C. A. Martinson, and H. Tachibana. Dept. of Plant Pathology, Iowa State University, Ames IA 50011-1020.

Water relations and growth parameters were evaluated in inoculated and uninoculated soybeans to determine the physiological effects of brown stem rot. A susceptible (Pride B216) and a resistant (BSR 201) cultivar were injection-inoculated with *Phialophora gregata* spores at vegetative stage 2. Disease decreased total leaf area and number of branches, nodes, leaves, and pods. The number of flowers was unaffected by disease. Transpiration rate and stomatal conductances were doubled in inoculated plants compared with uninoculated plants. Disease reduced stem conductances 83% and 36% in Pride B216 and BSR 201 plants, respectively. Leaf water potential was lower in inoculated Pride B216 plants than in uninoculated Pride B216 and inoculated and uninoculated BSR 201 plants. Disease severity was 83% in the inoculated Pride B216 and 66% in the inoculated BSR 201 plants. Yield reduction may result from xylem dysfunction and disruption of stomatal regulation leading to decreased production of branches, leaves, and pods.

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EARLY RESPONSES OF COWPEA TO INOCULATION WITH RHIZOBIUM FREDII 257. A.T. Trese and S.G. Pueppke, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Plant genes determining specificity in legume-rhizobium symbioses are likely to be expressed early in the sequence of nodule initiation and development. We have chosen the combination of cowpea and R. fredii 257 because the plant shows a very strong, early response to inoculation. Within 2 days numerous centers of cell division develop in the cortex, densely clustered around a point occupied by the root tip at the time of inoculation. After 4 days many of these centers have become minute protrusions on the root surface. To identify changes in gene expression in this tissue, seedlings were harvested 3.5 days after inoculation, and 3 cm. segments were taken from the most responsive region of the primary roots. The lateral roots and stele were removed, and mRNA was extracted. After in vitro translation the products were analysed by SDS-PAGE. Proteins of approximate MWs 50, 32, 24, 19, and 18.5K increased upon inoculation, while proteins of 22 and 21K decreased. Further analyses by 2-D gels is in progress, as well as construction of a cDNA library.

255. Withdrawn

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MOLECULAR CLONING OF CHITINASE GENES FROM SERRATIA MARCESCENS. L. Sundheim, A. R. Poplawsky, and A. H. Ellingboe. Agricultural University of Norway, 1432 As-NLH, Norway, and Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Chromosomal DNA from Serratia marcescens was partially digested with EcoRI to yield 15-25 kb fragments. A genomic library was prepared in the cosmid pLAFR3. Of 5600 clones, 21 expressed chitinase activity as indicated by clearing of a chitin-containing medium. The chitinase positive clones were of two classes, as shown by subcloning in pLAFR3 and pBR325. The chitinase activity was encoded by a 9.4 kb EcoRI fragment in 17 clones and by an 18 kb fragment in 4 clones. Triparental matings with the helper plasmid pRK2013 and fluorescent Pseudomonas spp. yielded transconjugants which expressed chitinase activity and inhibited hyphal growth of Rhizoctonia solani and Magnaporthe grisea.

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PHYSIOLOGY OF PEANUT GREEN MOSAIC DISEASE SUPPRESSION BY BAVISTIN. D.V.R.Sai Gopal, V. Siva Prasad, T. Satyanarayana, K. Gopinath, P. Sreenivasulu and M.V. Nayudu, Department of Botany, S.V. University, Tirupati-517 502, India.

Systemic benzimidazole fungicides, Bavistin and Benomyl, suppress the symptoms of certain viral diseases [Tomlinson, 1982, In: K.F. Harris and K. Maramorosch(eds,) Pathogens, Vectors, and Plant Diseases: Approaches to Control, 23-42]. Bavistin (50% w/w Carbendazim) when applied as foliar spray (0.3% and 0.5%) suppressed the disease symtoms due to peanut green mosaic virus, a potyvirus from India (Sreenivasulu et al., 1981, Ann. Appl. Biol., 98: 255-260), in sap inoculated peanut. Physiological parameters which could contribute to the symptoms masking include increased delta-amino levulinic acid, chlorophyll, lipids, increased incorporation of C^{14} from C^{14}-glutamic acid into chlorophylls and of ^{14}C from C^{14}-acetate into lipids, decreased proline and decreased rate of senescence.

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PHYTOTOXIC SUBSTANCES PRODUCED BY CERTAIN ISOLATES OF CERCOSPORA ARACHIDICOLA ARE NOT CERCOSPORIN. S. A. Fore, M. E. Daub, and M. K. Beute, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Cercosporin is a red, light-activated toxin produced by Cercospora spp. It has been isolated from infected leaf tissue of several hosts at levels of 1.8-10.1 µg/gm tissue. We investigated the production of cercosporin by C. arachidicola, causal agent of peanut early leaf spot. No evidence was obtained for cercosporin production in vivo or in vitro by C. arachidicola. At a detection limit of 75 ng/gm, cercosporin was not found in infected peanut leaves or lesions. Although three of four isolates of C. arachidicola produced a red pigment in culture, this pigment was not cercosporin. Culture extracts of the red pigment-producing isolates were phytotoxic, but toxicity was not light-activated. Two major bands, with absorption maxima

at 465 and 435nm, were separated from culture filtrates by TLC. The identity and possible role of the phytotoxic substances isolated from C. arachidicola are not known.

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VIRULENCE ASSOCIATED PEPTIDES AND POLY(A) RNAs OF CRYPHONECTRIA PARASITICA. C. P. Carpenter, P. J. Kazmierczak and N. K. Van Alfen. Utah State University, UMC 4500, Logan, UT. 84321.

Cryphonectria parasitica is a fungal pathogen that causes chestnut blight. Isogenic strains of C. parasitica are virulent or hypovirulent, correlating with the absence or presence of vesicle encapsulated dsRNA, respectively. Virulent C. parasitica produces large amounts of several polypeptides and poly(A) RNAs not present in hypovirulent fungi. We have isolated a virulence associated protein which comprises more than 1% of the dry weight and is excreted into growth media. The minimal molecular weight of the polypeptide from SDS-PAGE is 24K, corresponding to the expected size of a translational product of differentially expressed poly(A) RNA of virulent strains of this fungus. Antibodies against this protein will be tested for reaction with in vitro translation products of the differentially expressed poly(A) RNAs.

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INDUCED AUXOTROPHIC AND NON-SCLEROTIAL ISOLATES OF SCLEROTINIA SCLEROTIORUM. R. V. Miller, E. J. Ford and D. C. Sands, Department of Plant Pathology, Montana State University, Bozeman, MT 59717-0002.

Auxotrophic and non-sclerotial mutants of Sclerotinia sclerotiorum were isolated after exposure of ascospores to ultraviolet light or treatment with N-methyl-N'-nitro-N-nitrosoguanidine. Pathogenicity of all of the mutants was assayed on eight hosts. The nutritional requirements of the auxotrophs were determined by auxanography. In some cases virulence to the auxotrophs required application of an external source of the appropriate nutrient concurrent with inoculation. Both types of mutants may provide a method of limiting broad host-range pathogens to the area of application for use as biological control agents.

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ANTIBIOTIC SPECTRA OF FLUORESCENT PSEUDOMONADS ISOLATED FROM THE POTATO RHIZOSPHERE AND RELATIONSHIP TO DISEASE SUPPRESSION. D. J. Rhodes and D. C. Gross, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Inhibition of Geotrichum and eight potato pathogens on potato-dextrose agar (PDA) was used to resolve 136 rhizosphere-associated fluorescent Pseudomonas strains into approximately 25 antibiosis groups. Sensitivity to antibiotics ranged from Clavibacter michiganense subsp. sepedonicum (inhibited by 133 strains) to Fusarium sambucinum (18 strains). The four strains which inhibited all of the fungi tested (Group 4) suppressed decay of tuber slices caused by Pythium ultimum and F. sambucinum. Tuber yield in Pythium-infested field plots was increased over 18% by two strains in Group 4, one of which was a vigorous root colonist and produced a black diffusible pigment on PDA. Six similar pigment-producing strains were detected among 735 isolates from potato root surfaces in the Columbia Basin. These typically possessed wide-spectrum antifungal activity and were inhibitory to fungal decay of tuber tissue.

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PRODUCTION OF A VOLATILE ANTIBIOTIC BY ENTEROBACTER CLOACAE AND ITS POSSIBLE ROLE IN THE BIOLOGICAL CONTROL OF PATHOGENIC FUNGI BY THE BACTERIUM. C. R. Howell and R. D. Stipanovic. USDA, ARS, SCRL, P. O. Drawer JF, College Station, TX 77841.

Isolates of Enterobacter cloacae reported to be biological control agents of seedling diseases were found to symetrically inhibit fungal growth when grown in dual cultures on media lacking sugar. Inhibition of fungal growth in dual cultures with E. cloacae on partitioned plates indicated that the inhibitor was volatile. The addition of glucose to the growth medium completely suppressed inhibitor production by the bacterium. Fractional distillation of bacterial cultures and low temperature trapping of released volatiles resulted in the capture of a volatile fraction in aqueous solution that inhibited fungal growth when added to fresh media. Gas chromatography of the active fraction indicated the presence of one major peak. Isolation and chemical and biological characterization of this inhibitor is underway.

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MECHANISMS DETERMINING SENSITIVITY TO THE ANTIBIOTIC GLIOTOXIN. R. W. Jones and J. G. Hancock, Dept. of Plant Pathology, University of California, Berkeley, 94720.

Gliotoxin (mw 326) is a neutral, hydrophobic antibiotic, produced by many fungi, whose toxicity is based upon a bridged polysulfide region. Fungistatic concentrations differ three-fold between Pythium ultimum (9.3µg/mg dry wt) and Rhizoctonia solani AG4 (27.0). Both fungi showed rapid, concentration-dependent binding of 14C gliotoxin, but P. ultimum bound >2-fold more than R. solani. Sulfhydryl reagents inhibited 14C gliotoxin binding; conversely, gliotoxin inhibited binding of 3H iodoacetic acid. Intracellular glutathione levels could not account for differences in gliotoxin sensitivity. Hypersensitivity of "deep rough" Salmonella mutants to gliotoxin suggest a rate-limiting movement through the cell wall followed by reaction with membrane sulfhydryl groups.

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IDENTIFICATION AND CHARACTERIZATION OF A METABOLITE PRODUCED BY TALAROMYCES FLAVUS WHICH MEDIATES BIOCONTROL OF VERTICILLIUM DAHLIAE. K. K. Kim, D. R. Fravel, and G. C. Papavizas, USDA, ARS, Beltsville, MD 20705.

Talaromyces flavus produced a metabolite in culture filtrates which killed microsclerotia of Verticillium dahliae in vitro and in soil. The metabolite was isolated, identified, and selected properties were studied. The semipurified metabolite alone did not inhibit germination of microsclerotia of V. dahliae and inhibition of germination was restored in the presence of glucose. The interaction was optimum at pH 5.0. The molecular weight of the metabolite was estimated to be 200,000 daltons. The bioactivity profile of the metabolite on HPLC was coincident only with glucose oxidase activity. Therefore, the metabolite is assumed to be glucose oxidase. The actual inhibitor of the pathogen may be hydrogen peroxide which is a product of the reaction catalyzed by glucose oxidase.

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PARTIAL CHARACTERIZATION OF COMPOUNDS PRODUCED BY PSEUDOMONAS FLUORESCENS AND BACILLUS LICHENIFORMIS ANTAGONISTIC TO PYRENOPHORA TRITICI-REPENTIS, THE CAUSE OF WHEAT TAN SPOT. F. Mehdizadegan and F. J. Gough, Plant Pathology Dept., Oklahoma State Univ., and USDA-ARS, P.O. Box 1029, Stillwater, OK 74076.

Concentrated cell-free filtrates of King's B and nutrient broth media from cultures of P. fluorescens and B. licheniformis, respectively, were diluted in potassium phosphate buffer (0.05 M, pH 7.0) and divided into two aliquots. One aliquot was dialyzed against the buffer and one was ultrafiltered. Samples collected successively at approximate MW cut-offs of 50,000, 30,000, 10,000, 5,000, and 1,000 were tested for activity against P. tritici-repentis. Active materials were placed on Sephadex (G 50, G 25) columns (100 x 2.5 cm) and eluted with phosphate buffer (pH 7.0) at the rate of 0.5 ml/min at room temperature. Results of bioassay tests with the various fractions indicated that the P. fluorescens and B. licheniformis compounds have molecular weights <1,500 and about 32,000, respectively.

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BACTERIAL ANTAGONISM TO SEPTORIA TRITICI, CAUSAL AGENT OF SEPTORIA TRITICI BLOTCH. F. Mehdizadegan and F. J. Gough, Plant Pathology Dept., Oklahoma State Univ., and USDA-ARS, P.O. Box 1029, Stillwater, OK 74076.

Four bacterial antagonists to Septoria tritici were isolated from soil of wheat fields and from wheat leaves. Two bacteria isolated from soil were identified as Bacillus subtlis and B. pumilus, and two isolated from leaves were identified as B. subtlis and Pseudomonas fluorescens. All four bacteria and their cell-free culture filtrates inhibited growth on V-8 agar medium. Application of these antagonists to wheat seedlings in replicated greenhouse tests significantly (P=0.05) reduced the number of lesions per gram of leaf tissue. Spraying the Bacillus sp. onto flag leaves of wheat in the field significantly reduced the number of lesions that developed as compared with the numbers in plots sprayed with fresh sterile medium and with sterile tap water.

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CRICONEMELLA XENOPLAX POPULATIONS AS AFFECTED BY NONHOSTS INTERPLANTED WITH PEACH. D. P. Whittington and E. I. Zehr, Department of Plant Pathology and Physiology, and W. C.

Bridges, Department of Experimental Statistics, Clemson University, Clemson, SC 29634

The potential for poor or nonhost plants to control Criconemella xenoplax in peach was investigated. Selected plants were interplanted in field microplots containing peach trees with established C. xenoplax populations. Soil in each microplot was sampled monthly and populations per 100 cm^3 soil were determined. Similar interplantings were studied for 3 mo in the greenhouse to determine their effect upon increasing nematode populations. No plants suppressed established populations in the field or significantly delayed population increases. Lower populations sometimes were observed with buckhorn plantain, 'Janie Flame' marigold, sicklepod, and goosegrass. Centipedegrass resulted in significantly higher populations at some sampling dates.

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ATTEMPTS OF BIOCONTROL OF FUSARIUM WILT OF CARNATION IN ITALY. A. Garibaldi and M.L. Gullino, Istituto di Patologia vegetale, Via Giuria, 15, 10126 Torino - Italy.

Saprophytic Fusaria, isolated from Fusarium-suppressive soils, have been tested during the last 5 years in glasshouse experiments in order to reduce soil colonization by Fusarium oxysporum f.sp. dianthi. Soil infestation with high amount of antagonists inoculum (100/200 g of inoculated wheat kernels/m^2) effectively reduced soil colonization and plant infection by F. dianthi. Inoculation of carnations by dipping roots in a conidial suspension (10^7 conidia/ml) of the antagonists at transplanting protected plants about as well as soil inoculation. This method of application is simple and could be applied in the practice. The effectiveness of the antagonistic Fusaria can be improved by combining chemical (benomyl, 20 g/m^2) with biological measures. Competition for nutrients between F.dianthi and saprophytic Fusaria might explain soil suppressiveness.

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BIOCONTROL OF RHIZOCTONIA SOLANI IN TALL FESCUE TURFGRASS E.M. Sutker, and L.T. Lucas, Dept. of Plant Pathology, North Carolina State University, Raleigh, 27695.

Isolates of Rhizoctonia zeae and binucleate Rhizoctonia-like fungi with low virulence and an isolate of Laetisaria arvalis from turfgrass were studied as potential agents for biocontrol of brown patch caused by R. solani. In field plots, two year old KY31 tall fescue was inoculated with biocontrol agents one week prior to inoculation with R. solani. A total of 5 isolates, 3 appropriate controls and 4 replications were used; the experiment was repeated. Typical R. solani leaf lesions were evident in all treatments. Biocontrol isolates and R. solani were reisolated from leaves. There were significant differences in percent disease of live leaf clippings among treatments after one month. No treatment differences were evident by visual inspection of the turfgrass stand. Isolates in order of decreasing effectiveness were L. arvalis, R. zeae, and binucleate R.-like fungi. Thus, turfgrass isolates of L. arvalis and Rhizoctonia spp. have the potential for biocontrol of brown patch caused by R. solani.

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EFFECTS OF PLANT AGE AND PHOTOPERIOD ON YELLOW NUTSEDGE INOCULATED WITH PUCCINIA CANALICULATA IN THE GREENHOUSE. W. M. Dowler, W. L. Bruckart, and N. R. Huckle. USDA-FDWSRU, Ft. Detrick, Bldg. 1301, Frederick, MD 21701.

Successful biological control of yellow nutsedge (Cyperus esculentus) by P. canaliculata (P. c.) in field studies probably requires increase in disease development following early-season application of the fungus. In greenhouse studies, mean tuber biomass (number of tubers x weight/tuber) for plants inoculated once with P. c. 6 or 10 wk after planting was 18.2 and 15.2 g/pot, respectively, compared with 19.5 g from the controls. Inoculation of C. esculentus on a weekly basis beginning 4 wk after planting resulted in mean biomass values of 10.8, 8.1, 6.9, 5.9 and 5.9 g for 0, 1, 2, 3, and 4 inoculations, respectively. Reduction in tuber biomass from treatment with P. c. occurred under a 16-hr and not under a 13-hr photoperiod. These results suggest that plant age at the time of inoculation is an important factor in biocontrol of nutsedge.

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BENTAZON TREATMENT OF YELLOW NUTSEDGE REDUCES THE RATE OF DISEASE DEVELOPMENT BY PUCCINIA CANALICULATA IN THE FIELD. W. L. Bruckart, J. Ray Frank, and D. R. Johnson. USDA-FDWSRU, Ft. Detrick, Bldg. 1301, Frederick, MD 21701.

Sublethal rates of bentazon herbicide were found to reduce rate of disease development by P. canaliculata in field research to evaluate biological control of yellow nutsedge (Cyperus esculentus) in Maryland. Inoculation with P. canaliculata (0 and 70 g/ha urediniospores) was made in mid-June in each of two years either one week before or at the time of treatment with 0.0, 0.3, or 0.6 kg ai/ha bentazon. The experiment was a 2 x 3 factorial that included five replications. Percent disease was determined every other week throughout the growing season. Treatment with bentazon at 0.3 and 0.6 kg/ha resulted in 50 and 54% less disease, respectively, by mid-August in plots treated with P. canaliculata than in plots without bentazon. Higher concentrations of this herbicide may interfere with biocontrol of C. esculentus by P. canaliculata.

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METHODS AND MEDIA FOR DETERMINING MYCELIAL REACTION ZONES IN LECUOSTOMA CINCTA AND LEUCOSTOMA PERSOONII. S. A. Hammar, G.C. Adams. Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Isolates of Leucostoma cincta and Leucostoma persoonii were collected from cankers on peach. These isolates were paired on various media to determine the best media for the development of mycelial interaction zones (barrages) characteristic of vegetative incompatibility reactions. The media tested were Difco PDA, acidified Difco PDA, Endothia complete agar, Leonians agar, Difco cornmeal agar, Difco oatmeal agar, V-8 juice agar, malt agar, and Neurospora synthetic crossing medium. In contrast to Leucostoma kunzei, conditioning cultures by transfer to water agar prior to pairing did not affect the barrage reactions. Paired incompatible isolates consistantly formed dark barrages only on Difco oatmeal agar. Interaction lines were most distinct when plates were incubated in the dark. Pairings among isolates from an orchard segregated into several interaction groups.

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PRODUCTION AND REGENERATION OF ALTERNARIA CRASSA PROTOPLASTS. N.L. Brooker, and D.O. TeBeest, University of Arkansas, Dept. of Plant Pathology, Fayetteville, AR 72701.

Protoplasts were prepared from mycelia of Alternaria crassa using commercially available enzyme (Novozyme 234). Conditions for the production and regeneration of stable protoplasts were influenced by fungal age, incubation time, and by the osmotic stabilizer used in both production and regeneration. Optimal regeneration rates (68%) were obtained by treating 12-h-old hyphal tips with Novozyme 234 for 2 h. 1.2 M KCl was the optimal osmotic stabilizer for the production of protoplasts (4.9 X 10^6) while 1.2 M $MgSO_4$ was the optimal osmotic stabilizer for the regeneration of protoplasts. Protoplasts generated from multinucleate hyphal tips varied in size, and regeneration rates were inversely related to the protoplast generation time. Mercaptoethanol pretreatment of hyphae reduced both production and regeneration of protoplasts. Protoplasts can be used in transformation studies of this biocontrol fungus.

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STUDIES ON GREEN RING MOTTLE VIRUS. K. Zagula Haufler, N.M. Aref and D.C. Ramsdell, Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing.

Green ring mottle virus (GRMV) causes an important disease of Prunus species. Sweet cherry and peach are symptomless carriers of GRMV, which at present is detected in these hosts only by indexing budwood onto flowering cherry. In our studies, infected cherry and peach leaves yielded a dsRNA of 5.0 X 10^6 M_r in agarose gels. GRMV was partially purified by extraction in 0.5M Tris buffer, pH 8.2, containing 4% polyvinylpolypyrrolidone, 0.5% bentonite, 0.2% β-mercaptoethanol and 5% Triton X-100, followed by chloroform clarification and two cycles of polyethylene glycol precipitation. Further purification was achieved by cesium sulfate step gradient ultracentrifugation. Purified particles were 10 nm X 800-900 nm, with $A_{260/280}$ = 1.57. Using double antibody sandwich ELISA, polyclonal antisera were able to detect GRMV in 9/11 2-yr-old 'Montmorency' cherry trees previously slash-inoculated with GRMV and in 'Kwanzen' cherry trees grafted with infected budwood obtained from P. Fridlund.

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A VIRUS ASSOCIATED WITH A NEW BLIGHT DISEASE OF HIGHBUSH BLUEBERRY. P. R. Bristow, Washington State University, WWREC,

Puyallup, 98371 and R. R. Martin, Agriculture Canada, 6660 NW Marine Dr., Vancouver, British Columbia, V6T 1X2.

Blighting of blossoms and of new vegetative growth were the primary symptoms of a previously undescribed disease of highbush blueberry (Vaccinium corymbosum) in Washington. A second flush of leaves was usually produced and affected plants appeared nearly normal by late-summer but there was little or no fruit. Plants with symptoms one year were sometimes symptomless the next year, but the vigor of new growth was reduced. The cultivars 'Berkeley', 'Blueray', 'Collins', 'Herbert' and 'Pemberton' are particularly susceptible. Blight symptoms have been graft transmitted from infected to healthy plants. Bundles of virus-like particles in leaf tissue were associated consistently with infected plants and were present in plants after grafting. Purified particles were about 700 nm long by 14 nm in diameters, had a coat protein of 33,000 daltons and a RNA about 3.5 x 10^6 daltons.

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PURIFICATION AND SEROLOGY OF FLEXUOUS ROD PARTICLES ASSOCIATED WITH SHEEP PEN HILL DISEASE OF HIGHBUSH BLUEBERRIES. E.V. Podleckis and R.F. Davis, Dept. Plant Pathol., Cook College, N.J. Agric. Exp. Sta., Rutgers Univ., New Brunswick, NJ 08903.

Previously we reported flexous rod viral-like particles in extracts and ultrathin tissue sections of highbush blueberries with symptoms of Sheep Pen Hill Disease (Phytopathology 76:1065). The flexous rod particles were purified from blueberry leaves using a red ringspot virus purification scheme (Phytopathology 71:673). Frozen infected tissue was ground in 0.5 M phosphate buffer containing 0.75% sodium sulfite and the filtered homogenate clarified by adding 1 M urea and 2.5% Triton X-100. Two cycles of differential centrifugation produced partially pure preparations that were further purified by density gradient centrifugation. Density gradient purified virus was injected into six-week-old Swiss Webster mice to produce polyclonal ascites that reacted with purified virus in indirect ELISA and decorated only homologous virus particles in immunosorbent electron microscopy tests.

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MULTIPLE VIRUS RESISTANT YELLOW WAX CAPSICUMS, B. Villalon, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, Texas 78596.

Yellow wax pepper, one of about 20 cultivated Capsicum annuum L. types, has for many years been associated with the pickled pepper industry. The domestic market for pickled and canned peppers has grown rapidly during the past 15 years. Increased demand for the yellow wax, mildly pungent pepper has stimulated production in Texas. Most commercial yellow wax peppers are susceptible to viruses. The Texas Agricultural Experiment Station at Weslaco has developed several new yellow wax, multiple virus-resistant pepper breeding lines with different levels of pungency. Genetic resistance to tobacco etch virus, potato virus Y, pepper mottle virus, and 'Caloro', 'Hungarian Yellow Wax', 'Gold Spike', 'Cascabella', 'Pepperoncini' and 'Tabasco' types.

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RESISTANCE IN PEPPER TO TOBACCO ETCH VIRUS. C. W. Kuhn, G. B. Padgett, and F. W. Nutter, Jr., Dept. of Plant Pathology, University of Georgia, Athens 30602.

Three levels of resistance to tobacco etch virus (TEV) were identified in pepper genotypes. Resistance was evaluated by comparing symptom severity, incubation period, and virus concentration in 16 genotypes to the reaction in susceptible cultivar Yolo Wonder B. Moderate resistance, as in cultivar Tambel 2, was characterized by mosaic and mild stunting, a long incubation period, and low virus concentration for 2 to 3 wk after inoculation. Resistant genotypes, such as Florida breeding line XVR 3-25, were difficult to infect by mechanical inoculation; however, about 35% of the plants developed symptoms under field conditions. Two University of Georgia breeding lines, C44-NV and C44-CA, were extremely resistant. No infection occurred by mechanical inoculation, and less than 10% of the plants developed a mild mottle (no stunting) in the field. We conclude that resistance to TEV in pepper can be attributed to inability of the virus to infect plants and slow virus accumulation.

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OCCURRENCE OF WATERMELON MOSAIC VIRUS IN KUWAIT. Ali M. Jafri, H.F. Sharif, and K.H. Abu-Teen, Department of Botany and Micro-biology, Kuwait University, P.O. Box 5969, Safat, Kuwait.

Watermelon Mosaic Virus is wide-spread and endemic in Kuwait causing a mosaic disease of watermelon crops (Citrus vulgaris) in the field. Symptomatologically, only common mosaic type symptoms were observed in watermelon. Virus was transmitted by sap inoculation from watermelon to watermelon as well as to some other hosts. Cucurbita pepo, Cucumis sativus, C. melo, Daturastramonium, and Nicotiana rustica developed systemic infections after inoculation, no local lesion hosts were found in our trials. Electron microscopic observations revealed flexuous rods in dips and pin-wheel, scroll and laminated inclusions in ultra-thin sections. Host range, enzyme-linked immunosorbent assays (ELISA) and electron microscopic studies suggested that this virus was Watermelon Mosaic Virus (Type 2) and distantly related to Potato Virus Y (PVY).

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TOMATO FERN LEAF MOSAIC VIRUS AFFECTING TOMATO CROP IN KUWAIT. Ali M. Jafri, K.H. Abu-Teen and H.F. Sharif, Department of Botany and Microbiology, Kuwait University, Kuwait.

A virus causing a mosaic type disease on tomato is wide-spread in Kuwait. Based upon symptoms, we have named it the Tomato Fern Leaf Mosaic Disease. The virus predominantly causes typical mosaic symptoms accompanied by intermittent yellowing of interveinal leaf areas. In the later stages of infection, younger leaves become distorted with a "Fern-leaf" type appearance. The virus was transmitted by sap inoculation from tomato to several herbaceous hosts. Arachis hypogea, Nicotiana benthamina, Physalis floridana, and several cultivars of Lycopersicon esculentum (tomato) developed systemic infections after inoculation, while Chenopodium amaranticolor, C. quinoa, Datura stramonium, D. metel, Nicotiana tabacum, N. glutinosa, N. rustica and Solanum magic ball produced necrotic local lesions. Electron microscopy, agar gel double diffusion, and host range studies suggest that the tomato fern leaf mosaic disease in Kuwait is caused by a strain of tomato mosaic virus.

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SEROLOGICAL RELATIONSHIPS OF THREE PROTEINS OF PAPAYA RINGSPOT VIRUS TYPE W (PRSV-W) TO ANTIGENS OF ZUCCHINI YELLOW FLECK VIRUS (ZYFV). C. A. Baker and D. E. Purcifull, Dept. of Plant Pathology, University of Florida, Gainesville 32611.

Rabbit polyclonal antisera were prepared to SDS-PAGE purified capsid and cylindrical inclusion (CI) proteins of PRSV-W. In SDS-immunodiffusion tests, these antisera reacted with antigens from PRSV-infected leaves but not with sap from healthy plants. Sap from ZYFV-infected leaves (Phytopath. Medit. 20:123-128) cross-reacted with both antisera. Antisera to the amorphous inclusion (AI) protein of PRSV-W (Virology 142:34-43) also reacted with sap from leaves infected with ZYFV. Using immunofluorescent techniques, the CI and AI antisera reacted with corresponding inclusions in epidermal strips of tissue infected with either PRSV-W or ZYFV. These results indicate that the structural and two nonstructural proteins of PRSV-W and ZYFV are antigenically related.

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A SURVEY OF PROCESSING AND FRESH MARKET TOMATOES IN OHIO FOR THE PRESENCE OF TOBACCO MOSAIC VIRUS USING VIRAL-ASSOCIATED dsRNA ANALYSIS. S. T. Nameth and R. M. Riedel, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

Tomato fields from seven counties in Ohio were sampled and analyzed for the presence of tobacco mosaic virus (TMV). The method of virus detection was viral-associated double-stranded RNA (dsRNA) analysis. Fifty plants were sampled from each field surveyed. Sample size ranged from 4-7 grams of plant tissue. Plant samples were ground to a fine powder in liquid nitrogen and the dsRNA was purified by phenol extraction and cellulose column chromatography. Purified dsRNA was electrophoresed on a 5% polyacrylamide vertical mini gel. Up to eighty samples could be electrophoresed at one time. In all cases samples expressing symptoms of TMV infection were confirmed to be positive for the presence of TMV associated dsRNA (replicative form ~4.2 x 10^6 M.W.). Depending on sample size and time of sampling sub-genomic dsRNAs were also observed. Results indicate the validity of using viral-associated dsRNA analysis as a means of virus detection in a large scale disease survey.

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EFFECT OF TEMPERATURE ON ADULT-PLANT LEAF RUST RESISTANCE IN WHEAT. K. Kaul, and G. Shaner. Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Effect of temperature on eleven wheat breeding lines and on the newly released cultivar Compton was studied to determine the range within which the adult-plant leaf rust resistance of these lines was effective. Morocco, a susceptible cultivar was the check. Infection types (IT) were observed at temperatures from 15/12 C to 30/21 C (day/night). Morocco was consistently susceptible (IT 3+). Major differences in infection types were observed at both extremes in all but three lines: P75438, P76603, and P69195, which showed IT 0 or 0c at all temperatures. None of the lines showed susceptible types (IT 3+) at the lower extreme of 15/12 C, whereas at least a few IT 3+ were observed at the upper extreme of 30/21 C in all temperature sensitive lines. P77121 showed a sudden loss of resistance at high temperature. Coker 79, P751909, P76905, and P76779 showed a gradual shift to higher infection types as temperature increased. P751915 remained resistant only over a narrow range. P74140-6 and 7 showed a higher IT at both low and high temperatures. Cultivar Compton showed a few IT 3+ at 30/21 C when grown continuously at that temperature, but not when reared at a lower temperature until inoculation.

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CORRELATION BETWEEN LEAF AND NECK BLAST RESISTANCE IN RICE. J. M. Bonman, B. A. Estrada, and J. M. Bandong, International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Blast disease of rice, caused by Pyricularia oryzae, has two phases: leaf blast and neck blast. It is uncertain if vegetative stage resistance to leaf blast is correlated with resistance to neck blast. Twenty-nine lowland rice lines, representing a wide range of susceptibility to leaf blast, were tested in three upland nursery experiments using a mini-plot method. Neck blast resistance was measured in three lowland field plantings inoculated at heading with a mixed population of P. oryzae. Leaf blast resistance was expressed as the area under the disease progress curve (AUDPC). Mean AUDPC values from the nursery tests correlated with the percentage of severe neck blast recorded in the three field tests (r=0.63, 0.75, and 0.70) and with mean severe neck blast (r=0.82). Several lines that appear to be exceptions to the general relationship are being retested.

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POTENTIAL FOR RICE IMPROVEMENT THROUGH SOMACLONAL VARIATION FOR DISEASE RESISTANCE. Q.J. Xie, M.C. Rush, R. Massaquoi and J. Cao. Dept. Pl. Path. and Crop Physio., La. Agric. Expt. Sta., LSU Agric. Center., Louisiana State Univ., Baton Rouge, LA 70803.

Twenty-nine hundred T2 and T3 generation somaclonal lines from seven rice cultivars were grown in field tests in 1986. These lines were evaluated for variation including disease resistance. Ninety-three lines showed variation in blast (Pyricularia oryzae) resistance following natural infection and 38 lines showed variation in sheath blight (Rhizoctonia solani) resistance after inoculaton. Four somaclonal lines from the susceptible cultivar Labelle and one from the moderately susceptible cultivar Zenith appeared to be more resistant to sheath blight than the parent cultivars. They were evaluated for sheath blight resistance in greenhouse tests. The resistance of the four somaclonal lines was significantly greater than that of Labelle. The Zenith somaclone was more resistant than Zenith. The resistance of Labelle somaclonal line LR163-1 was equal to that of the highly resistant cultivars Tetep and Tadukan.

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NEW SOURCES OF RESISTANCE TO PUCCINIA HORDEI OTTH. IN BARLEY LAND RACE CULTIVARS. A. Yahyaoui and E. L. Sharp. ESAK, Boulifa, 7119 Le Kef, Tunisia and Plant Pathology Dept., Montana State University, Bozeman, MT 59717.

New genes for resistance to P. hordei appeared to be common in several collections of barley (Hordeum vulgare L.) land race cultivars originating in Central and Southern Tunisia. Response of five land race cultivars to a number of different isolates of P. hordei from the Mediterranean region differentiated them from the known genotypes. A dominant resistance gene that behaved as Pa_3 was found in Tu32. Three of the land race cultivars (Tu17, Tu27, and Tu34) each have a dominant resistance gene that is different from the previously known resistance genes. The dominant resistance genes identified in this study were as effective as Pa_3 and Pa_7, hence, should be considered as new sources of resistance. Further testing is needed to determine the genetic relationships between these genes.

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RELATIONSHIP OF QUANTITY OF EPICUTICULAR WAX TO RESISTANCE OF RICE TO SHEATH BLIGHT; R. C. Massaquoi and M. C. Rush; Dept. of Plant Path. and Crop Physio., La. Agric. Expt. Sta., LSU Agric. Ctr., Baton Rouge, Louisiana 70803.

Scanning electron microscope comparisons between resistant and susceptible cultivars suggested that the quantity of epicuticular wax on leaf blade and sheath surfaces may play a role in resistance of rice to sheath blight caused by Rhizoctonia solani. Gravimetric quantification of wax from cultivars with different resistant levels gave a significant positive correlation (r = .813) between wax concentration and resistance level. The resistant cultivars Tetep and Taducan averaged 65% and 48% more wax, respectively, than the susceptible cultivar Labelle. The number of infection cushions (IC) found on sheath surfaces was significantly higher in susceptible cultivars. A significant correlation (r = .987) was found between percent tissue diseased and IC no./sq.cm. The wax may prevent contact between the pathogen and compounds produced by rice that induce IC formation.

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THE ROLE OF DUVATRIENE-DIOLS IN THE SUSCEPTIBILITY OF BURLEY TOBACCO TO PERONOSPORA TABACINA. M. N. Rao, M. R. Siegel, M. Wiglesworth, R. S. Ferriss, and J. Kuć. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Greenhouse studies with tobacco (Ky-14) have indicated a relationship between 4,18,13 duvatriene-1,3-diols (DVT) and resistance to blue mold. However, studies with field-grown tobacco failed to demonstrate the existence of this relationship. It was found that 1) leaves dipped in acetone for 1 sec. to remove DVT are more susceptible than non-dipped leaves, 2) susceptibility of both acetone dipped and non-dipped leaves decreases with age to near zero at plant maturity, and 3) DVT levels increase with plant age. Although the association of decreases in susceptibility with increases in DVT might be interpreted as indicating that DVT affects susceptibility, statistical analysis does not indicate that this relationship is significant. The role of DVT in age related resistance and the nature of the mechanism(s) involved still remain obscure.

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HIGH LEVELS OF RESISTANCE TO POWDERY MILDEW IN BETA MARITIMA. E. D. Whitney, ARS, USDA, 1636 E. Alisal St., Salinas, CA 93905

Powdery mildew of sugar beet caused by Erysiphe polygoni became epidemic in the United States in 1974 and has reoccurred each year since. Resistance breeding has produced tolerant cultivars; however additional sources of resistance would be desirable. Fifty-five Beta maritima accessions from Europe were greenhouse tested for reaction to E. polygoni. Seven accessions did not show visible mildew on some plants. These accessions were field tested under natural infection. All of the accessions that had mildew free plants in the greenhouse also had mildew free plants in the field. Frequency of plants without mildew ranged from 13-88% in the greenhouse and 12-93% in the field. Susceptible sugar beets were heavily mildewed in both greenhouse and field tests. In a greenhouse test the most resistant sugar beet cultivar had a reading of 2.7 and 3.1 (scale 0-9) at 2 and 4 weeks after inoculation while F_1 plants from crosses between resistant B. maritima plants were 0.0 at both readings.

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ESTIMATION OF HERITABILITY OF SUGARCANE SMUT RESISTANCE IN LOUISIANA. C. P. Chao and J. W. Hoy. Dept. of Plant Pathology and Crop Physiology, Louisiana State University, Agricultural Center, Baton Rouge, LA 70803.

Heritability of resistance to sugarcane smut (Ustilago scitaminea) was estimated using 18 bi-parental crosses involving resistant, moderately susceptible, and susceptible parents.

Stalks of all parents and 10 F1 progeny of each cross were dip-inoculated with smut teliospores prior to planting. Susceptibility was measured as an average percentage of stalks infected. By regression of progeny means on mid-parent means, narrow-sense heritability was estimated to be 0.55. Narrow-sense heritability estimated by factorial ANOVA was 0.16. No dominance variance was detected. Based on the comparison of heritability standard errors estimated by the two methods, the mid-parent regression method estimated heritability more precisely. These results suggest that resistance to smut is heritable in the breeding population under Louisiana environmental conditions.

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INHERITANCE OF RESISTANCE TO POWDERY MILDEW RACE 1 AND RACE 2 IN MUSKMELONS. David Kenigsbuch and Yigal Cohen, Bar-Ilan University, Ramat-Gan 52100, Israel.

The inheritance of resistance to race 1 and race 2 of the powdery mildew agent Sphaerotheca fuliginea in Cucumis melo PI 124111 was studied in crosses with susceptible gynoecious C. melo WI 998. F1 was resistant to race 1 and moderately resistant to race 2. The F2 progenigies segregated 3 resistant:1 susceptible to race 1 and 1 resistant:2 moderately resistant:1 susceptible to race 2. BC progenies to the resistant parent were all resistant to race 1 and segregated 1 moderately resistant:1 susceptible to race 2, while BC progenies to the susceptible parent segregated 1 resistant:1 susceptible to race 1, and 1 moderately resistant:1 susceptible to race 2. We concluded that resistance in PI 124111 to race 1 of S. fuliginea is conferred by a single dominant gene, and to race 2 by an incompletely dominant gene. Breeding lines of C. melo carrying both resistance genes were developed. Research supported by BARD.

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RESPONSE OF SOYBEAN CALLI TO CULTURE FILTRATES OF Septoria glycines. H. Song, S. M. Lim, and L. E. Gray, Department of Plant Pathology, USDA-ARS, University of Illinois, IL 61801.

Williams and Corsoy 79 soybean calli were treated with culture filtrates of Septoria glycines grown in a modified Czapek's solution medium to determine callus reactions to the filtrates. Callus browning was observed in the two soybean cultivars 24 hrs after the fungus culture filtrate was added 1/3 (v/v) to the basal Murashige and Skoog's callus growth medium. Variation in callus browning response to the fungus filtrate was greater in Corsoy 79 than in Williams. Culture medium filtrate alone did not cause browning in both cultivars. Calli from nonhosts tobacco, carrot, cotton, and Datura spp. were not affected by Septoria culture filtrates.

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INHERITANCE OF RESISTANCE TO BLACK STAIN OF MALTING BARLEY. M. R. Miles, R. D. Wilcoxson, and D. C. Rasmusson, Depts. Plant Pathology, and Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108.

Barley black stain reduces the quality and market value of barley used in the malting and brewing industries. Populations from four crosses of resistant by susceptible barleys were evaluated for the disease in F2 and F3 generations on individual heads using a 1 to 5 scale: one indicated little black stain and 5 severe disease. Mean scores of resistant parents were 1.8 and 2.3 and susceptible parents 2.7, 2.9 and 3.7. F3 progeny means were 2.6, 2.4, 3.1 and 2.7 and indicated greater susceptibility than predicted by mid-parent values. Heritability estimates of 42% to 56% were obtained on F2 plant basis using a parent-progeny correlation. Distributions of F3 line means were nearly continuous and exhibited transgressive segregation, largely toward susceptibility. We concluded resistance to black stain was conditioned by few loci and inherited in a recessive manner.

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PEROXIDASE ACTIVITY - A POSSIBLE MARKER FOR RESISTANCE OF MELON AGAINST DOWNY MILDEW. R. Reuveni, Z. Karchi, Division of plant Pathology, and Field Crops respectively. ARO, Newe-Ya'ar Experiment Station Haifa Post 31-999, ISRAEL.

The possibility of using peroxidase as a marker for non-specific resistance has been investigated in melon cultivars and genetic lines with different reactions to Pseudoperonospora cubensis infection. The level of peroxidase activity in leaves of non-infected resistant plants was considerably higher than in susceptible plants. While the lowest activity was found in non-infected highly sensetive plants. Such correlation has been obtained under growth chamber as well as field inoculations and has also been demonstrated in populations derived from crosses of resistant X susceptible material.

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EVALUATION OF COMPONENTS OF PARTIAL RESISTANCE TO TOBACCO BLUE MOLD. R. C. Rufty and C. E. Main, Departments of Crop Science & Plant Pathology, North Carolina State University, Raleigh, 27695.

Two susceptible and 4 resistant tobacco (Nicotiana tabacum L.) genotypes were evaluated for components of partial resistance to tobacco blue mold (Peronospora tabacina Adam). Inoculation efficiency, incubation period, lesion diameter, latent period and sporulation capacity were measured in experiments conducted at the North Carolina State University Phytotron. Genotypes were significantly different for all components of resistance. Resistant genotypes produced fewer and smaller lesions, had a lower degree of sporulation and exhibited longer incubation and latent periods. Results corroborate field observation and indicate that deployment of blue mold resistant germplasm should greatly reduce onset and progress of blue mold epidemics. Furthermore, the probability of rapid development of new races of P. tabacina may be reduced because resistance interferes with several different stages of the pathogen's development.

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SEVERITY OF PEAR FRUIT RUSSETTING ASSOCIATED WITH EPIPHYTIC INDOLEACETIC ACID-PRODUCING BACTERIA. S. E. Lindow. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Russetting of Bartlett pear fruit at harvest was increased significantly compared to controls by application of bacterial producers of indole-3-acetic acid (IAA), but not non-IAA producing bacteria to pear flowers at full bloom. IAA-producing bacteria maintained population sizes in excess of 10^6 cells/g fr. wt. on pear tissue for over one month after inoculation. Application of tryptophan to trees increased the severity of fruit russetting on trees treated with IAA-producing bacteria but not on uninoculated trees. The severity of fruit russet was lower on trees treated with non-IAA producing bacteria or a mixture of streptomycin and oxytetracycline. The average amount of IAA produced in vitro by bacteria isolated from four orchards of high russet severity was higher than by bacteria from four orchards having low russet severity at harvest.

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ROLE OF A PHENAZINE ANTIBIOTIC IN DISEASE SUPPRESSION BY PSEUDOMONAS FLUORESCENS 2-79. L. S. Thomashow and D. M. Weller, USDA-ARS, 367 Johnson Hall, WSU, Pullman, WA 99164.

Pseudomonas fluorescens 2-79, which produces a phenazine antibiotic, is suppressive to take-all of wheat caused by Gaeumannomyces graminis var. tritici (Ggt). Genetic studies were undertaken to assess the importance of this antibiotic in disease suppression by 2-79. Eight transposon Tn*5* mutants defective in phenazine production were noninhibitory to Ggt in vitro, and were also less suppressive than 2-79 in greenhouse tests in which seeds treated with the bacteria were sown in soil infested with Ggt. Antibiotic synthesis and inhibition in vitro and on roots were fully restored in two mutants complemented with a cosmid from a 2-79 DNA library. These two mutants contain single Tn*5* insertions into adjacent *Eco*R1 fragments in the 2-79 genome, and the restriction map of the region flanking the insertions is colinear with that of the complementing 2-79 DNA. These results indicate that the phenazine antibiotic has an active role in take-all suppression.

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POPULATIONS OF XANTHOMONAS CAMPESTRIS PV. VESICATORIA (XCV) IN LESIONS ON RESISTANT AND SUSCEPTIBLE TOMATO LINES. G. E. Cameron, J. B. Jones, and J. W. Scott, IFAS, University of Florida Gulf Coast Research and Education Center, 5007 60th Street East, Bradenton, FL 34203.

Hawaii 7998 (H7998), a tomato line resistant to XCV, develops fewer bacterial spot lesions than susceptible lines and produces a hypersensitive response. XCV populations in leaf lesions on greenhouse grown tomatoes were quantified to determine if the lesions on H7998 developed populations comparable to those on other lines. Approximately 2-3 weeks after inoculation with 10^8 cfu/ml XCV, the diameters of individual lesions were measured. The lesions were then macerated, serially diluted, and plated on a selective Tween medium. Populations in lesions of 'Walter' (susceptible) were at least 100 times higher per cm^3 of lesion than populations in H7998 lesions. Lesion development on H7998, based on XCV populations, appears to be a hypersensitive response.

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MUTANTS OF ERWINIA HERBICOLA AFFECTED IN ANTIBIOTIC ACTIVITY. J. L. Vanneste, L. B. Smart, and S. V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Strain Eh 252 of E. herbicola is highly effective in reducing fire blight incidence under orchard conditions and it inhibits E. amylovora, in vitro, by antibiosis. To examine the relationship between these two properties, mutants of Eh 252 were produced using bacteriophage lambda as a vector for Tn5. Genetic analysis revealed that Tn5 insertion was random and stable. Of 1480 kanamycin-resistant colonies, two lacked antibiotic activity. Both mutants contain single Tn5 insertions, as revealed by Southern hybridization using the Tn5-containing plasmid pRZ102 as probe. The ability of one mutant to reduce the incidence of fire blight blossom infection, under controlled environment conditions, was much less than that of the wild-type strain; both strains multiplied, in planta, at the same rate. These preliminary data suggest that antibiotic activity is involved in the ability of strain Eh 252 to control fire blight.

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TRANSPOSON-INDUCED AVIRULENT MUTATION IN PSEUDOMONAS VIRIDIFLAVA: IDENTIFICATION OF PEL AND OUT GENES AS PATHOGENICITY DETERMINANTS. C. H. Liao, H. Y. Hung, and A. K. Chatterjee, East. Reg. Res. Ctr., USDA/ARS, Philadelphia, PA and Dept. of Pl. Pathol., Kansas St. Univ., Manhattan.

P. viridiflava SF312, a soft-rotting pathogen of harvested vegetables, constitutively synthesizes an extracellular pectate lyase (PL) with PI $\geq$ 10, MW 43K, and elutable with NaCl 0.05M in the DEAE-cellulose (Cl^-) column. Tn5 was successfully introduced into this pseudomonad by using pSUP1011 in E. coli SM 10 as a vector. Besides auxotrophs and EPS-altered strains, two types of avirulent mutants were obtained. The Pel^- failed to synthesize PL, whereas the Out^- failed to secrete PL and protease. Both Pel^- and Out^- mutants lost the ability to infect and macerate 5 tested plants. We conclude that:(1) this pseudomonad encodes a single PL for the virulence, and 2 genes (Pel and Out) for the expression of the pathogenicity; (2) the protease alone does not initiate infection or maceration; and (3) the protease and PL share a common route for transport.

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ISOZYME ANALYSIS TO IDENTIFY PATHOVARS OF XANTHOMONAS CAMPESTRIS. M. R. Bonde, USDA-ARS, Frederick, MD; Q. B. Kubicek and E. L. Civerolo, USDA-ARS, Beltsville, MD; and G. L. Peterson, USDA-ARS, Frederick, MD.

Fourteen of 46 enzymes tested using horizontal starch-gel electrophoresis were in sufficient quantity, easily resolved, and gave consistent results which could be used to identify Xanthomonas campestris pathovars. These included: fructose diphosphatase, galactose dehydrogenase, glucokinase, glucose-6-phosphate dehydrogenase, glucose phosphate isomerase, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, phosphoglucomutase, and phosphogluconate dehydrogenase. Of 11 pathovars (a total of 45 isolates), there was little intra-pathovar variation; X. c. pv. holcicola (4 isolates), X. c. pv. manihotis (7 isolates), X. c. pv. nigromaculans (4 isolates), and X. c. pv. pelargonii (3 isolates) exhibited none. The large number of isozyme differences between pathovars demonstrates their potential for identifing pathovars of this bacterial species.

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DETECTION, BIOVAR DISCRIMINATION AND VIRULENCE ASSESSMENT OF AGROBACTERIUM TUMEFACIENS FROM SOIL WITH A T-DNA ^{32}P LABELED PROBE AND SELECTIVE MEDIA. R.N. Goodman, G.L. Cleveland, X-A. Pu, I.U. Haque, and R. Alarcoa e Silva. Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Agrobacterium tumefaciens recovery from soil was improved with a modified Roy-Sasser medium, biovars, 1, 2, and 3 were differentiated using a galactose-glucose medium containing brome cresol purple and detection of all three biovars with a conserved common segment of ^{32}P-labeled T-DNA made it possible to accurately assess the numbers of virulent agrobacteria in a soil sample. Virulence in plants was rapidly established in inoculated grape leaf petioles of highly sensitive cultivar, Chancellor.

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MOTILITY OF PSEUDOMONAS SYRINGAE PV. GLYCINEA. D. R. Hattermann and S. M. Ries. Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801

Motility in Pseudomonas syringae pv. glycinea is oxygen dependent, decreases with increasing growth temperature (17-33 degrees centigrade), and is optimum at pH 6-7 and at an EDTA concentration of 10^{-5}M. High concentrations of EDTA drastically inhibit motility. Asparagine, sodium citrate, and glycerol stimulate motility when present in the motility medium, and the composition of the growth medium may also have an effect on motility. This organism is chemotactically attracted to leaf extracts of intercellular space fluid from both a susceptible ('Wells II') and a resistant ('Williams') soybean (Glycine max) cultivar. All observations above, except O_2 dependency, were made via capillary assay.

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MONOCLONAL ANTIBODIES USED TO CHARACTERIZE XANTHOMONAS CAMPESTRIS PV. DIEFFENBACHIAE. R.L. Bonner, A.M. Alvarez, J. M. Berestecky and A.A. Benedict. University of Hawaii, Honolulu, HI 96822.

Eight monoclonal antibodies (MAbs) were used to characterize 365 strains of Xanthomonas campestris pv. dieffenbachiae (Xcd) using ELISA. Strains were isolated from twelve genera of aroids collected from five Hawaiian islands, Florida, and California. Approximately 97% of the total Xcd strains and 99% of 190 anthurium strains reacted with one or more the MAbs. Thirteen distinct groups were identified; 94% of the anthurium strains formed 6 groups 3 of which were exclusively anthurium strains. Group 9 consisted predominantly of strains from Colocasia esculenta; other groups contained strains from a variety of aroids. One MAb, 97-2-1-1, reacted with 98% of 124 non-starch-hydrolyzing Xcd strains but none of the hydrolyzing strains. Pathogenicity, host range, and virulence of the groups were determined. These MAbs are useful for identifying and differentiating strains of Xcd and should be valuable in epidemiological studies.

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IDENTIFICATION OF XANTHOMONADS FROM CRUCIFER SEEDS WITH MONOCLONAL ANTIBODIES. A.M. Alvarez, A.A. Benedict, G. Or, and C.Y. Mizumoto. University of Hawaii, Honolulu, HI 96822

Monoclonal antibodies (MAbs) were used to identify Xanthomonas campestris pv. campestris (Xcc) and distinguish the pathogen from nonpathogenic xanthomonads and other bacteria recovered from crucifer seeds. Seed extracts were plated on semi-selective media, and 329 xanthomonas-like cultures from representative seed lots were purified and tested with selected MAbs. Of 89 cultures identified as Xcc, 87% caused black rot, and the remainder caused atypical lesions or were weakly virulent on cabbage. Most of the latter strains were negative for a new MAb, X21. All 240 cultures that failed to react with 3 Xcc-specific MAbs (X9, X13, or X17) were nonpathogenic on cabbage. Many of these reacted with Xanthomonas-specific MAbs (X1 and X11) and were identified as X. maltophilia by bacteriological and fatty acid analyses. MAbs were then generated to identify X. maltophilia.

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EPIPHYTIC PSEUDOMONAS SYRINGAE ON BARLEY: A QUANTITATIVE STUDY. D. G. Georgakopoulos, D. C. Sands and A. L. Scharen*, Department of Plant Pathology, *ARS, USDA, Montana State University, Bozeman, MT 59717-0002.

Epiphytic populations of P. syringae from 24 barley cultivars were determined by dilution plate assay of 10-leaf samples on BCBRVB, a modified Kings B selective medium. Leaf symptoms were recorded at each sampling. P. syringae colonies were

tested for ice nucleation activity (INA) by a drop-freezing technique and the percentage of INA+ bacteria determined. Populations were log 0-3 cfu/leaf before heading for all cultivars except one and reached log 3-6 cfu/leaf by the end of the growing season. Populations from some cultivars were consistently 100% INA+ bacteria. There was no correlation between leaf symptoms and population levels. Five high and one low population cultivars were reexamined in the field during Winter, 1987. Two supported populations of log 3-4 cfu/leaf and one supported only log 1 cfu/leaf. Three supported lower population levels than they had in the field during Summer, 1986.

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LIPOSOME AND THE GROWTH OF SPIROPLASMAS. Meghnad Konai and C. J. Chang, Department of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

Liposome was prepared from a mixture of cholesterol, palmitic acid, oleic acid, and egg phosphatidylcholine using the modified method of Kahane and Razin (Biochim. Biophys. Acta 471:32). Liposome was used to replace horse serum in R_2 medium for the growth of Spiroplasma floricola (23-6), S. melliferum (AS 576) and S. apis (SR 3). All maintained their helicity and continued to grow in the liposome medium. Six passages were completed for S. floricola and 5 passages for S. melliferum and S. apis. Thirty µl was transferred into 2 ml fresh medium in each passage. The data suggest that liposome can replace serum in R_2 medium for spiroplasma growth. Further replacement of bovine serum albumin and lipid with liposome in a chemically defined medium will give an opportunity for precise study of amino acid requirements of spiroplasmas.

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PURIFICATION OF ASTER YELLOWS AGENT FROM DISEASED LETTUCE USING AFFINITY CHROMATOGRAPHY. Y. P. Jiang, J. D. Lei and T. A. Chen, Plant Pathology Department, NJAES, Rutgers University, New Brunswick, NJ 08903

An affinity chromatographic procedure has been developed for purification of the mycoplasma-like organism (MLO) from lettuce infected with aster yellows (AY) agent. The affinity column consisted of Staphyloccus protein A (SpA) covalently linked to a 6MB Sepharose matrix and coupled with monoclonal antibody (MA) specific against AY-MLO. Crude sap from symptomatic lettuce was centrifuged at low and high speeds to remove plant debris. The resuspended pellet was then loaded on the column where the AY-MLOs were retained. After an incubation period, extraneous unbound plant materials were washed from the column. The AY agent was released from the column by mechanical shaking and then eluted. Intact and undamaged cells were observed by electron microscopy and their purity was confirmed by electrophoresis. In addition, further confirmation of AY-MLO was made in Western blots and in electron microscopy probing with MA following by protein A-gold labeling.

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IDENTIFICATION OF SURFACE PROTEINS OF SPIROPLASMA CITRI. J. Fletcher, S. E. Denman, J. W. Wills, and A. C. James, Depts. of Plant Pathology and Microbiology, Oklahoma State Univ., Stillwater, OK 74078-0285.

Spiralin (29 kd) has been the only membrane protein of S. citri shown to have a surface component. Intact cells of S. citri (BR3) were treated with trypsin, chymotrypsin, or proteinase K and subjected to SDS-PAGE. All three enzymes cleaved a 56 kd protein, the first two also cut one of 90 kd, and chymotrypsin degraded one of 45 kd, indicating probable surface location. Only proteinase K cleaved spiralin. In a second procedure, BR3 cells were treated with anti-S. citri membrane serum and lysed. Staph A protein was added and antigen-antibody complexes were pelleted. Four S. citri proteins with presumed surface components were identified at approximately 90, 80, 55, and 29 kd by Western blotting. In similar experiments the 90 kd protein was present in pathogenic strains BR6 and BR18, but was missing from nonpathogenic strain R8A2 and from nonphytopathogenic S. melliferum and S. floricola.

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RESISTANCE TO KABATINA JUNIPERI WITHIN JUNIPERUS VIRGINIANA AND J. SCOPULORUM PROGENIES. Glenn W. Peterson, Rocky Mountain Forest and Range Exp. Stn., Forestry Sciences Laboratory, Univ. Nebr., Lincoln 68583.

Kabatina juniperi kills branch tips of 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 progenies of 131 J. virginiana and 23 J. scopulorum trees were arrayed in a randomized block design. Initial infection was by spores from infected older Juniperus spp. nearby. Infection was extensive in 1985 and epidemic in 1986. Disease evaluations were made in late March 1987, when symptom development was optimum, by rating each tree on basis of percentage of branch tips infected: 1=0-3%; 2=4 to 20%; 3=21 to 40%; 4=>40% of tips infected. The average disease rating for 131 J. virginiana progenies was 2.5 (range 1.5 - 3.6) and for 23 J. scopulorum progenies was 2.7 (range 1.6 - 3.6). There were several progenies of each species that had high resistance.

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FACTORS AFFECTING INFECTION OF ASPEN BY CYTOSPORA CHRYSOSPERMA. A. W. Ramaley, G. A. McIntyre, and W. R. Jacobi. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Cytospora chrysosperma (Pers.) Fr. infected all seed-propagated Populus tremuloides Michx. trees inoculated 2 but not 7 days after chisel wounding in the greenhouse. Cankers were larger after 2 weeks on water-stressed than well-watered trees. Inoculation of excised aspen branches with moisture > 90% saturation showed significant ($P = < 0.03$) clonal differences in susceptibility. Canker size was also related to pathogen isolates. Each month bark samples were collected from and wounds inflicted on field aspen. The pathogen was isolated more frequently from bark between June and November than from December to May. C. chrysosperma was not isolated from the interior of healthy bark. Nearly all infections occurred on wounds made in July, suggesting the importance of environmental factors.

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MULTIPLE SYMPTOMS AS SIGNATURES OF FUSIFORM RUST RESISTANCE IN LOBLOLLY AND SLASH PINES. C. H. Walkinshaw and R. L. Anderson, Southern Forest Experiment Station, Gulfport, MS 39505, and Resistance Screening Center, Asheville, NC 28804.

The Resistance Screening Center provides a valuable service to tree improvement specialists by identifying loblolly (Pinus taeda L.) and slash (P. elliottii var. elliottii Engelm.) pines resistant to fusiform rust. Over one million seedlings (about 8,500 seedlots) have been screened since 1975. During this time, a number of symptoms have been evaluated for resistance indicies. Gall shape, rough bark, symptoms without swelling, shoots, reaction wood, and height of seedlings are examples. Some of these indicies have dramatically improved correlations between greenhouse and field tests. Current emphasis is to develop criteria for grouping pines with similar resistance. Ratios for incidences of symptoms are given as signatures for loblolly and slash pines. These may serve for classifying resistance types at the Center.

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NEEDLE DISEASES OF SPRUCE IN SWITZERLAND. Heiniger Ursula and Schmid Manuela. Swiss Fed.Inst.For.Res., CH-8903 Birmensdorf, Switzerland.

In recent years spruce (Picea abies Karst.) showed a remarkable loss of needles, a symptom of the widely observed forest decline. In order to study the time course of the needle cast and the possible involvement of needle diseases we hung buckets in 27 stands to collect falling needles. Brown needles were mainly shed in fall, green needles in spring. Fruiting bodies of three fungal species were found: Tiarosporella parca (Berk. et Br.) Whitney (on up to 40% of brown needles), Rhizosphaera kalkhoffii Bubak (20%) and Lophodermium piceae (Fuckel) Höhn. (20%). The role of T.parca is not known. It is often associated with brown secondary branches during winter. L.piceae is found on older red needles in fall and R.kalkhoffii on very old needles mainly during spring and summer.

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CRYSTALS OF CALCIUM OXALATE ASSOCIATED WITH THE HYPHAE OF ENDOTHIA PARASITICA. A.R. Bennett and D.F. Hindal Dept. of Plant Path. & Agr. Micro. 401 Brooks Hall, and P.A. Allender, Dept. of Pathology, B.S. 176, West Virginia Univer. Morgantown, WV 26506.

Crystals associated with the hyphae of 2 virulent and 3 hypovirulent strains of Endothia parasitica were observedby scanning electron microscopy and polarized light microscopy after the strains were grown for 10 days on a defined glucose-yeast extract agar medium containing .025 M calcium acetate. Crystal density was greatest at the center of the colonies and decreased

progressively towards the leading edge but none were observed at the hyphal tips. Crystals occurred as aggregates of flattened, tetragonal bipyramids and were identified as calcium oxalate by x-ray diffraction and birefringence under crossed polarizers. Crystals did not form on cultures grown without the addition of calcium. The hypothesis that calcium oxalate crystals are produced by virulent and hypovirulent strains of Endothia parasitica with the addition of calcium to the agar medium is supported by these observations.

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CYLINDROCLADIUM SCOPARIUM ISOLATED FROM NURSERY SOIL IN WASHINGTON. Walter G. Thies; Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, OR 97331.

In the spring of 1976, soil samples were collected from the USDA Forest Service Wind River Nursery in southern Washington. Each sample was a composite of 12 plugs collected with a 2.5-cm diameter tube-type sampler from the top 20 cm of soil. Samples were evaluated for propagules of Cylindrocladium scoparium Morgan by using the direct isolation technique reported by Thies and Patton (Phytopathology 60:599-601). The fungus was recovered from 12 of 16 samples collected from various areas within the nursery and from 0 of 15 samples of forest soil collected from points around the periphery of the nursery. When found, concentrations were low and ranged from 0.3 to 1.2 propagules per gram of soil (dry wt.). Analysis of spring 1986 samples showed that low concentrations of the propagules were still present. Seedlings infected by C. scoparium have not been found in the nursery. This is an extension of the known geographic range of this pathogen in the United States.

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A TISSUE CULTURE SYSTEM FOR ASSESSING EFFECTS OF MOISTURE STRESS ON HYPOXYLON MAMMATUM ASCOSPORE INFECTION OF ASPEN CLONES. R. Belanger, P. Manion and D. Griffin. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210.

Six aspen clones were tissue cultured to produce plantlets from dormant buds. One to 2 cm plantlets were moisture stressed by adding various concentrations of mannitol to the medium. H. mammatum ascospore inoculation of unwounded plantlets resulted in visible signs of mycelium after 3-4 days. After 10 days, mycelial growth on controls and moderately stressed plantlets remained superficial; in contrast, highly stressed plantlets were invaded by the mycelium and exhibited necrotic lesions at the site of inoculation. The level of moisture stress needed for mycelium invasion and lesion development varied (-6 to -12 bars) among the clones. The tissue culture system can provide insight into the interaction of water stress and aspen clone susceptibility to hypoxylon canker.

319. Withdrawn

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COMPARISON OF TALC, OIL, AND WATER AS SPORE DILUENTS FOR INOCULATING LODGEPOLE PINE WITH ENDOCRONARTIUM HARKNESSII. P.V. Blenis and H.D. Pinnell, Department of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

Talc, water, and oil were compared as diluents for dispersing Endocronartium harknessii (J.P. Moore) Y. Hiratskuka spores onto 10-week-old lodgepole pine (Pinus contorta var. latifolia Engelm) seedlings. Approximately 50 seedlings per diluent were inoculated in eight replicated experiments. Spore deposition was determined for 360 seedlings; the coefficients of variation did not differ among the treatments. SEM was used to determine germination on the surface of 70 trees. The percentages of germination (and standard deviations) for the oil-, talc-, and water-suspended spores were 31% (23), 57% (25), and 52% (16), respectively. The average numbers of seedlings infected after 3-4 months were 50%, 80%, and 81%, for the oil, talc, and water treatments, respectively. Oil caused some phytotoxicity.

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MYCOPLASMALIKE ORGANISMS IN DECLINING ASH THAT LACK DIAGNOSTIC MORPHOLOGICAL SYMPTOMS OF ASH YELLOWS. W. A. Sinclair. Dept. Plant Pathology, Cornell University, Ithaca, NY 14853.

Mycoplasmalike organisms (MLOs) causing ash yellows (AY) have been suggested to be responsible for decline of white ash (*Fraxinus americana*) and red/green ash (*F. pennsylvanica*) in the eastern United States, but most declining trees lack the witches'-brooms and deliquescent branching that are diagnostic for AY. I used the fluorochrome DAPI (4'6-diamidino-2-phenylindole · HCl) and epifluorescence microscopy to detect DNA (presumably in MLOs) in phloem sieve tubes of twigs and roots collected from white ash that lacked witches'-brooms. Frequency of MLO detection increased successively in the following four categories of trees: vigorous, slow growing, exhibiting dieback alone, and exhibiting deliquescent branching (often with dieback). MLOs were detected more than twice as often in roots as in twigs. In twigs, diseased sieve tubes degenerated and MLO were detected less readily during autumn-spring than in summer. Diseased sieve tubes sometimes displayed autofluorescence in addition to DAPI-enhanced fluorescence. Healthy sieve tubes did not autofluoresce. MLO are common in declining ash that lack diagnostic external symptoms of AY.

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GROWTH REDUCTIONS IN YOUNG DOUGLAS-FIR INFECTED WITH VERTICICLADIELLA WAGENERI. T. T. Lawson and F. W. Cobb, Jr., Department of Plant Pathology, University of California, Berkeley, CA 94720.

Growth increments of thirteen Douglas-fir (Pseudotsuga menziesii) trees infected with V. wageneri were compared with fifteen uninfected trees in a 20-year-old plantation. Between 1981 and 1985, terminal growth averaged 4 m in infected trees and 5.5 m in controls. Radial growth over that time averaged 2.9 cm per year in infected trees and 3.2 cm in controls. The ratio of height to diameter, radial growth index, and terminal growth index were 73.4, 0.27, and 0.34 respectively in infected trees as compared to 82.9, 0.67, and 0.88 in uninfected Douglas-fir. Differences were significant at the 0.05 level.

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PATHOLOGICAL HEARTWOOD FORMATION IN POLE-SIZED DOUGLAS-FIR INFECTED WITH VERTICICLADIELLA WAGENERI. T. T. Lawson and F. W. Cobb, Jr., Department of Plant Pathology, University of California, Berkeley, CA 94720.

Twenty-eight Douglas-fir (Pseudotsuga menziesii) trees (13 infected with V. wageneri, 15 uninfected controls) were measured for water conduction and heartwood formation at ground level and at 1.5 m above the ground. These trees ranged in age from 10 to 16 years and averaged 12 m in height. Pathological heartwood was identified by tissue inability to conduct fast-green dye and reaction to a potassium-iodide stain for starch. Physiologically normal heartwood in control trees occupied an average of 23 and 18 percent of the disk at ground level and 1.5 m respectively. Pathological heartwood in V. wageneri-infected trees occupied an average of 64 percent of the disk area at ground level and 51 percent of the area at 1.5 m. The ratio of volume of heartwood to volume of bole averaged 0.21 for controls and 0.58 for diseased trees.

324. Withdrawn

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EFFECT OF OZONE ON COLONIZATION OF DISTURBED FOREST SOIL BY MOSS. G.R. Stanosz, V.L. Smith, and R.I. Bruck. Dept. Plant Pathology, N.C. State Univ., Box 7616, Raleigh, NC 27695.

Mixed forest floor and upper mineral soil from a 1580 m conifer-hardwood stand in the Pisgah National Forest, NC was placed in 4 cm diam. x 21 cm deep "Leach conetainers" and exposed to 0, .08, .16, .24, or .32 uL O_3/L air (ppm). Twelve containers in each of 3 reps/treatment were fumigated in continuously-stirred tank reactors for 6 hrs/day on 4 consecutive days/wk.

Soil was watered 3 days/wk with deionized water adjusted to pH 4.3 with H_2SO_4 + HNO_3 (70 meq SO_4^{--}:30 meq NO_3^-). After 10 wks the amount of soil surface covered by moss (predominantly Ditrichum pusillum, but also D. lineare and Pohlia nutans) was evaluated on a scale of: 1=0-25%; 2=26-50%; 3=51-75%; 4=76-100%. Mean ratings were negatively correlated (p=.01) with O_3 concentration and surface area colonized in conetainers exposed to .32 or .24 ppm was about half of that for 0 ppm (mean ratings of 1.1, 1.4, and 2.6, respectively). Coverage differences appeared to be due in part to O_3 suppression of plant numbers. Also, height of D. pusillum plants (measured after 12 wks treatment) was negatively correlated (p=.01) with O_3 concentration.

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SELECTION PRESSURE IMPOSED BY TWO- AND THREE-WAY OXADIXYL MIXTURES IN PHYTOPHTHORA INFESTANS. Yigal Cohen and Yair Samoucha, Bar-Ilan University, Ramat-Gan 52100, Israel.

Potatoes were grown in ten 10x5 m polyethylene houses, 5 rows in each. Middle row (spreader) was inoculated with 9:1 S:R sporangial suspension of P. infestans while other 4 rows were sprayed with either mancozeb (weekly intervals) SAN-518 (oxadixyl+mancozeb, 1+7), Pulsan (oxadixyl+cymoxanil+mancozeb, 1+0.4+7), or Sandocur-M (oxadixyl+cymoxanil+mancozeb, 1+2+7) (biweekly intervals). Frequency of R subpopulation was determined for a 2 mo period in infected leaves collected from fungicide-treated rows. Late blight severities (0=no disease, 5=plants fully blighted) were 5,5, 2.8 and 1.6 in plants treated with mancozeb, SAN-518, Pulsan and Sandocur-M, respectively. Average frequencies of R subpopulation were 81,28,28 and 19% in plants treated with SAN- 518, Pulsan, Sandocur-M and mancozeb, respectively. 3-way oxadixyl mixtures have a higher control efficacy and a lower selection pressure compared to the 2-way oxadixyl mixture.

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CHEMICAL SUPPRESSION OF PEANUT GREEN MOSAIC DISEASE IN PEANUT. D.V.R. Sai Gopal, V. Siva Prasad, K. Gopinath, T. Satyanarayana, P. Sreenivasulu, and M.V. Nayudu, Department of Botany, S.V. University, Tirupati - 517 502 India.

Peanut green mosaic virus, a potyvirus (Sreenivasulu et al., 1981, Ann. Appl. Biol., 98: 255-260), induce initial chlorotic spots followed by severe mosaic and stunting of peanut plants. Bavistin (50% w/w Carbendazim) when applied as a foliar spray (0.5%) prevented the virus induced decrease in the length of mainshoot, root; the number of pegs, pods, leaves; the leaf area; the dry weight of shoot, root and pods in peanut. It also decreased abscission of leaves.

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PENICILLIUM DIGITATUM BIOTYPES WITH REDUCED SENSITIVITY TO IMAZALIL. J. W. Eckert. Department of Plant Pathology, University of California, Riverside, CA 92521.

Imazalil has been applied intensively in citrus packinghouses for the past four years to control biotypes of Penicillium digitatum and P. italicum resistant to benzimidazole fungicides. During 1986, several isolates of P. digitatum were collected in packinghouses which grew well on PDA containing imazalil 1 µg/ml that completely inhibited the usual isolates. Lemons treated with imazalil in storage wax were collected in two packinghouses, inoculated deeply with isolates of P. digitatum differing in imazalil sensitivity, and stored at 15 C for three weeks. Sporulation of three typical isolates of P. digitatum was completely suppressed on lemons containing 1.6-1.8 ppm imazalil, an acceptable residue level, but six isolates with reduced sensitivity to imazalil in vitro produced medium to heavy sporulation on lemons of the same fruit lots. Three isolates sporulated heavily on lemons with a 5.8 ppm residue, resulting from a 1 g/L imazalil dip treatment.

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INTERACTION OF INSECTS AND SEEDLING DISEASES ON COTTON STAND ESTABLISHMENT. P. D. Colyer and S. Micinski, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Red River Research Station, Bossier City, LA 71113

Nine in-furrow treatments directed at specific components (i.e. diseases, insects, and nematodes) of the early season pest complex of cotton were evaluated in replicated field plots. Data collected on thrip populations, disease ratings and nematode galling were related to stand establishment, seedling vigor and yield of seed cotton. High thrip counts and disease ratings were associated with poor stand development and reduced yields. Seedling vigor as determined by plant weight was also reduced by thrips and high disease ratings. This research demonstrates the importance of managing both insects and diseases associated with cotton seedlings to produce a vigorous stand and to improve seed cotton yields.

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FUNGICIDES FOR ROSE BLACKSPOT CONTROL. A. K. Hagan, Dept. of Plant Pathology, C. H. Gilliam, and D. Fare, Dept. of Horticulture, Auburn University, AL 36849.

Myclobutanil (0.04, 0.08, and 0.16 g. a.i./L), pyrifenox (0.05 and 0.10 g. a.i./L), and diniconazole (0.06, 0.13, and 0.26 g. a.i./L) were evaluated for control of blackspot on field-grown roses with chlorothalonil (1.35 g. a.i./L) and triforine (0.18 g. a.i./L) from 1984 to 1986. Fungicides were applied weekly from April to September. Myclobutanil at 0.16 g. a.i./L and pyrifenox at 0.10 g. a.i./L maintained excellent season-long protection from blackspot equal to that obtained with chlorothalonil and triforine. Lower rates of pyrifenox and myclobutanil reduced blackspot damage but did not provide adequate disease control when compared to the highest application rate of either fungicide. Despite significant reductions in disease severity, diniconazole did not control blackspot as effectively as chlorothalonil and triforine. Diniconazole retarted shoot elongation at all rates while myclobutanil and pyrifenox did not.

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INFLUENCE OF WASHING ON RESIDUAL ACTIVITY OF TWO RYEGRASS SEED TREATMENT FUNGICIDES. P. F. Colbaugh. Texas Agricultural Experiment Station, Texas A&M University Research and Extension Center, Dallas, Texas 75252.

Overseeding operations with ryegrasses during the fall planting season are followed by heavy irrigation to encourage rapid seedling establishment. Inoculation studies with Pythium aphanidermatum were used to determine residual disease protection by two seed treatment fungicides following washing of treated ryegrass seed. Both fungicide-treated and untreated seed were rinsed in running water to approximate leaching by irrigation following field planting operations. Leaf canopies of one to three week old seedlings established from metalaxyl (Apron 25W)-treated seed were not susceptible to foliar blighting, while seedlings established from ethazole (Koban 30W) treated seed did not reduce foliar blighting during any stage of seedling development. These results suggest systemic activity of metalaxyl in developing ryegrass seedlings gave longer periods of protection against Pythium diseases.

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EFFECT OF FUNGICIDE RATES AND APPLICATION TIME ON CROWN RUST PREVALENCE OF MANHATTAN PERENNIAL RYEGRASS. John E. Watkins, Department of Plant Pathology, Robert C. Shearman and Leonard A. Wit, Jr., Department of Horticulture, University of Nebraska, Lincoln, Nebraska 68583-0722.

One protectant and three systemic fungicides were evaluated for control of Puccinia coronata on 'Manhattan' perennial ryegrass. The systemic fungicides more effectively controlled crown rust than the protectant chlorothalonil. This was accomplished at lower rates of active ingredient and with fewer applications. A single late July application of triadimefon at a 2 oz/1000 sq ft rate was more effective than either two or three 1 oz applications beginning early July. The experimental fungicide diniconazole (XE-779) significantly reduced crown rust prevalence at either the 1 oz or 2 oz rate; however, the 1 oz rate applied only twice was not as effective as three applications at 1 oz or two applications at 2 oz. Another experimental systemic fungicide, PP 523, was less effective at 3 oz than at 4 or 6 oz.

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SPRING DEAD SPOT RESEARCH IN NORTH CAROLINA. L. B. McCarty, L. T. Lucas, and M. R. Newnam, North Carolina State University, Raleigh, NC 27695-7616.

Spring dead spot (SDS), the most important disease of bermudagrass in NC, may be predisposing the grass to low temperature kill. Experiments were conducted to (i) isolate the SDS causal agent, (ii) examine fungicide/fertility interactions on bermudagrass low temperature stress survival, and (iii) evaluate fungicides for SDS control. Leptosphaeria-like fungi were isolated from SDS infected bermudagrass in NC. Benomyl, applied in the fall, increased regrowth of Tifway bermudagrass following exposure to low temperature stress while other fungicides as well as potassium and nitrogen sources did not. SDS infected bermudagrass was damaged following low temperature stress while non-infected bermudagrass was not. SDS control with fungicides is being evaluated throughout the year.

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ISOLATION, IDENTIFICATION AND CHEMICAL CONTROL OF CYLINDROCLADIUM MUSAE SP. NOV. ASSOCIATED WITH TOPPLING DISEASE OF BANANA. C. R. Semer, IV, D. J. Mitchell, M. E. Mitchell, F. N. Martin and A. C. Alfenas. Univ. of Florida, Dept. of Plant Path., Gainesville, 32611.

Toppling disease of banana was severe in several plantations in Costa Rica during 1985/1986. A Cylindrocladium sp. was recovered from ca. 10% of the affected roots. Pathogenicity of isolates was confirmed. Morphological characteristics of these isolates differed from those of all previously described Cylindrocladium spp. with globose vesicles. Conidia of Cylindrocladium musae sp. nov. were 37% longer than the largest previously reported conidia of any Cylindrocladium sp. Attempts to produce the perfect stage were unsuccessful. This fungus grew 6 mm at 36C on PDA while four other tested species with globose vesicles did not grow at 36C. Protein and isoesterase profiles of C. musae differed when compared to all other described species with globose vesicles. Benomyl and prochloraz showed 100% control of wilting and mortality of banana plants caused by C. musae in greenhouse studies.

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THE METEOROLOGICAL ASPECTS OF THE 1985 OCCURRENCE OF BLUE MOLD IN KENTUCKY. J. M. Davis and C. E. Main, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, and W. C. Nesmith, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Plant pathologists confirmed the mid-June occurrences of primary blue mold lesions in six south-central Kentucky counties which are aligned in a north-south direction from Monroe County on the Tennessee border to Mercer County in central Kentucky. Infected Nicotiana repanda plants located in south-central Texas were hypothesized to be the inoculum source. On-site inspection in Texas confirmed moderate to heavy sporulation on these plants from 10 May to 30 May. Atmospheric trajectory analysis supports the south-central Texas source region. Evidence supplied by trajectory analysis, weather radar composite charts, and the alignment of county first occurrence dates supports a late May-early June arrival date for the inoculum and the importance of wet deposition processes in bringing the spores to the leaf surfaces.

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SPORE DEPOSITION GRADIENTS NEAR A SOURCE. Donald E. Aylor, Connecticut Agricultural Expt. Station, New Haven, CT 06504.

The shapes of spore deposition gradients are influenced by the combined effects of loss by deposition, dilution by atmospheric turbulence, and height of release. Physics suggests that gradient shape will have both an exponential and a power law mathematical component; which component dominates the shape of the gradient depends on how rapidly spores escape from the crop canopy. Deposition gradients of urediniospores of Puccinia recondita were studied by counting the number of pustules which developed on potted trap plants exposed at several distances between 0.1 and 3.7 m downwind of a line source within a 1-m tall crop. These data were fitted well by a power law. The escape from the canopy of tracer particles released simultaneously from two heights in the canopy was also measured. Within 2 m from the source, 20-30% and 45-55% of the particles, released at 0.4 m and 0.65 m height, had escaped the canopy. The results will be explained using a mathematical model which can be generalized for a variety of conditions.

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ICE NUCLEATION AND DISEASE CAUSED BY PSEUDOMONAS SYRINGAE PV. SYRINGAE IN TOMATO TRANSPLANTS. W. G. Bonn and R. D. Gitaitis, Research Station, Harrow, Ontario NOR 1G0 and Coastal Plain Experiment Station, Tifton, Georgia 31793.

Pseudomonas syringae pv. syringae (PSS) which causes leafspotting similar to bacterial speck on Georgian tomato transplants and ice nucleation in plants when temperatures fall slightly below freezing was studied for its ability to survive long distance transport and initiate disease and frost damage in transplants shipped north in the spring. Transplants shipped with epiphytic PSS and planted in the field at Harrow did not express disease symptoms even though bacteria were present for up to 14 days after planting. Consecutive light frosts of -2.3°C and -1°C killed 70% of the transplants from Georgia with PSS but only 34% of the check plants without PSS. A repeat of the experiment using greenhouse-grown transplants emphasized the ability of PSS to survive on tomato leaf tissue without inciting disease symptoms. Frosts of -4.7°C and -3.2°C on consecutive nights killed all plants inoculated with PSS, however 50% of the uninoculated transplants survived.

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SPACIAL AND TEMPORAL SPREAD OF ASIATIC CITRUS BACTERIAL CANKER DISEASE IN REPLANT ORCHARDS IN ARGENTINA. T. R. Gottwald, U.S. Department of Agriculture, 2120 Camden Rd., Orlando, FL 32803, R. G. McGuire and S. Garran.

Two plots of 187 trees each were established in Concordia, Argentina to study the spread of citrus bacterial canker disease on grapefruit and sweet orange from a known source. Single trees were inoculated with Xanthomonas campestris pv. citri (Xcc) and planted in the center of each plot. Disease was estimated as the number of diseased leaves over the total number of leaves per plant. Disease spread was first detected 49 days after inoculum was placed in the plots. Initial spread was in the direction of prevailing winds and followed a blowing rain in mid-January. Subsequent spread was less dramatic and generally nondirectional. The rate of disease increase (Gompertz rate parameter k) was less in orange, .0052, than in grapefruit, .0090. Disease gradients varied over time from -0.713 to -1.237 and from +0.048 to -1.856 for orange and grapefruit, respectively, and were affected by disease-induced defoliation.

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COMPETITIVE AND NON-COMPETITIVE FITNESS OF PHYTOPHTHORA INFESTANS RESISTANT AND SENSITIVE TO METALAXYL. Yigal Cohen, David Kadish and Masha Grinberger, Bar-Ilan Univ., Israel.

Three metalaxyl-sensitive (MS) and three metalaxyl-resistant (MR) field isolates of P. infestans, were compared in the absence of metalaxyl. At a constant environment of 20°C MR isolates produced significantly larger lesions (LA), and had a larger daily increment in disease severity (DS) compared to MS isolates, but were similar in sporulation capacity (SC) and infection efficiency (IE). Compound fitness index (CFI) calculated as CFI = LAxDSxSC/IE was significantly larger for MR than for MS isolates. Competitive fitness was measured in 9:1 MS:MR inoculated plants, growing in plastic houses by monitoring the frequency of MR. Frequency of MR reached about 90% when disease severity was 4 (0 = no disease, 5 = plants fully blighted) and declined to about 25% when plants were fully blighted. Results explained the severe MR-induced late blight epidemic outbreaks in potatoes in Israel in the absence of selection pressure.

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A SIMULATION ANALYSIS OF FUNGICIDE RESISTANCE DYNAMICS IN FUNGAL PATHOGEN POPULATIONS. M. G. Milgroom, and W. E. Fry, Dept. of Plant Pathology, Cornell Univ.,Ithaca, NY 14853.

The ratio of fungicide resistant to fungicide sensitive individuals in a fungal population is influenced by the initial frequency of resistance and the relative growth rates of the two populations. A system of simulation models involving potatoes, metalaxyl, chlorothalonil, and Phytophthora infestans was used to estimate effects of initial frequency of resistance, weather, protectant fungicide, host resistance, metalaxyl (dose, frequency, weathering) and fitness of resistant individuals on suppression of disease and fungicide resistance. Factors which suppressed pathogen growth rates retarded changes in the ratio of resistant to sensitive individuals. Eradicant use of metalaxyl suppressed resistance, but not disease.

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A GENERAL METHOD FOR QUANTIFYING HOW CLIMATIC FACTORS AFFECT VARIATION IN PLANT DISEASE SEVERITY FROM YEAR TO YEAR. S.M. Coakley, L.R. McDaniel, NCAR, P.O. Box 3000, Boulder, CO 80307 and R.F. Line, Washington State University, Pullman, WA 99164.

The computer program WINDOW was written to analyze for correlation between climatic factors and disease severity. WINDOW is used to sequentially examine daily meteorological data in 25, 30,..,and 65 day segments for the growing season. WINDOW is advanced in five day increments, e.g., begins on day of year 5, 10,...etc,. For time periods highly correlated with disease, the length and beginning dates of segments are subsequently incremented one day at a time. Climatic factors examined include average minimum and maximum temperature, precipitation total and frequency, and additional factors defined for each disease. Factors highly correlated with disease severity are used in multiple regression analysis and the resultant models are validated. Models with two variables explained 70-76% of the variation in stripe rust (Puccinia striiformis) severity on three winter wheat cultivars at Pullman, WA.

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DETECTION OF PUCCINIA RECONDITA f. sp. TRITICI UREDINIOSPORES IN LOUISIANA. K.V. Subba Rao, and L. Anzalone, Jr., Dept. of Pl. Path. & Crop Phys., La. Agric. Exp. Sta., La. State Univ. Agricultural Center, Baton Rouge, La 70803.

KC 7d spore samplers were operated continuously at two locations, Baton Rouge (BR) and Bossier City (BC) and daily urediniospore counts were taken. Least number of spores trapped was in summer. The maximum number of consecutive days that no spores were trapped was 5 at BR and 11 at BC. The viability of the trapped inoculum varied from 20% in Aug. to 81% in Jan. at BR and 11.5% in July to 75% in Sept. at BC. McNair 1003, a leaf rust-susceptible cultivar, was planted from May 1, through Sept. 18, 1986, at 15d intervals. Leaf rust appeared in many plantings and survived temperatures of upto 37 C for 82d. McNair 1003 plants in pots were exposed for 2d in a field at BR at 15d intervals and then incubated in a greenhouse at 21 C. Rust incidence on plants varied from 25-100%. The results demonstrate that viable leaf rust inoculum is present throughout the summer and may provide primary inoculum for winter wheat in Louisiana.

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SPREAD AND INCREASE OF SUGARCANE SMUT IN LOUISIANA. J. W. Hoy and M. P. Grisham. Dept. of Plant Pathology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, and U. S. Sugarcane Research Unit, Houma, LA 70361.

Sugarcane stools showing smut whips resulting from infection by Ustilago scitaminea were counted and mapped in a field of cultivar CP 65-357 (357) as plant cane (PC) in 1984 and in PC fields of 357 and CP 74-383 (383) in 1985. These stools were treated as inoculum point sources and new stool infections in the first ratoon (FR) crop were counted, the distance to the nearest PC infection was determined, and disease gradients for each field were fitted to Gregory's model. Model r values for the 1985 357 and 1986 357 and 383 fields were -0.88, -0.83 and -0.90, and the slopes were -1.02, -1.44 and -1.81, respectively. Following the severe 1984-85 winter, the no. of new infections produced/ infected PC stool of 357 was 0.8, and after the mild 1985-86 winter, the no. of new infections/PC stool was 5.3 for 357 and 2.1 for 383. Total whips/ha changes between PC and FR were -29% for 1985 357, +498% for 1986 357 and +161% for 383.

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QUANTIFICATION OF TILLETIA INDICA TELIOSPORES IN MEXICAN WHEAT FIELD SOIL. L. E. Datnoff, M. H. Royer, M. R. Bonde, USDA-ARS, Frederick, MD; T. T. Matsumoto, Calif. Dept. Food Agr., Sacramento; and J. M. Prescott, CIMMYT, Mexico.

The number of T. indica teliospores (TS) in soil from Karnal bunt-infested wheat fields in Northwest (NW) Mexico was determined using the bubbling-flotation-sieving method (Phytopathology 76:1144, 1986). A standard curve was developed to estimate the amount of TS recovered from soil artifically infested (AI) with 50 to 5000 TS/10 g AI soil. A log transformation [$\log_{10}$ (TS # + 1)] stabilized the variances which allowed the development of a prediction equation from the standard curve. This equation was used to estimate the number of TS naturally infested (NI) in NW Mexican soil (X) based on the number of TS recovered (Y): $\log_{10} Y = -2.0 + 1.47 \log_{10} X$. The mean number of TS in NI soil (and 95% confidence limits) was estimated to be between (411)-476-(551) and (1394)-1617-(1877) TS/10 g soil. These data will be used to study the relationship between inoculum density and disease.

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RELATIONSHIP BETWEEN ENVIRONMENT, INOCULUM DENSITY OF TWO PATHOGENS, AND DEVELOPMENT OF ALFALFA LEAFSPOT DISEASES. K. Von Chong and C. Lee Campbell, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695.

To better understand alfalfa leafspot epidemics, environmental parameters and density of Leptosphaerulina briosiana and Pleospora herbarum were measured continuously in or above the canopy and disease severity, stem height, defoliation, and amount of debris were assessed weekly at 3 sites in 1985. Mean defoliation was correlated with rain (-,P=0.15) and spores of L. briosiana/m^3 of air (+,P=0.05) at 3 sites, and with mean debris (+,P=0.10) and total spores of P. herbarum (+,P=0.10) at 2 sites. Mean debris was correlated (+,P=0.05) with mean disease the previous wk. Mean disease was correlated (+,P=0.05) with number of L. briosiana spores and mean debris the previous wk at 2 sites. Correlations were found between change in mean disease (+,P=0.15) and mean temperature at 2 sites and weekly change of mean debris (+,P=0.10) with maximum temperature at 3 sites. These associations provide an initial view of the interactions present in alfalfa leafspot epidemics.

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EFFECT OF ANTHRACNOSE AND OTHER FACTORS ON SURVIVAL OF A SEGREGATING STYLOSANTHES GUIANENSIS POPULATION IN SEVERAL PASTURE ENVIRONMENTS. J. M. Lenné, A. Vargas de Alvarez and J. W. Miles, Tropical Pastures Program, CIAT, Cali, Colombia.

From 1985 to 1987, reaction to anthracnose, caused by Colletotrichum gloeosporioides, other biotic factors and plant survival were evaluated in an F_2 population of the promising tropical pasture legume Stylosanthes guianensis in association with both Andropogon gayanus and Trachypogon-dominant savanna under three stocking rates in Carimagua, Colombia. After 12-mo, S. guianensis survival was 16.2, 13.8 and 8.4% in the savanna and 17.0, 6.8 and 4.2% in A. gayanus at high, medium and low stocking rate, respectively. After 18-mo, survival was 3.6, 6.2 and 1.0 %, respectively, in the savanna and zero at all stocking rates in A. gayanus. Anthracnose was associated with greater plant death at low and medium stocking rates. Stemborer (Caloptilia sp.) incidence was greater in the savanna association. Lack of survival of S. guianensis in A. gayanus was related to anthracnose stress and grass competition.

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RELATIONSHIP BETWEEN ENVIRONMENT AND BLACK SIGATOKA OF BANANA. W. Thal, T. Arroyo*, and H. W. Spurr, Jr. USDA-ARS Research Laboratory, Oxford, NC 27565 and Dept. Plant Pathology, N. C. State University, Raleigh 27695, and Banana Development Corporation of Costa Rica, San Jose*.

Environmental conditions favorable to black Sigatoka (caused by Mycosphaerella fijiensis var. difformis) were investigated. Environment (daily temperature, hourly rainfall, daily relative humidity, hours cloudiness/day, daily evaporation) and disease (ascospore counts, severity) were measured on a banana plantation in the Atlantic zone of Costa Rica. Daily spore count had the highest correlations with hours cloudiness (r=0.64), hours rainfall/day (r=0.61) and evaporation (r=-0.56). The correlation between hourly spore counts and hourly rainfall was 0.38 for measurements from the same hour, but was near zero between spore count and rainfall in preceding hours. Disease severity correlated best to maximum temperature (r=-0.35) and hours rainfall (r=0.33). Models are being developed incorporating these data to improve selection and timing of fungicides and to develop alternative controls.

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EFFECT OF LIGHT AND TEMPERATURE REGIMES ON CIRCADIAN LEAF MOVEMENTS IN HEALTHY AND VIRUS-INFECTED SOYBEAN LEAVES. J. Fetzer and B. W. Kennedy, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Soybean (Glycine max L. 'Bansei') seedlings were grown in 12-hour light at 400 μ E.m-2.s-1 (L) followed by 12-hour dark (D) at various constant or fluctuating temperature regimes and inoculated with tobacco streak virus 5 days after planting. In healthy plants, a 24-hour (circadian) leaf movement rhythm characterized all L/D regimes at all temperature combinations (20C L/20C D, 25/25, 30/30, 20/30 and 30/20). If plants were placed in continuous light (L/L) for 2 days the period increased to 27 hours at constant temperatures and at 20/30; however, leaf movements tended to be disrupted (did not consistently fit a circadian cosine curve) at 30/20. Inoculated plants lost significant circadian leaf movement rhythm under all temperature and light regimes within 3 days after inoculation. Cessation of leaf movement was coincident with appearance of symptoms.

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PRIMARY INFECTION AND SPREAD OF MAIZE DWARF MOSAIC VIRUS IN SORGHUM IN CENTRAL TEXAS. J. D. Alexander, R. W. Toler, Dept. Plant Path. & Micro., and F. R. Miller, Dept. Soil & Crop Sci., Tex. Agr. Exp. Sta., College Station 77843.

A two-part experiment was conducted to measure the amount of secondary spread of maize dwarf mosaic virus (MDMV) strain A in sorghum. Part one consisted of six insecticide treatments on a susceptible sorghum entry. The first treatment was the application of insecticide starting after emergence and continuing until "boot" stage. Treatments 2 through 5 began sequentially at 10-day intervals after the first and continued until "boot" stage. The sixth treatment was a no-insecticide control. Part two consisted of 20 sorghum entries alternately planted with a susceptible entry in one treatment and a resistant entry in a second treatment. Infection frequencies were not significantly different among treatments in either test suggesting that most of the MDMV infections were from incoming viruliferous aphids rather than secondary spread. The greenbug (Schizaphis graminum) arrived late in the growing season; however, the corn leaf aphid (Rhopalosiphum maidis) was present throughout the season and was probably the primary vector.

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SECONDARY AND PRIMARY VIRUS INFECTIONS OF POTATOES IN THE JORDAN VALLEY. Mani Skaria and Mohammed A. Khudair, JVASP, (USAID-Washington State Univeristy), Ministry of Agriculture, P.O.Box 2099, Amman, Jordan.

In the Jordan Valley, in 1985-87 seasons, the earliest potato plantings were from uncertified local seed. The farmers who planted later used certified seed which was not available for early plantings. We compared the incidence of PLRV,PVY,PVX,PVA,PVS, and PVM viruses by ELISA, symptomatology, and indicator plants. Also we assayed several weed species. Secondary infections by PLRV,PVY,PVX,PVA and PVS were very high among potatoes of non-certified origin. Primary infections among potatoes from certified seeds increased over time. Several insect vectors and weeds were shown involved in spreading potato viruses in the Jordan Valley.

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EFFECTS OF SENSITIVE AND TOLERANT MAIZE GENOTYPES ON ACCUMULATION OF MAIZE DWARF MOSAIC VIRUS. M. D. Law, J. W. Moyer, G. A. Payne. Plant Pathology Department. North Carolina State University, Raleigh, NC 27695-7616.

The effects of sensitive and tolerant dent corn genotypes on maize dwarf mosaic virus (MDMV) infection was determined using ELISA and infectivity assays. A 4 cm section at the center of the fourth leaf of 13 day old plants was mechanically inoculated. The temporal accumulation of coat protein (CP) and infectious virus was determined in the inoculated area of the leaves, areas proximal and distal to the inoculated area and in the non-inoculated leaves. Infectious virus was present in the inoculated sections of each genotypes. Sensitive and tolerant genotypes did not differ significantly in accumulation of CP in different sections of the inoculated leaves. Proximal and distal movement of virus was detected in sensitive genotypes. Significantly greater amounts of CP accumulated in the non-inoculated leaves of the sensitive genotypes. These investigations suggest that the resisitance acts by interfering with systemic movement rather than directly on virus multiplication.

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OCCURRENCE OF BARLEY YELLOW DWARF VIRUS IN THE JORDAN VALLEY. Mani Skaria and Mohammed Zoubi, JVASP(USAID-Washington State University),Ministry of Agriculture, P.O.Box 2099, Amman, Jordan.

Barley yellow dwarf virus symptoms were observed on soft wheats in the Jordan Valley in 1986-87. Four vector aphid species, namely- Rhopalosiphum padi (Linnaeus), R. maidis (Fitch), Schizaphis graminum (Rondani), and Sitobion avenae (Fabricius) - have been found feeding on cereals. We assayed several symptomatically infected wheats, barleys, and some wild oats by aphid transmission on to 'Clintland 64' oats. Enzyme linked immunosorbent assay (ELISA) was done against the PAV and the RPV types of BYDV. So far, we have found only the RPV type of BYDV in the Jordan Valley. Aphid transmission tests which were done early in the seasons were negative in ELISA. Wild oats are hosts of the RPV type of BYDV in the Jordan Valley.

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A VIRUS CAUSING CHLOROTIC RINGSPOT OF PEANUT. E. E. Wagih and H. A. Melouk, Dept. of Plant Pathology, College of Agriculture, Alexandria, Egypt, and USDA/ARS, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Symptoms of a virus isolated from an Erectoides (E) peanut hybrid growing in a greenhouse at Stillwater consisted of a wide chlorotic ringspot associated with chlorotic line pattern and mottling. This virus differed in symptomology, host range, and serological properties from other peanut viruses tested. The virus was transmitted mechanically and by grafting to cultivated peanut causing similar symptoms to those on the E hybrid. The virus produced a mosaic in Pisum sativum 'Little marvel', and severe malformation and reduction in leaf area in Lupinus albus 'Tiftwhite'. The virus did not infect Vigna unguiculata 'California blackeye', and at least five cultivars of Glycine max, which are known hosts of peanut mottle virus. The virus elicited necrotic local lesions and chrolotic ringspot, in Phaseolus vulgaris 'Topcrop' and Chenopodium amaranticolor, respectively.

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POTATO CHLOROTIC STUNT, A DISEASE OF POTATOES IN IRAN CAUSED BY A PREVIOUSLY UNDESCRIBED PLANT RHABDOVIRUS. D. Danesh and B. E. L. Lockhart, Department of Biology, University of Isfahan, Isfahan, 81745, Iran, and Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

A mechanically transmissible plant rhabdovirus with particles measuring 60 x 240 nm was identified as the cause of a chlorotic stunt disease of potatoes in the Iranian provinces of Isfahan, Hamedan, Damavand and Shahrekord. Field symptoms were: chlorosis, stunting, leaf-curling and systemic necrosis. Disease incidence was normally 1-8%, but reached 50% in the cv. Spunta. In addition to solanaceous species, the virus infected Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, and Phaseolus vulgaris cv. Red Kidney. The virus had a nuclear maturation site and was related serologically to tomato vein-yellowing virus, but not to potato yellow dwarf, Sonchus yellow net, broccoli necrotic yellows, Australian Datura or Euonymus vein-clearing viruses. The name potato chlorotic stunt virus (PCSV) is proposed for this rhabdovirus.

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DETECTION OF A DOUBLE-STRANDED RNA ASSOCIATED WITH GROUNDNUT ROSETTE. E. Breyel, R. Casper, O. A. Ansa, C. W. Kuhn, S. M. Misari, and J. W. Demski. Biologische Bundesanstalt fur Land- und Forstwirtschaft, Braunschweig, W. Germany; Ahmadu Bello University, Zaria, Nigeria; University of Georgia, Athens 30602.

The presence of a low-molecular weight double-stranded (ds) RNA (900 base pairs) associated with groundnut rosette can be used as a diagnostic tool for groundnut rosette virus, the symptom inducing agent. A simple procedure has been developed that is rapid, reliable, and requires minimal equipment. Using this procedure, the dsRNA was detected only in peanut plants with green rosette or chlorotic rosette symptoms. It was not found in uninoculated peanut plants, in symptomless peanut plants with groundnut rosette assistor virus alone, or in peanut plants infected with several other known peanut viruses. More than 2 µg of the dsRNA can be isolated and purified from each gram of rosetted tissue. Therefore, the dsRNA can be detected in 0.1 g or less of diseased tissue.

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MULTIPLE REGRESSION ANALYSIS OF THE RELATIONSHIP AMONG COMPONENTS OF "RESISTANCE" IN SORGHUM GENOTYPES WITH DIFFERENT REACTIONS TO MDMV-A. L. M. Giorda, R. W. Toler, Dept. Plant Path. & Micro., Tex. Agr. Exp. Sta., College Station, TX 77843, and M. J. Jeger, Tropical Dev. & Res. Inst., London, WC1X 8LU.

Virus concentration, incidence and severity of the disease, and yield reduction measured on 12 cultivars were used as parameters for regression analysis. The model that best explained the variation in yield was $Y=bo + b_1x_1 + b_2x_2 - b_3x_3$ where x_1= virus concentration; x_2= square of symptom severity; x_3= interaction of symptom severity and virus accumulation; Y= proportion of decrease in yield/panicle (probit transformation). There were significant positive correlations between decrease in yield and 1) increase of virus concentration and 2) symptom severity. Variation in symptom severity was the dominant factor that accounted for the variation of the dependent variable. Necrotic reactions caused an interaction with virus accumulation, giving a negative correlation with x_3.

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BEET DISTORTION MOSAIC VIRUS--A NEW SOIL-BORNE VIRUS OF SUGARBEET. H. Y. Liu, J. E. Duffus, and J. S. Gerik. USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905.

A new soil-borne virus which causes leaf distortion and mosaic symptoms on sugarbeet has recently been found from sugarbeet in Texas. The infectious agent, termed beet distortion mosaic virus (BDMV), is mechanically transmissible. In limited host range studies, it mechanically affects Beta vulgaris, B. macrocarpa, Spinacia oleracea and some chenopodiaceous weed hosts. The virus particles are long flexuous rods c. 12 nm in width and 200-2400 nm in length. The particles are similar in size to those of wheat spindle streak mosaic virus (WSSMV). In preliminary tests, however, pinwheel inclusion bodies have not been found in sugarbeet tissue infected with BDMV and no serological relationship to WSSMV has been demonstrated. The virus is soil borne and some evidence indicates that the vector is the fungus Polymyxa betae Keskin.

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OCCURRENCE OF RED CLOVER VEIN MOSAIC VIRUS (RCVMV) IN ALFALFA BREEDING LINES. Z. Pesic and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

RCVMV, a member of the carlavirus group, was isolated from alfalfa breeding lines maintained in the greenhouse and in the field. The virus was identified on the basis of host range, symptomatology, serology and transmission electron microscopy. RCVMV caused yellow vein mosaic and leaf necrosis in alfalfa (Medicago sativa), systemic vein clearing in Alsike clover (Trifolium hybridum), stunting of peas (Pisum sativum), cvs. Alaska and Rondo, and chlorotic mottling in Vicia faba. In leaf-dip and purified preparations, virus particles were about 640 nm long and 12 nm wide. Homologous reaction with the RCVMV isolate from alfalfa was observed by immonosorbent electron microscopy using two RCVMV antisera. Extensive premature death of alfalfa plants occurred after the breeding lines were transferred from the field into the greenhouse. At least 60% of 80 clones were found to be infected with RCVMV.

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NON-SPECIFIC APHID TRANSMISSION OF AN RPV SEROTYPE OF BARLEY YELLOW DWARF VIRUS. J. R. Creamer and B. W. Falk, Department of Plant Pathology, University of California, Davis, CA 95616.

A barley yellow dwarf virus (BYDV) isolate (CA-RPV-1) was recovered from Kombar barley grown in Davis, California. CA-RPV-1 was transmitted to California Red Oats by Rhopalosiphum padi (15/15), Sitobion avenae (13/16) and Schizaphis graminum (4/5) using ca. 10 aphids per plant. These plants, which showed characteristic reddening and severe stunting, were tested by DAS ELISA with polyclonal antisera made against NY-PAV, NY-RPV, and NY-MAV, and reacted positively only with the NY-RPV sera. Plants infected by CA-RPV-1 also reacted positively with three monoclonal antibodies made against RPV-NY (RPV-1, -2, and -3) in an indirect ELISA when ELISA plates were coated first with polyclonal antibodies to NY-RPV or NY-MAV. No reactions were obtained when monoclonal antibodies to NY-PAV or NY-MAV were used. The ds-RNA banding pattern from plants infected by CA-RPV-1 was identical to that of NY-RPV. CA-RPV-1 transmitted by R. padi or S. avenae was indistinguishable.

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CYTOPATHOLOGY AND SITES OF RHABDOVIRUS ASSEMBLY AND ACCUMULATION FOR IRANIAN (SHIRAZ) MAIZE MOSAIC VIRUS INFECTED MAIZE. E. D. Ammar, R. G. Gomez-Luengo, D. T. Gordon, The Ohio State Univ., OARDC, Wooster, OH 44691.

Ultrastructural studies were conducted on Iranian (Shiraz) maize mosaic virus (IMMV) infected maize leaf cells. Rhabdoviruslike particles (RVLP) assembled on membranes of the inner nuclear envelope and dilated cisternae of the endoplasmic reticulum; RVLP accumulated in the perinuclear space and within the dilated cisternae, respectively, for the two sites of assembly. Massive numbers of RVLP sometimes filled the cytoplasm. Enlarged nuclei with surrounding masses of RVLP were detected in cells of phloem, vascular parenchyma, mesophyll and epidermis. Enlarged nuclei were seen by light microscopy primarily in cells of IMMV-infected leaves in or near vascular bundles. The cytopathology of cells with RVLP included a highly vesiculated cytoplasm, tubular structures apparently originating from a modified endoplasmic reticulum, and degenerated chloroplasts.

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FINE, HELICAL, FILAMENTOUS STRUCTURES ASSOCIATED WITH MAIZE YELLOW STRIPE, A LEAFHOPPER-BORNE DISEASE AGENT FROM EGYPT. E. D. Ammar, Faculty of Agric., Cairo Univ., Giza, Egypt; The Ohio State Univ., OARDC, Wooster, OH 44691.

Maize yellow stripe (MYStp), a disease infecting maize and wheat plants, transmitted persistently by the leafhopper Cicadulina chinai (Cicadellidae, Homoptera), has been recently reported from Egypt. Electron microscopy of thin sections from maize leaves, naturally or experimentally infected with the agent of MYStp, revealed large intracytoplasmic masses of fine filaments ca. 5-6 nm thick. In most cases, these filaments appeared to be helically wound to form long, flexuous tubular structures ca. 30 nm in diameter. Masses of these structures were detectable by light and electron microscopy in the phloem elements, vascular parenchyma and mesophyll of infected leaves, but were never found in healthy leaves. The above described structures apparently have not been previously associated with any virus or viruslike disease in plants.

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A NEW DISEASE OF FITTONIA VERSCHAFFELTII CAUSED BY A PATHOVAR OF XANTHOMONAS CAMPESTRIS. J. H. Blake. Dept. of Plant Pathology, University of Florida, Gainesville, 32611.

A foliar blight of Fittonia verschaffeltii (nerve plant) was observed in several central Florida nurseries. Watersoaked, marginal, brown lesions occurred on fully expanded leaves of all varieties. Lesions often extended along the white veins of the affected leaves. A yellow bacterium was consistently isolated from these lesions. Nerve plants inoculated with this organism developed typical symptoms 7-14 days after inoculation. In contrast, Aphelandra squarrosa (zebra plant) developed black, necrotic lesions both marginally and within the leaf lamina. The bacterium was reisolated from symptomatic tissue of both genera and was identified as a pathovar of X. campestris based on a series of physiological and biochemical tests.

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DISTRIBUTION AND CONTROL OF PSEUDOMONAS CICHORII ON CHRYSANTHEMUM LEAVES. P.S. Randhawa, Yoder Brothers Inc., P.O. Box 68, Alva, FL 33920

Asymptomatic leaves (5th and 8th from shoot apex) of chrysanthemum grown in south west Florida under field conditions were detached. Both surfaces of each leaf were pressed for 10 min against an agar medium, selective for Pseudomonas cichorii. Leaf shape and location of P. cichorii colonies were marked on Petri plate lid and the pattern transferred onto a tracing paper. Of 565 leaves examined, 84 and 128 leaves contained the pathogen on the upper and lower leaf surfaces, respectively. The number of cfu were 1-42 per upper surface and 1-245 per lower surface. Bacterial density was highest along leaf margins on 56% upper surfaces and 40% lower surfaces. Pathogen recovery was independent of time (10 AM, 1 PM, 3 PM) of leaf detachment. Twice weekly sprays with Cuproxat at 2.5 ml/L inactivated the pathogen by 42 and 36% on upper and lower leaf surface, respectively.

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Effect of host nutrition on severity of Xanthomonas blight of Syngonium podophyllum. A. R. Chase, University of Florida, IFAS-Central Florida Research and Education Center - Apopka, 32703.

Syngonium podophyllum 'White Butterfly' are susceptible to a pathovar of Xanthomonas campestris which is systemic and causes a foliar blight. Plants were grown for 2 mo in a steam-treated potting medium top-dressed with Osmocote 19:6:12 at one of the following rates: 0.8, 3.4, 5.9, 8.4, 10.9, 13.5, 15.9, and 18.4 g/12.5-cm pot. Plants were inoculated with 1 x 10^8 cfu/ml of X. campestris to runoff and placed in plastic bags for 3 d. Plants received intermittent misting (5 sec/30 min, 12 hr daily) starting 24 hr prior to inoculation and continuing until test completion. Fertilizer rates above 0.8 g/pot did not affect plant quality or height but number of leaves responded quadratically as rate increased. Mean percentage of foliage with disease symptoms was reduced linearly as fertilizer rate increased ($y=10.9-0.5x$, $R^2=0.71$). This test was performed three times with Osmocote and three times with 20:20:20 applied as a liquid feed giving the same response each time.

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RELATIONSHIP OF SUSCEPT NUTRITION, AIR TEMPERATURES, AND DURATION OF LEAF WETNESS TO THE DEVELOPMENT OF HELMINTHOSPORIUM LEAF SPOT OF CREEPING BENTGRASS AND KENTUCKY BLUEGRASS. H. B. Couch and B. D. Smith. Dept. of Plant Path., Physiol., and Weed Sci., Virginia Tech, Blacksburg, VA 24061.

Helminthosporium leaf spot, incited by Bipolaris sorokiniana, is a major disease of bentgrass (Agrostis palustris) and Kentucky bluegrass (Poa pratensis) in sections of the North American continent characterized by warm, humid summer months. Bentgrass and bluegrass plants grown under nutritional regimes of either high imbalanced N or high balanced N, P and K were more susceptible to the disease that those grown under either low imbalanced N or low balanced N, P and K. Plants grown at a soil moisture regime of cyclic -0.033 MPa → -1.5 MPa → -0.033 MPa, were more susceptible to the disease than those grown at continuous -0.033 MPa. At a leaf surface temperature of 21 C, 48 hr leaf wetness was required for a high incidence of infections. However, when leaf surface temperatures were 26 -32 C, the same magnitude of infections occured within 24 hr.

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EFFECTS OF GROWER ACTIVITY AND ENVIRONMENTAL MODIFICATION ON BOTRYTIS CINEREA CONIDIAL CONCENTRATION IN A GREENHOUSE. M. K. Hausbeck and S. P. Pennypacker. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Botrytis cinerea conidial concentration in a commercial greenhouse was monitored using a Burkard recording spore trap during February to August, 1986. In a geranium (Pelargonium x hortorum) stock system that had been established for five months (planted in October, 1985), increased conidial concentrations were associated with grower activity including watering, pesticide application, and harvesting of cuttings. When plants were eleven months old, diurnal fluctuations of conidial populations occurred. The environment of plants on a 4' x 60' bench was modified by forcing warm air into the plant canopy. This treatment created an environment with a reduced relative humidity compared to that monitored in a control bench and during grower activities, conidial concentrations were also reduced for the sampling period of 26 February to 24 March, 1986.

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CAUSE AND CONTROL OF A CANKER DISEASE OF ULMUS PARVIFOLIA. A. H. McCain and H. Shull, Dept. of Plant Pathology, University of California, Berkeley, CA 94720

A serious canker disease of Ulmus parvifolia (Evergreen Chinese Elm) occurs in the San Francisco Bay area of California. Lenticular shaped, perennial cankers occur on trunks and branches. The cankers originate from twigs infected with anthracnose caused by Stegophora ulmea. The 'Drake' cultivar is resistant to S. ulmea and does not develop anthracnose or cankers whereas 'Truegreen' and 'Evergreen' are susceptible and develop anthracnose and cankers. Excision of a narrow band (6 mm) of living bark at the edge of the cankers eliminates the infection and healing takes place. Benomyl is effective in controlling the anthracnose phase of the disease.

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BOTRYODIPLODIA THEOBROMAE, A STRESS PATHOGEN OF DOGWOOD (CORNUS FLORIDA). J. M. Mullen, Plant Pathology Dept., C. H. Gilliam, Horticulture Dept.; A. K. Hagan, and G. Morgan-Jones, Plant Pathology Dept.; Auburn University, AL 36849.

In 1985 several field-grown 3-yr-old pink dogwoods in a nursery setting began to wilt and die. Declining trees showed evidence of large, inconspicuous trunk cankers slightly darker than the normal bark color. Botryodiplodia theobromae was consistently isolated from canker margins. Pathogenicity of B. theobromae was compared on non-stressed and drought-stressed white seedling dogwoods, 4-5 ft tall. Drought stress was applied before, after, or before and after fungal inoculation. Cankers developed only on drought-stressed trees. Largest cankers developed on trees subjected to preinoculation stress or a combination of pre- and post-inoculation stress. Smaller cankers developed on trees subjected to a post-inoculation stress only. Non-stressed trees did not appear to be susceptible to this disease. B. theobromae was consistently isolated from all inoculated trees.

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PROGRESS IN THE DEVELOPMENT OF PERENNIAL RYEGRASS CULTIVARS WITH IMPROVED RESISTANCE TO RHIZOCTONIA BROWN PATCH. B.B. Clarke, J.M. Johnson-Cicalese, and C.R. Funk, Rutgers University, New Brunswick, NJ 08903.

Brown patch, caused by the fungus Rhizoctonia solani, is a widespread disease of perennial ryegrass (Lolium perenne) in regions of the United States receiving extended periods of warm humid weather. Turf trials were conducted to select and develop perennial ryegrasses with improved resistance to brown patch under field conditions in NJ. Perennial ryegrass cultivars and selections differed substantially in disease reaction. Most ryegrass cultivars originating from the cool summer climates of northwestern Europe, New Zealand or Canada (i.e.-Pelo, Ruanui and Norlea) were highly susceptible under turf maintenance. Germplasm from the continental climates of central Europe, however, produced cultivars (Loretta and Elka) with moderate levels of brown patch resistance. Cultivars (i.e.-Palmer, Prelude and Premier) developed from naturalized ecotypes in the Mid-Atlantic region of the U.S. exhibited the most significant improvements in resistance to this disease.

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EFFECT OF AIR TEMPERATURE AND HERBICIDE INDUCED STRESS ON THE SUSCEPTIBILITY OF CREEPING BENTGRASS TO CURVULARIA LUNATA. B. D. Smith and H. B. Couch. Dept. of Plant Path., Physiol., and Weed Sci., Virginia Tech, Blacksburg, VA 24061.

Separate experiments were conducted to determine the effects of heat stress, 2,4-D, dicamba, bensulide, MSMA, and MCPP on the capacity of Curvularia lunata to infect and colonize "Penneagle" creeping bentgrass (Agrostis palustris), and on the relative health of bentgrass plants in the absence of C. lunata. Individual treatments consisted of plants which were untreated, herbicide treated, and/or heat stressed. Subgroups were then sprayed with an aqueous suspension of spores and mycelium of C. lunata or left uninfested. All plants were then placed in a dew chamber for 72 hours at 30 C. Plants were evaluated for chlorosis, leaf tip dieback, and the extent to which they were colonized. Stress induced by heat and/or the herbicides resulted in greater colonization by C. lunata only to the extent that these treatments produced moribund plant tissue.

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CHARACTERIZATION OF BROWN PATCH ON CENTIPEDE AND ST. AUGUSTINE GRASS IN SOUTH CAROLINA. R. A. Haygood and S. B. Martin, Department of Plant Pathology and Physiology, Clemson University, Clemson SC 29634-0377 and Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, New Haven, CT 06504.

Centipede and St. Augustine grass specimens displaying symptoms of brown patch were submitted frequently in November and December of 1986 to the Clemson University Plant Problem Clinic from the sand hills and the coastal plains of South Carolina. Rhizoctonia solani anastomosis group 2 type 2 was consistently isolated from the soft, basal sheaths of both grasses. Optimum temperatures for growth of isolates from both grasses on PDA was approximately 26 C. Isolates from Centipede grass were pathogenic to both Centipede and St. Augustine grass. Brown patch is apparently very destructive on both grasses as they enter dormancy in the fall and early winter. The authors are unaware of a previous report on the completion of Koch's postulates on brown patch of Centipede grass.

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ROOT ROT OF BAMBOO PALM CAUSED BY Phytophthora arecae. R.C. Ploetz and D.J. Mitchell, TREC and IFAS, Univ. of Florida, 18905 S.W. 280 St., Homestead, 33031, and Dept. Plant Pathology, Fifield Hall, Univ. of Florida, Gainesville 32611, respectively.

Phytophthora arecae (Coleman) Pethybridge was isolated from brown to black, necrotic roots of declining bamboo palms (Chamaedorea seifrizii X erumpen cv. 'Florida Hybrid') grown in a nursery in Homestead, Fla. In artificial inoculation studies, this fungus was shown to infect apices and naturally injured portions of first- and second-order roots and to cause the symptoms above. Drenches of metalaxyl (Ridomil 2 EC) at 0.015 g a.i./L or phosetyl-Al (Aliette 80 WP) at 1.15 g a.i./L reduced infection and symptom development in seedlings of this ornamental palm essentially to levels observed in noninoculated control plants.

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ETHYLENE RESPONSE AND CHLOROPHYLL LOSS IN SEQUENTIALLY SENESCENT LEAVES OF POA PRATENSIS INFECTED BY BIPOLARIS SOROKINIANA. C. F. Hodges, Department of Horticulture, Iowa State University, Ames, IA 50011.

The upper epidermis of 4 sequentially older leaves of a P. pratensis shoot were inoculated with B. sorokiniana. Plants were grown under ambient (1000 mbar) and hypobaric (233 mbar, O_2, CO_2 supplemented) pressures. Each older leaf was analyzed for ethylene and Chlorophyll (Chl) at 24, 48, 72, and 96 hr after inoculation. Endogenous ethylene of inoculated leaves peaked at 48 h and then declined. Peak ethylene of the 2 youngest leaves was 3.25-3.75 $\mu l\ l^{-1}$ and the two oldest leaves about 2.5 $\mu l\ l^{-1}$. The younger leaves with greater ethylene under ambient pressure had less Chl loss (25-30%) than older leaves (35-40%) with a smaller increase in ethylene. Hypobaric pressure reduced Chl loss in younger (8-10%) and older (19-20%) infected leaves. The results suggest that older tissues are more sensitive to ethylene and that tissue age may be a factor in the dual role of ethylene as an inducer of resistance and as a contributor to symptom expression.

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Production of monoclonal antibodies against an isolate of apple mosaic virus from rose. S.-M. Wong and R. K. Horst. Dept of Plant Pathology, Cornell University, Ithaca, NY 14853.

An isolate of apple mosaic virus (ApMV) was isolated from rose cultivar 'Sweet Surrender' in New York. After screening a total of 584 hybridomas, 20 ApMV specific monoclonal antibody secreting cell lines were obtained from 2 fusions between immunized spleen cells of BALB/c strain mouse and murine myeloma cell line Sp 2/0-Ag.14. Seventeen Mabs were of IgG_1 type, 1 was IgG_{2b}, and 2 were IgM. Ascites fluid was produced from 10 selected cell lines. When tested with indirect ELISA, 8 out of the 10 Mabs gave maximum dilution end points of 1:100,000 to 1:1,000,000. None of the Mabs reacted to 12 isolates of prunus necrotic ringspot virus from rose in New York. Seven out of the 20 Mabs would precipitate the homologous virus. This is the first report of ApMV specific Mabs that possess the virus precipitation property which may have applications in virus indexing and diagnosis programs.

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A MAGNAPORTHE SP. WITH A PHIALOPHORA CONIDIAL STATE CAUSES SUMMER PATCH DISEASE OF POA PRATENSIS L. AND P. ANNUA L. P.J. Landschoot and N. Jackson, University of Rhode Island, Kingston, R.I. 02881.

Poa pratensis L. and P. annua L. exhibiting symptoms of summer patch disease in Rhode Island were surveyed for the presence of brown, ectotrophic fungi on the roots and crowns of affected tillers. A fungus with a Phialophora conidial state was consistently isolated and fruited in culture to produce the teleomorphic state. According to Mr. John Walker of the Biological and Chemical Research Institute, Rydalmere, Australia, the fungus is a member of the genus Magnaporthe Krause and Webster, similar to M. rhizophila Scott and Deacon. Two-year-old P. pratensis sod inoculated with the Magnaporthe sp. resulted in roughly circular patches (26x18 cm in diam.) of stunted, reddish-brown to tan colored tillers. A tuft of apparently unaffected turf remained in the center of these patches. Results of these studies indicate a causal relationship between this Magnaporthe sp. and summer patch disease.

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INFLUENCE OF ACIDIC IRRIGATIONS ON ELEMENTAL CONCENTRATIONS IN WOODY ORNAMENTALS. J. T. Walker and J. B. Melin, University of Georgia, Georgia Station, Experiment, GA 30212.

Acidic irrigations (pH 2 or 3), were applied either 6 or 12 times from June through September to 8 species of container-grown woody ornamentals. Sulfuric and nitric acids (0.5 N, 2:1) were used to adjust the pH of the irrigation water. Twelve irrigations at pH 2 or pH 3 increased the sulfur levels 55% and 33%, respectively, above the levels for 6 applications. Plants exposed to 6 irrigations at pH 3 were similar in sulfur levels to those of controls, except for three species. No significant differences occurred in manganese or magnesium levels as a result of irrigations. Species differed from each other in elemental composition. There was species X irrigation interaction with iron at pH 2 but not at pH 3. Visible injury to all species resulted from irrigations at pH 2 but not from those at pH 3.

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RESPONSE OF LOBLOLLY AND SHORTLEAF PINE ROOT TIPS TO INFECTION BY PHYTOPHTHORA CINNAMOMI. J. C. Jang and F. H. Tainter. Department of Forestry, Clemson University, Clemson, SC 29634-1003.

Six-week-old loblolly and shortleaf pine seedlings were inoculated with high (5000 spores/ml) and low (2000 spores/ml) concentrations of zoospores of Phytophthora cinnamomi. Electron and light microscopy revealed that vegetative hyphae penetrated mostly intercellularly into root tips of both pine species. Penetration hyphae colonized cortex tissue of shortleaf pine root tips very rapidly, within 2 hr after inoculation, especially with high conc treatment. Less fungus establishment in loblolly pine root tissue was observed. After 24 hr incubation, the extent of colonization was not different between loblolly and shortleaf pine with the high conc treatment. Although no apparent differences in penetration and colonization between high and low conc treatments were found in shortleaf pine, fewer penetrations and slower hyphal development were observed in the low conc treatment of loblolly pine. Electron microscopy showed no hypersensitive reaction.

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ANALYSES OF ^{14}C TRANSLOCATION THROUGH COTTON PLANTS TO SCLEROTIA OF PHYMATOTRICHUM OMNIVORUM. S. D. Lyda and J. Z. Riggs, Dept. Plant Path. & Micro., Tex. Agr. Exp. Sta., College Station 77843.

Basipetal translocation of ^{14}C through cotton plants to sclerotia of Phymatotrichum omnivorum was studied in field and greenhouse tests. Ten cotton plants in ten locations were fumigated for 20 min with $^{14}CO_2$ generated by acidification of 100 mCi $NaH^{14}CO_3$. The fumigated plants were harvested weekly for 10 wks and their root systems were removed via extracting a 15x90-cm soil core. Plants were divided into bolls, leaves, stems and roots, oven dried, ground and analyzed for total ^{14}C. A similar test was conducted under greenhouse conditions with 3 mCi of the isotope. Nine inoculated cotton plants and three pots of soil were analyzed weekly over a 10-wk period. All ^{14}C analyses were conducted with a Tri-Carb Tissue Oxidizer. High levels of isotope activity were found in all plant parts but minimal activity was noted in sclerotia from the field. Sclerotia were recovered in 18% of soil cores. Radioactivity was found in sclerotia coincident with plant death in the greenhouse test.

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EFFECT OF MONOCULTURE OF RADISH ON RHIZOCTONIA DISEASE IN DIFFERENT SOILS FROM HAWAII. L. L. Chern and W. H. Ko, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI 96720

Monoculture of radish in the presence of Rhizoctonia solani caused a decrease in disease incidence in some but not all replicates of the five Hawaiian soils tested. Suppressiveness could not be induced by monoculture of radish in the presence of R. solani in Volcano (pH 5.1) or Kawaihae (pH 7.9) soil. Although monoculture of radish induced suppressiveness to Rhizoctonia disease in Hamakua (pH 4.5) and South Kohala (pH 6.5) soils, disease decline following successive monoculture in different replicates was not directly correlated with suppressiveness when fresh inoculum of R. solani was added. These results suggest that in the study of induction of suppressive soil by monoculture in the presence of the pathogen, the result of each replicate should be treated separately, and the measurement of suppressiveness should be based on the disease severity with fresh inoculum added after the last replanting.

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POLYETHYLENE MULCHING OF COTTON FOR ERADICATION OF VERTICILLIUM DAHLIAE: MECHANISMS OF ACTION. J. A. Liebman, O. C. Huisman, and L. J. Ashworth, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

When clear polyethylene mulch was applied around cotton, microsclerotia (MS) of Verticillium dahliae were killed to a depth of 120cm in soil between but not within plant rows. A significant portion of MS were killed within 8 days after tarps were applied. In mulched soil shaded with fiberglass insulation, MS survived as well as in the control plots. High temperatures (45C) were sufficient to kill MS in the upper 30cm beneath mulch. At lower depths (75-120cm) temperatures were not high enough to kill MS; thus other mechanisms operated. MS suspended in a gas space at 75cm were not killed by mulching, while MS in adjacent field soil were killed. No differences were detected between mulched and non-mulched soil for oxygen or carbon dioxide content, oxygen diffusion rates, or mobilization of manganese. There was no evidence that gases directly toxic to MS accumulated in tarped soil.

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THE DEBILITATION OF COCHLIOBOLUS SATIVUS CONIDIA IN SOIL AT HIGH MATRIC POTENTIALS. D. J. O'Leary and J. L. Lockwood, Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312

Membrane filter envelopes containing C. sativus conidia were buried in loam soil at 0, -50, -150 and -300 mb matric potentials (ψ_m). Conidia incubated at 0 mb for 7, 14 and 28 days were 89, 24 and 8% viable (fresh conidia=98±1%), had 42, 19 and 9% germination on a salts solution (fresh=72±5%), and caused 75, 47 and 2% necrosis of wheat seedlings inoculated with 100 conidia (fresh=78±16%), respectively. Viability did not differ among ψ_m less than 0 mb, and on days 7 and 14 viability of conidia at these ψ_m was not significantly ($P=0.05$) different from that of fresh conidia; however, on day 28 conidia at -50, -150, and -300 mb were significantly less viable (79, 71, 70%, respectively) than fresh conidia (99%). On day 28 conidia incubated at -150 (31%) and -300 (44%) germinated significantly less in the salts solution than did fresh conidia (73%). Aggressiveness did not decline at ψ_m less than 0 mb.

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ROOT-INFECTING, RHIZOSPHERE, AND NONRHIZOSPHERE FUNGI ASSOCIATED WITH CITRUS IN FLORIDA. G. Smith and S. Nemec. U. S. Department of Agriculture, 2120 Camden Road, Orlando, FL 32803.

Mean population densities (MPD) of root-infecting, rhizosphere, and nonrhizosphere fungi were enumerated from a Florida coastal flatwood and a central ridge citrus site. Fourteen root-infecting fungi were isolated from rough lemon feeder roots; Botriodiplodia theobromae, Cylindrocarpon destructans, Fusarium equiseti, F. oxysporum, F. semitectum, F. solani, Macrophomina phaseoli, Neocosmospora vasinfecta, Phoma sp., Phytophthora parasitica, Pyrenochaeta sp., Rhizoctonia solani, Thielaviopsis basicola, and an unidentified pycnidial fungus (UPF). After 1 year of tree growth, rhizosphere MPD's of F. solani, T. basicola, and the UPF were, respectively, 99,500, 3,736, and 33,952 cfu/g soil in the coastal flatwood site and 30,395, 2,271, and 56,911 cfu/g soil in the central ridge site. Nonrhizosphere MPD's of F. solani, T. basicola, and the UPF were, respectively, 700, 17, and 367 cfu/g soil in the coastal flatwood site and 737, 111, and 4,367 cfu/g soil in the central ridge site.

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THE INFLUENCE OF PRATYLENCHUS PENETRANS AND TEMPERATURE ON ROOT ROT OF STRAWBERRY BY BINUCLEATE RHIZOCTONIA SPP. J. A. LaMondia and S. B. Martin, Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504.

The influence of P. penetrans (PP) and temperature on root rot of strawberry by binucleate Rhizoctonia spp. was examined in a factorial experiment. Sixteen-week-old strawberry runner plants (cv. Honeoye) were inoculated with 3 isolates of binucleate Rhizoctonia spp. (AG A, AG G, and AG I, sensu Ogoshi), 3 levels of PP from monoxenic culture in suspension at rates of 0, 17 or 170 PP/cm^3 of soil, and incubated 8 wk at 10 or 24 C. A line intercept method was used to estimate the length of healthy and Rhizoctonia rotted roots. In all cases, the high PP level increased the amount of fungal root rot, with greater root rot at 24 than at 10 C. Final nematode populations were higher at 24 C (39 - 98 PP/g root) than at 10 C (12 - 97 PP/g root) and were not affected by AG. There were no differences in disease severity among the AG at 24 C, but at 10 C, AG I induced more root rot than AG A or AG G.

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PATHOGENIC PYTHIUM SPP. ISOLATED FROM SUGARBEETS GROWN IN THE TEXAS PANHANDLE. C. M. Rush and E. M. Baker. Texas Agricultural Experiment Station, Texas A&M University, Bushland, TX 79012.

Approximately 38,000 acres of sugarbeets (Beta vulgaris L.) are grown in four counties of the Texas Panhandle. During the summer and fall of 1986, diseased beets were sampled to determine etiology. Three Pythium spp. were subsequently isolated, P. deliense Meurs, P. irregulare de Bary, and a heterothallic species in the P. sylvaticum Campbell and Hendrix complex. Soil samples were then taken from five fields in each of the four beet growing counties to determine the prevalence of each species. Pythium deliense was recovered from 25% of the sampled fields, the heterothallic species from 75%, but P. irregulare was not recovered from the soil samples. Koch's postulates were performed to determine the pathogenicity of selected isolates from beets and soil dilution plates. All isolates of the three species were pathogenic. The economic importance of these pathogens is as yet undetermined.

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ROLE OF HYLASTINUS OBSCURUS AND FUSARIUM AVENACEUM IN RED CLOVER ROOT DECLINE. Xixuan Jin and J. Morton. Dept. Plant Pathology, West Virginia University, Morgantown, WV 26506-6057.

Fumigated (methyl bromide) and nonfumigated field plots of red clover were established with the following treatments added to soil: unamended, 340 kg P ha^{-1}, 2% (v/v) F. avenaceum-infested oat kernels, and 2% sterilized oat kernels. In 1985, F. avenaceum was isolated from 3-25% of fibrous roots but from < 2% of tap roots. Infection was highest in low P treatments. No insect damage was evident. In 1986, colonization by F. avenaceum ranged from 10-25% in fibrous roots. Infection in tap roots (31%) was restricted mostly to sites of root injury. Feeding injury by H. obscurus (clover root borer) occurred on tap roots, with severity increasing with greater root diameter and higher soil fertility. Incidence and extent of root decay was similar in fumigated and nonfumigated plots with and without added Fusarium inoculum. Thus, the primary cause of red clover root decline appears to be insect-related, with infection by F. avenaceum of secondary importance.

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EFFECTS OF THIRAM AND ALUMINUM ON IN VITRO GROWTH OF SCLERODERMA CITRINUM AND S. MACRORRHIZON ISOLATES. P.M. Flemington, J.N. Bruhn, D.L. Richter. Michigan Tech. University, Houghton, Michigan.

Ectomycorrhizal Scleroderma spp., common to mesic and xeric sites in the northern Lake States, show potential use for inoculation of conifer seed. Treatments at the Wyman (Mich. DNR) Nursery employ thiram (protectant) and powdered Al (lubricant). In vitro effects of these substances on S. citrinum (1 isolate) and S. macrorrhizon (2 isolates) were assessed. Nursery application rates for thiram and Al were extrapolated to 0.04 g thiram/l and 0.02 g Al/l for use in MMN agar medium. Scleroderma isolates were grown on MMN agar + thiram or Al at 1/2X, 1X, and 2X the base rate. Radial growth progress was measured at 5 day intervals for 55 days. Using α=.05 for all tests, the S. citrinum isolate grew fastest, unaffected by Al. The slower growing S. macrorrhizon isolate was inhibited only by the 2X rate of Al while the faster growing isolate was inhibited by all levels of Al. The S. macrorrhizon isolates were inhibited by 1/2X thiram basal rate; the S. citrinum isolates by 1X. The S. citrinum isolate appears to be most compatible with nursery applications of thiram and Al. Elimination of thiram from nursery use may increase seedling colonization by all three Scleroderma isolates.

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PHYTOPHTHORA ROOT ROT DEVELOPMENT ON MYCORRHIZAL AND PHOSPHORUS-FERTILIZED NON-MYCORRHIZAL SWEET ORANGE SEEDLINGS. J. H. Graham and D. S. Egel, University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850.

Sweet orange seedlings were inoculated with Glomus intraradices (VAM) or fertilized with P (NM). Seedlings were transplanted into non-infested soil or soil infested with 1 or 10 chlamydospores/cm^3 of Phytophthora parasitica. In Experiment 1, non-infested VAM plants were larger than NM plants which were nearly deficient in P. P. parasitica reduced leaf P of VAM and NM plants, but reduced growth of only VAM plants. The percentage of root tips with rot was lower on VAM root systems. With the loss of root tips, VAM colonization was reduced. In Experiment 2, NM and VAM seedlings were similar in size and had sufficient leaf P. P. parasitica reduced leaf P and root weight irrespective of VAM treatment. P. parasitica reduced total and healthy root tips to about the same extent on NM and VAM root systems. The reduction of root rot by VAM was due to effects on P nutrition.

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CHARACTERISTICS OF GERMINATION, HYPHAL GROWTH AND ROOT PENETRATION BY FOUR SPECIES OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI. A. W. Barkdoll and N. C. Schenck, Plant Pathology Department, University of Florida, Gainesville, 32611.

Germination, hyphal growth and root penetration by four species (Glomus manihotis, G. mosseae, Acaulospora longula, and Gigaspora pellucida) of vesicular-arbuscular mycorrhizal (VAM) fungi were documented. Spores were buried in the soil on millipore filters near germinated seeds; soil (pH4.2 - 6.0) was infested with VAM fungi. After 21 days filters were removed to observe germination and hyphal growth; host roots were sampled for penetration. All species formed "vegetative spores" during hyphal growth without root penetration except G. pellucida which formed auxillary cells. Hyphae of G. pellucida often branched immediately before root penetration while the other three species penetrated without branching. Glomus manihotis grew along the root before penetrating while G. mosseae approached and penetrated at right angles. All species formed hyphal anastomoses on filters with A. longula and G. pellucida anastomosing more frequently.

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INCIDENCE OF VA MYCORRHIZAL FUNGI IN NATIVE AND CULTIVATED BRAZILIAN SOILS. N. C. Schenck, J. O. Siqueira, and E. Oliveira. Univ. of Florida, Gainesville, and ESAL, Lavras, MG, Brazil.

Soil samples from native "cerrado" vegetation and adjacent cultivated agricultural areas in southeastern Brazil were wet sieved for spores of mycorrhizal fungi. The relative incidence of each species of mycorrhizal fungi varied with location but average spore numbers per liter of soil were higher in cultivated (784 spores) than native (543 spores) soils. However, there was a greater diversity of species in native than cultivated soils. The number of predominant species in coffee monoculture was the lowest of the various agroecosystems sampled and consisted of species that were not plant growth stimulators. The occurrence of many species was influenced by the presence or absence of specific soil components as determined by soil analysis, e.g. P, Al, Fe, Ca, Mg, et al. Implications of these results in regard to mycorrhizal fungi and their possible involvement in "decline"-type diseases are discussed.

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ANASTOMOSIS GROUPS AND PATHOGENICITY OF RHIZOCTONIA SOLANI FROM POTATOES IN PERU. C. Martin and R. Anguiz. The International Potato Center (CIP), Apartado 5969, Lima, Peru.

Isolates of Rhizoctonia solani were recovered from stems, stolons and tubers of potatoes in three different agroecological zones of Peru: Coastal Valleys (150-270 m elevation), highland valleys (2400-3600 m elevation), and on the eastern slopes of the Andes (850 m elevation). Isolates were confronted against tester isolates of anastomosis groups (AG)-1,-2,-3,-4, and -5. AG-3 and AG-4 were found; the first in the highland isolates (cool environment), and the second in isolates from the coast and eastern slopes of the Andes (warm environments). A few isolates from the three agroecological zones did not anastomose with any of the tester AG isolates. Pathogenicity of AG-3 and AG-4 was determined by the percent damping-off of seedlings. These were grown from seed sown in soil artificially infested with a mixture of five isolates per each AG. Seedlings were kept at low (16-18°C) and high (15-34°C) temperatures for 30 days. The highest percentage of damping-off occurred in soil infested with isolates of AG-4 at both temperature regimes. However, percent seedling damping-off in soil infested with AG-4 isolates was 50% higher at high temperature than at low temperature (72.3 and 53.4% mortality). The same type of response at high and low temperatures was obtained with AG-3 isolates.

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A NECROSIS-INDUCING FACTOR PRODUCED BY PSEUDOMONAS SOLANACEARUM IN CALLUS TISSUES OR EXTRACTS OF INCOMPATIBLE CLONES OF SOLANUM PHUREJA. Y. Huang, J. P. Helgeson and L. Sequeira, Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Callus tissues derived from wilt-resistant or -susceptible clones of Solanum phureja respond differentially to inoculation with strains of Pseudomonas solanacearum. In incompatible combinations, tissues are rapidly killed, as is typical of a hypersensitive response. We now report that a necrosis-inducing factor was produced when bacteria (strain B1) were placed in contact with callus tissues or were grown in tissue extracts of clone C-3. The factor caused rapid browning of C-3 callus tissues and was heat and pronase sensitive. The factor was purified by ion exchange, gel permeation and affinity chromatography, and appears to be a protein of about 60 KD, as determined by gel electrophoresis. Low concentrations of this purified protein induced a necrosis of intact C-3 leaf tissues and rapid (24 hr) death of C-3 suspension-cultured cells, as determined by staining with fluorescein diacetate.

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INDUCTION OF SOYBEAN CHALCONE SYNTHASE mRNA IN RESPONSE TO PATHOGEN INOCULATION. D. N. Kuhn, G. Souciet, S. Dhawale, C. B. Jonsson. Biochemistry Dept., Purdue University, West Lafayette, IN 47907

Soybeans respond to a variety of stresses by producing the phytoalexin glyceollin. Chalcone synthase (CHS), a key enzyme in the glyceollin biosynthetic pathway, increases in activity and amount in soybean roots and leaves challenged with phytopathogens. We have measured the increases in chalcone synthase mRNA in leaves after inoculation with pathogenic and nonpathogenic races of *Pseudomonas syringae* pv *glycinea* (Psg) and in roots after inoculation with *Phytophthora megasperma* f.sp. *glycinea* (Pmg) races. In the incompatible Psg-soybean leaf interaction, CHS mRNA is induced 10 fold more than in the compatible interaction. CHS mRNA induction is identical in the compatible and incompatible Pmg-soybean root interactions. CHS mRNA is more abundant in the roots than in the leaves of uninoculated soybeans. CHS gene expression may be different in the roots and leaves. We have identified four different CHS genomic clones from soybean. With gene specific regions of these clones, we will determine the regulation of expression of each of these genes in the leaf and root under normal and pathogen stress conditions.

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EXTRACELLULAR PROTEASE OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA: CHARACTERIZATION AND INVOLVEMENT IN SOFT-ROT PATHOGENESIS. J.W. Willis, J.K. Engwall, J.E. Leach, and A.K. Chatterjee. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

E. carotovora subsp. carotovora strain Ecc71 produces an extracellular protease (Prt) which can be detected in culture supernatant using nutrient gelatin agar, an azocasein assay, or isoelectric focusing followed by gelatin activity overlay. The protease is heat stable in 10 mM Tris-HCl (pH 7.0) to 100°C, has a pI of ca. 5.1, and is a metallo-protease requiring Ca^{+2} for maximum activity. A previously cloned prt gene was inactivated by Tn*5* insertion and used to construct Ecc71 Prt^- mutants by marker exchange. Virulence on potato tubers was not detectably altered in the Ecc71 Prt^- mutants. The Prt digested hydroxyproline-rich glycoproteins (HPRG) isolated from potato tubers and tomato plants, whereas, culture supernatants from Ecc71 prt::Tn*5* mutants did not.

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EVALUATION AND INDUCTION OF RESISTANCE TO BLUE MOLD IN TOBACCO CULTIVARS DIFFERING IN LEVELS OF DUVATRIENEDIOLS. M. N. Rao, M. D. Wiglesworth, M. R. Siegel, and J. Kuč, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The 4,8,13-duvatriene-1,3-diols (DVT) are fungitoxic leaf surface components of tobacco. Tobacco Introductions TI 1068, TI 1112, TI 1406 and Ky 14, with different DVT levels, were evaluated for resistance to blue mold caused by Peronospora tabacina Adam. In greenhouse and field, TI 1112 was very resistant, closely followed by TI 1068. Ky 14 was susceptible and TI 1406 most susceptible. In the field, susceptibility decreased with age. TI 1068 had 1.5-5.0 X and TI 1112 and TI 1406 1/3-1/4 X the DVT level in Ky 14. DVT levels increased significantly with age in TI 1068 and Ky 14, and very little in TI 1112 and TI 1406. Systemic protection was induced in all cultivars by stem injection with sporangiospores of P. tabacina although DVT levels did not change. DVT was not responsible for induced systemic protection and it is not the sole factor contributing to resistance in tobacco.

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DIFFERENTIAL PROTEIN EXPRESSION IN WATERMELON XYLEM FLUID. C. L. Biles and R. D. Martyn, Dept. Plant Path. & Micro., Tex. Agri. Exp. Sta., College Station 77843.

Xylem fluid and cotyledon tissue of six watermelon (Citrullus lanatus) cultivars differentially susceptible to races 0, 1, and 2 of Fusarium oxysporum f. sp. niveum (FON) were assayed for general proteins and specific enzymes using IEF-PAGE and starch gel electrophoresis (SGE). SGE showed no polymorphic loci in the six enzyme systems examined (GOT, MDH, PGI, IDH, PGM, PER); however, differences were detected in the xylem fluid of the cultivar susceptible to both races after silver staining of IEF-PAGE when compared to the other cultivars. The most significant difference was that the susceptible cultivar lacked a large band corresponding to approximately 30,000 Da. No difference was observed in cotyledon tissue using IEF-PAGE. Preliminary experiments indicated that germination of FON race 0 was inhibited when microconidia were incubated for 8 hr in xylem fluid of the cultivars resistant to both races; however, variable results were obtained after incubation with other resistant and susceptible cultivars.

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SPECIFIC CHEMOTAXIS OF PYTHIUM DISSOTOCUM ZOOSPORES TO ROOT CAP CELLS OF GOSSYPIUM SPECIES. M. C. Hawes[ab], N. P. Goldberg[a] and M. E. Stanghellini[a]. Departments of Plant Pathology[a] and Molecular and Cellular Biology[b], University of Arizona, Tucson, AZ 85721.

Root cap cells of two cotton species elicited a specific chemotactic response in zoospores of Pythium dissotocum. When roots of cotton seedlings were placed into a suspension of P. dissotocum zoospores, there was immediate attraction, accumulation and encystment exclusively in the root cap cell region. Furthermore, root cap cells remained attractive when isolated from the root and placed into a zoospore suspension: attraction, accumulation, and encystment on individual root cap cells occurred within seconds after contact. Zoospores penetrated and killed isolated root cap cells within 15-30 minutes, and seedlings died within 24 hours. In contrast, zoospores of P. catenulatum, which exhibited a chemotactic response to roots of Agrostis palustris (Bentgrass), were not attracted to and did not infect seedlings or isolated root cap cells of cotton.

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RESPONSE OF NEAR-ISOGENIC PEA CULTIVARS TO INFECTION BY FUSARIUM OXYSPORUM F. SP. PISI RACES 1 AND 5. M. J. A. Charchar and J. M. Kraft, USDA-Agricultural Research Service, IAREC, P.O. Box 30, Prosser, WA 99350.

Paired pea cultivars, differing by a single dominant gene for resistance or susceptibility to F. oxysporum f. sp. pisi race 1 (M410 = susceptible, Vantage = resistant) or race 5 (Sundance = susceptible, Sundance II = resistant) were compared for their response to infection. Scanning electron microscopy revealed that vascular plugs sealed off xylem elements in the lateral roots, epicotyl, and above ground stems of the race 1 and 5 resistant cultivars. Intense mycelial invasion, without the formation of vascular plugs, occurred in the susceptible cultivars. Xylem fluids collected from the susceptible cultivars stimulated conidial germination and germtube growth of both races 1 and 5. Xylem fluids from the resistant cultivars did not stimulate or inhibit conidial germination, but inhibited germtube growth. Resistance was due to physical containment and reduced fungal growth.

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SURFACE COLONIZATION AND INTERNAL INFECTION OF RESISTANT AND SUSCEPTIBLE PEA CULTIVARS BY FUSARIUM OXYSPORUM F. SP. PISI RACES 1 AND 5. M.J.A. Charchar and J.M. Kraft, USDA-Agricultural Research Service, IAREC, P.O. Box 30, Prosser, WA 99350.

Two sets of cultivars, each differing by a single dominant gene for resistance or susceptibility to F. oxysporum f. sp. pisi race 1 or 5, were compared for their response to infection. The response of both resistant (Vantage = resistant to race 1, Sundance II = resistant to race 5) and susceptible (M410 = susceptible to race 1, Sundance = susceptible to race 5) cultivars were similar. Races 1 and 5 were isolated from tap and lateral root apices and excised lateral root ends of resistant and susceptible cultivars. Internal tap root and hypocotyl invasion occurred in all cultivars tested. However, lateral roots and stems of both resistant cultivars were not infected as compared to the susceptible cultivars. Epicotyls from both susceptible cultivars were severely infected as compared to the resistant cultivars. Resistance was expressed in lateral roots, epicotyls, and stems.

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EVIDENCE THAT THE AMOUNT OF "IMMUNITY SIGNAL" AVAILABLE TO A LEAF AFFECTS INDUCED SYSTEMIC RESISTANCE TO ANTHRACNOSE IN CUCUMBER. L. Matthews, and J. Kuč. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Inoculation of the lower leaves of cucumber plants with conidia of Colletotrichum lagenarium (induction) protected leaves above against disease caused by subsequent inoculation (challenge) with this fungus. Protection was dependent on plant age and the number of leaves between the induced and challenged leaves. Protection in the terminal leaf of a younger plant was better than that of an older plant. Regardless of plant age, protection in a challenged leaf was directly related to its distance (number of internodes) from the induced leaves. Removing intervening leaves between induced leaves and leaves to be challenged significantly enhanced protection (22-83%), and the terminal leaf of young plants was still better protected than older plants.

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EFFECTS OF TEMPERATURE AND LIGHT ON REACTION OF CORN TO RACE 3 OF EXSEROHILUM TURCICUM. S. Leath, R. P. Thakur*, and K. J. Leonard, USDA-ARS, Plant Pathology Dept., NCSU, Raleigh 27695, and *ICRISAT, Patancheru, P.O., Andhra Pradesh 502324, India.

Race 3 of Exserohilum turcicum was described in 1980 as virulent to corn with resistance genes Ht_2 or Ht_3 and avirulent to Ht_1 plants; we were unable to confirm this in greenhouse tests. Near-isogenic lines with Ht_1, Ht_2, or Ht_3 and their recurrent parent (RP) were grown in environmental chambers at day/night temperatures of 26/22 C with a light intensity of 640 umol/m^2/s (PAR) or 22/18 C with either 640, 320 or 160 umol/m^2/s (PAR) and then inoculated with E. turcicum race 3 or race 1. At 26/22 C, race 3 was avirulent to Ht_2 and Ht_3 but virulent on the RP; virulence of race 3 to Ht_2 or Ht_3 was clearly expressed at 22/18 C particularly at low light. This more conducive environment resulted in more lesions and greater sporulation (P=0.05). Race 3 was confirmed and was different from race 1, although resistance of Ht_3 to race 1 was partially negated at 22/18 C under the lowest light intensity. The differential reaction was clearest at 22/18 C with a light intensity of 320 umol/m^2/s.

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EFFECTS OF OXALIC ACID CONCENTRATION ON CALLUS INDUCTION AND GROWTH OF FOUR ALFALFA CLONES IN TISSUE CULTURE. P. E. Pierson, L. H. Rhodes and D. K. Myers, Departments of Plant Pathology and Agronomy, The Ohio State University, Columbus, Ohio 43210.

Four alfalfa (Medicago sativa L.) clones from the cultivar Vernal selected for their ability to produce somatic embryos in tissue culture were examined for tolerance to oxalic acid (OA), a toxic metabolite produced by Sclerotinia spp. Petiole tissue from each clone was placed on callus induction medium containing 0, 1.0, 2.5, 5.0, 7.5 or 10.0 mM OA. After four weeks significant differences in callus fresh weight were noted for both the effects of clone and OA concentration. Two clones, 3B and 6B, were 4 times more tolerant at 5 mM OA than clones 3A and 5B. Callus induction for all clones was completely inhibited by 10.0 mM OA. Tolerance to OA in vitro may be a criterion for selection of alfalfa plants resistant to Sclerotinia trifoliorum.

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EFFECTS OF RIDOMIL TREATMENTS ON YIELD OF CORN IN TILLED VS. NO-TILLED PLOTS ON POORLY DRAINED SOIL. I. W. Deep, P. E. Lipps and D. P. Miller, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

In the poorly drained soil in Northwest Ohio, corn yields have been lower in no tilled than in fall plowed plots. Ridomil treatments were applied in 1984 and 1985 to determine whether Pythium infection may be responsible for the lower yields. In 1984, yield with Ridomil treatment was higher than the control in no tilled plots but was the same as the control in fall plowed plots suggesting activity by Pythium. In 1985 mean yields were higher following Ridomil treatment in both no tilled and fall plowed plots, but the differences were not significant. In 1986, Apron seed treatment and three levels of Ridomil were tested in plots which varied in tillage, drainage and rotation. Yields in the control were lower in no tilled than in fall plowed plots. However, none of the Ridomil treatments increased yield suggesting that Pythium was not responsible for these yield differences.

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WINTER AND EARLY SPRING SURVIVAL OF P. recondita IN WHEAT FROM 1981-86. M.G. Eversmeyer, C.L. Kramer, and L.E. Browder, USDA-ARS, Dept. Plant Path., Kansas St. Univ., Manhattan, KS 66506

P. recondita survived the winter and early spring in date of planting plots at Manhattan, KS 4 out of 6 yr from 1981-86. Biweekly winter and spring observations on plots were recorded. During winter periods in which no uredinia were observed, plants were transplanted into the greenhouse at intervals to allow for development of latent infections. In 1982 and 1983 uredinia survived on early planted wheat (Aug and Sep) but in 1985 and 1986 on late planted wheat. Losses of less than 1.5% occurred in years with no fungal survival. Losses were greater than 2.5% in years with fungal survival. Yield reduction was greater in date of planting plots in which P. recondita survived even though final severity recorded was identical to severity in plots where the fungus had not survived. Maximum severities were reached up to 2 wk earlier in plots where survival occurred. Pathogenicity of surviving inoculum may be very different from exogenous inoculum.

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PATHOGENICITY OF PYRENOPHORA TRITICI-REPENTIS FROM THE NORTHERN GREAT PLAINS. J.M. Krupinsky USDA, ARS, Northern Great Plains Research Lab, P.O. Box 459, Mandan, ND 58554.

Isolates of Pyrenophora tritici-repentis obtained from diseased wheat leaves collected from local experimental plots and from wheat fields in North Dakota, South Dakota, and Montana were tested for pathogenicity. Detached seedling leaves of wheat cultivars were inoculated to compare five to seven isolates in each inoculation. Percent necrosis was estimated and lesion length was measured. Although all isolates caused symptoms on wheat, the level of disease symptoms produced by the isolates varied. Differences among isolates obtained from the northern Great Plains region were more easily detected than differences among the isolates obtained from the local experimental plots. Cultures from local plots that were selected for high pathogenicity were comparable to selected cultures from the regional area.

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HISTOLOGY OF INFECTION OF WHEAT BY TILLETIA INDICA, THE KARNAL BUNT PATHOGEN. B. J. GOATES. USDA-ARS, Crops Research Laboratory, Logan, UT 84321-6300.

Infection of wheat by Tilletia indica (Mitra) was investigated using scanning and transmission electron microscopy and light microscopy. Spikes were inoculated beneath inverted petri dish cultures which discharged abundant secondary sporidia. Germ tubes penetrated only through stomatal openings. Hyphal growth toward stomata was common and apparent hyphal anastamosis occurred on the glume surface. Often, germ tubes penetrated beyond the stomatal ledge but did not pass between the guard cells which prevented entry into the substomatal chamber. Intercellular hyphae were present in the upper portions of the glume, lemma and palea among parenchyma and chlorenchyma cells, but were not present in the basal portions during the early stages of infection. The hyphae grew intercellularly toward the floret base and eventually entered the pericarp of the ovary through the funiculus. Hyphae were not present in the endosperm or embryo and penetration of the ovary wall did not occur despite direct in situ inoculation of ovaries.

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PATHOGENICITY OF TWO DIAPORTHE SPP. ISOLATES FROM SOYBEAN PLANTS WITH "SUDDEN DEATH SYNDROME" DISEASE SYMPTOMS. B. L. Keeling, USDA-ARS, Jamie Whitten Delta States Research Center, Stoneville, MS 38776

Two Diaporthe spp. were isolated from soybean plants with "Sudden Death syndrome" disease symptoms. Plants exhibited inter-veinal chlorosis, discoloration of root and lower stem tissue, and lateral root rot. Both Diaporthe spp. were isolated from all plants examined. Colony growth characteristics of one type is that of Diaporthe phaseolorum var. sojae (Dps) and the second type is that of D. p. var. caulivora (Dpc). The Dps type isolates caused seed and seedling rot and stunting. Emergence and plant height was reduced 40% to 50% when seed were planted in infested soil. The Dpc type isolates did not cause seed and seedling rot. Both types were pathogenic when seedling hypocotyls were inoculated using the toothpick method. Cultivar resistance to seed and seedling rot caused by the Dps type isolates has been demonstrated

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PATHOGENIC VARIATION IN PYRENOPHORA TERES ON BARLEY. B. J. Steffenson and R. K. Webster. Department of Plant Pathology, University of California, Davis, CA 95616.

Pathogenic variation in Pyrenophora teres was studied by inoculating 22 barley genotypes with single-spore isolates of the fungus in the greenhouse. At least 10 pathogenic races were identified from over 75 isolates evaluated from California. Five isolates from Mexico were similar to races found in California. In contrast, two isolates from Minnesota and England were markedly different in their virulence combinations. For all isolates, the most complex race was virulent on six of the differentials, whereas the simplest race was not virulent on any of the genotypes. The most common race found in California was virulent on Atlas, Kombar, Prato, Hazera and Beecher. Algerian, Rojo, Coast, Ming, Canadian Lake Shore, CI 9819, CI 5791, CI 7584, and CI 5822 were resistant to all isolates. These genotypes have proven useful as a standard set of differentials for typing pathogenic variation in the net blotch fungus.

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DEVELOPMENT OF NORTHERN CORN LEAF BLIGHT LESIONS AS AFFECTED BY POSITION AND DENSITY OF LESIONS ON LEAVES. Y. Levy and K. J. Leonard, Dept. Life Sci., Bar Ilan Univ., 52 100 Ramat Gan, Israel, and USDA-ARS, N. C. State Univ., Raleigh, NC 27695.

The sixth expanded leaves of Seneca 60 sweet corn plants were inoculated with 15-ul droplets containing an average of 15, 75, and 150 conidia of Exserohilum turcicum. Lesions developed at 20, 28, and 45%, respectively, of the inoculation sites in the proximal (younger) part of the leaf blade. Lesions formed at all inoculation sites in the middle or distal portions of the leaf blade. At 15 days after inoculation, areas of lesions in the proximal, middle, and distal portions of leaf blades averaged 28, 90, and 120 mm^2, respectively. Lesion extension in leaves with 7 lesions within 10 cm of the tip was 85% less than in leaves with a single lesion. Lesions in the proximal and distal portions of the leaf blade supported production of an average of 1,450 and 20,000 conidia/mm^2, respectively. These data show that proximal portions of leaf blades are less susceptible than distal portions and that lesions of E. turcicum compete for resources within a leaf.

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DEVELOPMENT OF COMMON SMUT GALLS IN SWEET CORN ARTIFICIALLY INOCULATED WITH Ustilago maydis. R. P. Thakur and K. J. Leonard, ICRISAT, Patancheru P.O., Andhra Pradesh 502324, India, and USDA-ARS, N. C. State Univ., Raleigh, NC 27695.

Sweet corn plants injected in the leaf whorl with 1-2 ml of a suspension of 10^4 to 10^6 sporidia/ml of compatible isolates of Ustilago maydis 3-13 days before tassel emergence developed tassel galls; plants inoculated between the leaf sheath and stalk 6-8 internodes below the tassel at 0-8 days before tassel emergence developed ear and stalk galls. Galls appeared 13-19 days after inoculation in hybrids grown in greenhouse soil beds; gall development was less consistent in plants in pots. Across 24 hybrids in soil beds, 45% of inoculated plants developed ear galls, 20% had stalk but not ear galls, and 13% had only tassel or leaf galls. Gall incidence was negatively correlated with number of normal ears/plant (r=-0.51), but ear development was reduced by 29% even in inoculated plants with no galls. There were 75, 77, and 68% fewer ears on plants with ear galls, galls on stalks but not on ears, and only leaf and tassel galls than on check plants injected only with water.

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EFFECTIVENESS OF THREE ASSAYS IN DETERMINING THE HOST SPECIFICITY OF A PHYTOTOXIN PRODUCED BY PYRENOPHORA TRITICI-REPENTIS. D. A. Brown and R. M. Hunger, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285.

Three assays were used to determine the response of six wheat cultivars and one non-host, barley, to a phytotoxin produced in vitro by Pyrenophora tritici-repentis, which causes tan spot of wheat. A detached leaf assay was designed which involved placing 10µl of phytotoxin on a wound; this resulted in the induction of chlorosis within 72-96 hours. Injecting 0.2ml of phytotoxin into the culms of adult plants mimicked the chlorosis induced by the pathogen within 72 hours for susceptible cultivars. A seedling assay based upon percent inhibition of coleoptile elongation during exposure to the phytotoxin was sensitive and quantitative. The six wheat cultivars varied in their response to the phytotoxin in the seedling assay, and barley was inhibited to approximately the same degree as the resistant wheats.

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Fine Structure of Apple Leaves Treated with the Sterol-Inhibiting Fungicide, Bitertanol. S. V. Overton, L. D. Moore and O. K. Miller, Departments of Plant Pathology, Physiology & Weed Science and Biology, VPI&SU, Blacksburg, VA 24061.

Ultrastructural observations were made of leaves of the apple cultivar Red Delicious 12, 24 and 72 hours following foliar application of the sterol-inhibiting fungicide, bitertanol. Thylakoids of chloroplasts from treated leaves appeared swollen and irregular 12 hours after treatment which resulted in a loss of integrity of the chloroplasts. Occasionally mitochondria appeared washed-out; although no other changes in membrane or organelle structure were observed. Within 24 to 72 hours after treatment, however, thylakoids of chloroplasts from treated leaves appeared to be similar to those of the controls. The number of starch granules in the chloroplasts of treated leaves increased throughout the 72-hour period and remained high relative to levels in controls. This suggests that bitertanol does not have a lasting effect on apple leaves.

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Ultrastrucure of Spilocaea Pomi in Apple Leaves Treated with Bitertanol. S.V. Overton, L.D. Moore and O.K. Miller, Departments of Plant Pathology, Physiology & Weed Science and Biology, VPI&SU, Blacksburg, VA 24061.

The effect of treatment of apple leaves with the sterol-inhibiting fungicide, bitertanol, on Spilocaea pomi was examined. Although hypae from untreated leaves were enclosed in an extracellular matrix, those in treated leaves had none. In treated leaves, fungal nuclei and mitochondria were affected 12 to 72 hr. following application of bitertanol. Nuclear envelopes were not well defined and mitochondrial matrices were dissolved as were the normally plate-like cristae of mitochondria. This was accompanied by the accumulation of minute electron dense bodies around their periphery. Invaginations and proliferations of the hyphal plasmalemma and increased vacuolization were also observed. Mesosome-like structures were found in hyphae 12 hr. following application of the fungicide. The various stages of ultrastructural changes, including lysis, were dependent upon the treatment period.

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CHARACTERIZATION OF THE POLYMERASE PRODUCTS OF THE dsRNA OF CRYPHONECTRIA PARASITICA. P. J. Kazmierczak, D. R. Hansen and N. K. Van Alfen. Utah State University, UMC 4500, Logan, UT. 84321.

Cryphonectria parasitica is a fungal pathogen that causes chestnut blight. Hypovirulent variants of this fungus have been isolated. Cytoplasmically transmissible dsRNA is associated with hypovirulence. The dsRNA does not have a protein coat, but is contained within membranous particles. We have evidence that an RNA polymerase associated with these particles produces both single-stranded and double-stranded RNA products. The strandedness of the ssRNA polymerase products has been determined by hybridization to plus and minus copies of the dsRNA cloned into the phage M13. The ssRNA products hybridize strongly to M13 DNA containing the insert of the minus strand. Further characterization of the dsRNA products will indicate which strand/strands are being labeled in vitro.

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ACCUMULATION AND COMPARTMENTALIZATION OF SANGUINARINE UPON FUNGAL ELICITATION OF PAPAVER BRACTEATUM SUSPENSION CULTURES. S. D. Cline and C. J. Coscia, St. Louis University School of Medicine, St. Louis, MO. 63104

A fungal elicitor preparation from conidia of Verticillium dahliae was added to suspension cultures of Papaver bracteatum maintained without hormone for 14-days. Elicited cultures accumulated the benzophenanthridine alkaloid, sanguinarine, in cells and media in an elicitor-dose-dependent manner. Sanguinarine (10^{-5}M) has biostatic activity against various fungal pathogens. Maximum cellular sanguinarine levels produced (0.6 mg/g fr. wt.) represented a 12-fold increase over the controls. A major portion (61-74%) of the total sanguinarine accumulated was found in the media. Levels of the alkaloid precursor, dopamine, were not changed. Subcellular fractionation of cultured cells on renografin step gradients revealed sanguinarine to be enriched 3-fold over the original cells at the 4-8% interface, suggesting that sanguinarine is compartmentalized in vesicles. Supported by NSF & NIH grants PCM 83-14368:HL-07050.

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ALTERNATIVE INDUCED DISEASE RESISTANCE PATHWAYS IN SOYBEAN AND THEIR REGULATION. M. R. Lambert and T. L. Graham, Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210.

The phytoalexin response of soybeans to Phytophthora megasperma (PMG) is well characterized. Results will be presented that indicate the existence of non-phytoalexin resistance mechanisms which may also be induced upon treatment with PMG wall glucans. The non-phytoalexin pathway was uncovered through a differential time course of induction by biotic elicitors and through the use of two classes of synthetic "elicitors" which may allow induction of the two pathways independently. Triphenylmethyl amphiphiles induce phytoalexins, but not the underlying resistance response, whereas 1-aminocyclopropane carboxylic acid (ACC) induces a characteristic set of early stress proteins and resistance to infection without the accumulation of phytoalexins. The differential expression of these two pathways may be linked to the status of S-adenosyl methionine metabolism and modulating effects of ethylene and the polyamines on PMG wall initiated events.

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CHANGES IN MAIZE PEROXIDASE ASSOCIATED WITH VARIATION IN SUSCEPTIBILITY TO BIPOLARIS MAYDIS RACE T. M. Akhtar and M. O. Garraway, Department of Plant Pathology, Ohio Agr. Res. Dev. Centr. and The Ohio State University, Columbus, OH 43210.

Detached maize (Zea mays) leaves were exposed either to high temperature stress (HTS) i.e. 6 h in dark at 42 C or to sodium bisulfite (500 mg/l), a reducing agent. Such leaves were inoculated with Bipolaris maydis race T (BMT) then incubated for 24 or 48 h in the dark at 28 C. Control leaves were similarly inoculated but not exposed to HTS or bisulfite. Peroxidase activity in buffer and salt extractable fractions decreased in tissues subjected to HTS while it increased in bisulfite treated ones. Similarly, HTS decreased while bisulfite increased activity of cathodic isoperoxidases from these extracts. Leaves exposed to HTS prior to inoculation were significantly more susceptible to BMT, as indicated by electrotyle leakage, than non-treated leaves. In contrast, leaves exposed to bisulfite were significantly less susceptible. Thus, the activity of peroxidase may play a mediating role in the variation in susceptibility of maize to BMT infection.

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THE INDUCTION AND ROLE OF A POLYSOME-ASSOCIATED PROTEIN IN PLANTS RESPONDING TO PATHOGEN INFECTION OR HEAT SHOCK. C. H. Wu[1], H. L. Warren[2], C. Y. Tsai[3], and S. D. Lyda[4], Dept. Plant Path. & Micro., Tex. Agr. Exp. Sta., College Station 77843[1,4], Dept. Bot. & Plant Path., Purdue Univ., W. Lafayette 47907[2,3].

A 57 kDa polysome-associated protein (PAP) was induced within 12 to 48 hr in several maize cultivars under compatible interactions with various fungal pathogens, including Bipolaris maydis, B. zeicola, Exserohilum turcicum, and Colletotrichum graminicola. The 57 kDa PAP was also induced in maize by heat shock at 36° to 42° C for 2 hr and by paraquat treatment. Similary, a 54 kDa PAP was induced in cotton by heat shock treatment, and a 52 kDa PAP was induced in soybean by infection of Phytophthora megasperma var. sojae and by heat shock. The purified 57 kDa PAP of maize reassociated with polysomes in vitro and inhibited polysomal translation. Since this PAP is rapidly induced in response to various biological and environmental stresses and may affect protein synthesis, it may play an important role in stress response and disease development. The conservation of the stress-induced PAPs in maize, cotton, and soybean remains to be investigated.

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EVIDENCE FOR THE INVOLVEMENT OF MOLECULAR COMPONENTS OF PAPILLAE IN ML-O RESISTANCE TO BARLEY POWDERY MILDEW. J. R. Aist, R. E. Gold, and C. J. Bayles. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

In coleoptiles of a resistant isoline (R), penetration attempts were typically unsuccessful and papillae contained a light-absorbing component (LAC), whereas in a susceptible isoline (S) the reverse occurred. Chlortetracycline inhibited both the resistance to penetration and the incorporation of the LAC into papillae in R but had little or no effect in S. Overall, the penetration efficiency was 5% where papillae contained the LAC, 99% where they did not, and 47% where the amount of light absorbance was intermediate. Autofluorescence, UV absorbance and lacmoid staining all suggested that the LAC contains phenylpropanoids. Acid fuchsin revealed a basic staining material (BSM) in papillae in R but not in S, and in R the BSM was specifically associated with penetration failures. Thus, the LAC and BSM in papillae may be molecular components of the ml-o resistance mechanism.

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PHOTOSYNTHESIS OF HEALTHY AND LEAF RUST INFECTED WHEAT. G. D. Statler, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

The apparent rate of photosynthesis in healthy and Puccinia recondita infected wheat leaves was determined with a LI-6000 portable photosynthesis system. Plants were inoculated at anthesis. A line with Lr19 was resistant, Lr16 was moderately resistant, and Thatcher was suceptible when inoculated with race 1, P. recondita. Photosynthesis was measured on alternate days to day 20 after inoculation. The rate of photosynthesis changed very little for inoculated resistant plants. Photosynthesis was reduced on days 8, 12, 15 and 20 for susceptible wheat plants and on days 12, 15 and 20 for moderately resistant plants. Greater reductions were detected at days 15 and 20 for both susceptible and moderately resistant plants.

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COMPARISON OF COAT PROTEIN AMINO ACID SEQUENCES OF THE FOUR AUSTRALIAN STRAINS OF SUGARCANE MOSAIC POTYVIRUS. D.D. Shukla, K.H. Gough and C.W. Ward, CSIRO, Division of Protein Chemistry, Parkville 3052, Australia.

A comparison of the complete coat protein amino acid sequence of the Johnsongrass (JG) strain of sugarcane mosaic virus with the partial amino acid sequences from three other Australian strains of the virus, sugarcane (SC), Queensland blue couch grass (BC) and sabi grass (Sabi), showed that the JG coat protein was substantially different from the other three strains, the sequence homology being 66%. This is in marked contrast to the high sequence homology between SC, BC and Sabi strains (95-100%) but similar to that (51-62%) found between coat proteins of distinct members of the potyvirus group. On the basis of these findings and other information on major differences in serological, biological and biochemical properties, we believe that the Australian JG strain should be considered an independent member of the potyvirus group. The name 'Johnsongrass mosaic virus' is proposed for this new member.

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EXPRESSION OF ALFALFA MOSAIC VIRUS (AMV) RNA4 IN TRANSGENIC PLANTS CONFERS VIRUS RESISTANCE. L.S. Loesch-Fries, E. Halk, D. Merlo, T. Zinnen, L. Burhop, K. Hill, K. Krahn, N. Jarvis, and S. Nelson. Agrigenetics Advanced Science Company, 5649 E. Buckeye Road, Madison, WI USA.

Transgenic Nicotiana tabacum plants that expressed AMV coat protein under control of the cauliflower mosaic virus 19S promoter were produced by Agrobacterium-mediated transfer of the gene from a binary vector. RNA transcripts that were of the expected size as well as coat protein of the M_r and antigenicity of AMV coat protein accumulated in transgenic plants. Plants that expressed the highest levels of coat protein developed fewer primary infections following inoculation with AMV and developed stemic infections slower than plants that did not express coat protein. Resistance was specifically against AMV virions. AMV RNA and an unrelated virus, tobacco mosaic virus, were as infectious on plants that expressed coat protein as they were on plants that did not.

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PHYSICAL CHARACTERIZATION OF TN5 INSERTIONS IN CHLOROSIS-DEFECTIVE MUTANTS OF P. SYRINGAE PV. TOMATO. C. L. Bender and D. K. Malvick, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285.

P. syringae pv. tomato PT23 produces the chlorosis-inducing phytotoxin, coronatine. Ten chlorosis-defective mutants of PT23 were previously induced using the transposon Tn5. Two of these mutants, PT23.20 and PT23.21, were further characterized and were shown to be defective in production of coronatine (Phytopathology 76:1100). In the present study, total genomic DNA was isolated from the ten mutants and digested with EcoRI. Southern blot analysis of the restricted DNA was carried out using a 3.3 kb internal fragment of Tn5. After subtracting 5.7 kb, which represents the size of Tn5, the size of EcoRI fragments having homology to the probe was estimated to be 18 kb (five mutants), 16 kb (two mutants), 14 kb (one mutant), 10 kb (one mutant) and 4 kb (one mutant). These mutants may be useful in elucidating the coronatine biosynthetic pathway.

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TRANSFORMATION OF *FUSARIUM SOLANI PISI* USING THE CUTINASE PROMOTER. **Martin B. Dickman and P.E. Kolattukudy.** Institute of Biological Chemistry, Washington State University, Pullman, WA 99164 and Ohio State Biotechnology Center, O.S.U., Columbus, OH 43210

Cutinase, an extracellular enzyme secreted by *Fusarium solani* f.sp. *pisi*, is an essential determinant for the compatible colonization of its host, the pea plant. Cutinase gene expression in the fungus is induced by the host cutin monomers. In order to study cutinase gene regulation, transformation vectors were constructed containing a promoterless gene for hygromycin resistance, which was translationally fused to flanking sequences of the cutinase structural gene. Either protoplasts or mycelia were transformed with the vectors and hygromycin resistant transformants were obtained. Successful transformation was assessed by Southern analysis with probes for the hygromycin resistance gene and the putative promoter. Results indicated that DNA representing both of these components had integrated into the *Fusarium* genome and that the antibiotic resistance was a manifestation of promoter activity of cutinase flanking sequences. Such analysis revealed that this promoter activity resided within the 750 nucleotide segment immediately 5' to the initiation codon. Deletion analysis of the fragment was used to further define the promoter sequence. Supported by NSF grant # DMB-8306835.

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TRANSFORMATION OF FUNGAL PROTOPLASTS BY ELECTRIC-PULSE TREATMENT. E. T. Marek, M. G. Richey, C. L. Schardl, and D. A. Smith. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Electric-pulse treatment was used to mediate DNA-transformation of protoplasts of the filamentous fungi, Fusarium solani f. sp. phaseoli and Aspergillus nidulans (UCD1). Antibiotic resistance was conferred to Fusarium, with 2.6 stable transformants per µg of DNA obtained. UCD1 was converted from trp^- to trp^+ with 6.0 stable transformants per µg of DNA obtained. For UCD1, this represented a 2-3 fold increase in transformation frequency as compared to polyethylene glycol (PEG)-mediated DNA uptake. The average number of viable protoplasts used was 10^7 per sample. Electroporation resulted in 30-50% survival of the protoplasts. This technique has several advantages for DNA-mediated transformation. It avoids the use of toxic chemicals (such as PEG and lithium acetate), it is faster and it can give greater transformation frequency.

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RELATIONSHIPS AMONG YIELD COMPONENTS OF WINTER WHEAT AS INFLUENCED BY STRIPE RUST (PUCCINIA STRIFORMIIS TRITICI WEST). X.B. Yang and S.M. Zeng, Department of Plant Protection, Beijing Agricultural Univ. Beijing, People's Republic of China.

Research was conducted in 1983, 1984, and 1985 at two locations to study the relationships among yield components of wheat infected with stripe rust. Epidemics in 3 by 6 m plots of were manipulated by whole-plot inoculation and interval spraying with colloidal sulphur. Various epidemic patterns and uniform disease within plots were obtained. Samples (five sites/plot, 10 plants/site) were taken to determine head number per plant, grain per head, weight/1000 seeds, and yield per plant. The relationship of yield components was analyzed with pathway coefficient analysis. Positive correlation coefficients among yield components indicated no compensation among the yield components. The value of correlation coefficients between yield components and the area under disease progress curve accumulated for each growth stage increased as the plant developed.

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THE EFFECTS OF EARLY-SEASON INFECTION OF WHEAT BY ERYSIPHE GRAMINIS f. sp. TRITICI ON LATE-SEASON LEAF AREA AND YIELD. S. Leath, USDA-ARS and Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616

Five wheat cultivars differing in resistance to Erysiphe graminis f.sp. tritici were planted in 1985 in the upper coastal plain of North Carolina. Triadimefon foliar sprays were applied all season to exclude mildew or from flag leaf emergence until maturity; an unsprayed check also was included. Full season control of mildew resulted in lower disease severities on penultimate leaves, lower area under disease progress curve values, and larger yields than other treatments. Full-season disease control did not consistently increase total leaf area (TLA) at heading. Nevertheless, mildew severity values in early April were negatively correlated (-0.24 to -0.32, p=0.05) with TLA in mid to late-May for Saluda[1], Knox 62[2] and HW3021[1] (susceptible[1] and slow-mildewing[2]). Correlations for a resistant cultivar, Coker 983, and a susceptible cultivar, Roy, did not indicate this. Early-season mildew infection may reduce TLA and yield of wheat in the Southeast.

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YIELD RESPONSE OF WHEAT TO STEM RUST AS DESCRIBED BY THE WEIBULL FUNCTION. M. T. McGrath, S. P. Pennypacker, and C. H. Kingsolver. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

The Weibull dose response model, a modification of the Weibull probability distribution function, was used to investigate the relation between area under disease progress curves (AUDPC) and yield response, expressed as 1000 kernel wt. The model was fit to data from 35 experiments on the epidemiology of Puccinia graminis f. sp. tritici on spring and winter wheat conducted between 1960 and 1968 at 12 locations in the central U.S. The varieties Cheyenne or Baart and rust race 56 were used in most treatment plots. Significant differences in the exponential term of the model indicated the existence of an interaction between location and proportional yield response. The parameter describing the rate of yield decrease was usually less than or equal to 1, therefore the response resembled a negative exponential curve. The predicted AUDPC value associated with a 63% yield reduction ranged from 341 to 908 percent-days.

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EFFECTS OF WATER DEFICIT AND BLAST DISEASE ON YIELD COMPONENTS IN THE UPLAND RICE CULTIVAR C22. J. M. Bonman, L. M. Sanchez, and A. O. Mackill. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Blast disease, caused by Pyricularia oryzae, and water deficits are two constraints to stable production of upland rice worldwide. To quantify possible interactive effects of blast and water deficit on yield components in cv C22, a factorial design was used with two blast levels (fungicide protected or inoculated with P. oryzae) and two water levels (fully irrigated or stressed from 42 to 58 d after seeding). No interaction between water level and blast level was detected. Highest disease incidence occurred in stressed, inoculated plots with 4% diseased leaf area and 44% severe neck blast. Blast and water deficit caused additive reductions in grain weight, spikelet fertility, total dry matter, and grain yield. Microclimate was more favorable for disease in the irrigated plots than in the drought-stressed plots. After full irrigation was restored, however, disease increased fastest in plots that had been stressed.

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EFFECT OF SYNCHYTRIUM DESMODII ON SEEDLING RECRUITMENT AND ADULT PLANT YIELD OF DESMODIUM OVALIFOLIUM. J. M. Lenné and C. Torres G., Tropical Pastures Program, CIAT, Cali, Colombia.

Desmodium wart, caused by Synchytrium desmodii, is a damaging disease of the promising tropical pasture legume Desmodium ovalifolium in the eastern plains of Colombia. From 1985 to 1986, the effect of wart on seedling survival and adult plant yield of D. ovalifolium (CIAT 350), the latter in flooded and non-flooded situations, was determined. In wart-free areas, seedling recruitment was 16/m and 21/m in 1985 and 1986, respectively. In wart-affected areas, no seedlings survived more than 5-mo in both years even though initial populations were greater than 500/m . Under flooded conditions, adult plant yield was reduced by 72.6% while under non-flooded conditions there was no yield reduction and no significant difference between wart affected and wart-free areas. The effect of wart on pasture productivity and stability is under investigation.

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LATE BLIGHT, POTATO YIELD AND TOTAL INTERCEPTED LIGHT. Francis J. Ferrandino, Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504.

The relationship between yield and late blight damage was studied in two 20 m x 20 m potato fields (cv. Superior) infected with Phytophthora infestans L. Most previous models of yield loss are based on disease severity and make no allowances for plant size or solar insolation. Yield is the end product of photosynthesis and depends on the interception of light by healthy tissue. Healthy leaf area was assessed weekly within 1.5 m x 1.5 m quadrats and total daily isolation was monitored. Tuber yields from these quadrats were not significantly reduced as long as less than 40% of the foliage was destroyed. Yields decreased rapidly with increasing disease and 70% defoliation caused about a 70% reduction in yield. These results will be explained in terms of a mathematical model which relates yield to the amount of light intercepted by healthy potato foliage over the growing season.

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EFFECTS OF BLACK SHANK DISTRIBUTION ON TWO FLUE-CURED TOBACCO CULTIVARS WITH DIFFERENT LEVELS OF BLACK SHANK RESISTANCE. C. S. Johnson, VPI & SU, So. Piedmont Agr. Exp. Sta., P.O. Box 448, Blackstone, VA 23824.

Six of the twenty plants in field plots of 'K 326' (low resistance) and 'K 394' (high resistance) were inoculated with 3 ml of a mycelial suspension of Phytophthora parasitica var. nicotianae in groups of 1, 2, 3, or 6 adjacent plants either 4 or 6 wks after transplanting. Yield, value, quality index, and average price were determined for plants adjacent to disease foci, as well as for whole plots. Whole plot yields dropped from 3963 (for untreated control plots) to 3309, 3198, 3082, and 3097 kg/ha for plots with disease foci containing 1, 2, 3, or 6 adjacent plants, respectively. Yields from plots with disease foci containing 3 or 6 adjacent inoculated plants were not significantly different. Black shank distribution also reduced quality index and average price of individual plants adjacent to disease foci but not when whole plots were considered. Economic value was unaffected when groups of inoculated plants were increased above 2 adjacent plants.

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IMPACT OF LEAFSPOT DISEASES ON YIELD OF ALFALFA VARIES AMONG HARVESTS. C. Lee Campbell and J. A. Duthie, Dept. of Plant Pathology, North Carolina State University, Raleigh, 27695.

In North Carolina, alfalfa is harvested 4-6 times/year; the importance of leafspot diseases (caused by Leptosphaerulina briosiana, Pleospora herbarum, Phoma medicaginis, and Cercospora medicaginis) at each harvest is understood poorly. In 1986, five harvests were compared in 24 3mx3m plots of alfalfa (cv. Arc), 12 of which were sprayed weekly with chlorothalonil (0.13 a.i. ml/m^2). Disease severity and dry weight yield, measured at each harvest in a central 1 m^2 portion of each plot, were significantly correlated only at harvests 4 and 5. The effect of fungicide treatment on both disease severity and yield varied significantly among harvests. At harvests 1 to 5, respectively, mean final disease was 6, 14, 8, 6, and 13% in unsprayed plots, and 3, 5, 4, 2, and 5% in sprayed plots. Over the year, 19% of the mean yield in sprayed plots was lost in unsprayed plots. Harvests 1 to 5, respectively, accounted for 18, 7, 12, 23, and 38% of yearly yield loss in unsprayed plots and 18, 27, 15, 28, and 12% of yearly yield in sprayed plots.

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FIELD LOSSES IN SUGARCANE FROM THE RATOON STUNTING DISEASE. M.P. Grisham, USDA/ARS, Sugarcane Research Unit, P.O. Box 470, Houma, LA 70361

In a series of field experiments at Houma, LA, yield effects of the ratoon stunting disease (RSD) caused by Clavibacter xyli subsp. xyli on sugarcane (Saccharum interspecific hybrids) were determined. In each experiment, the main (treatment) plots of the split-plot design had either RSD-infected or check plants, and the subplots had various sugarcane cultivars. Each plot was 5 m long and 6 m wide. There were four replications of each treatment X cultivar combination. Cane was harvested in three successive years, and yield per plot calculated as sugar per hectare. Yield losses were greatest for infected cane in the third year. Over the 3-year cycle, average yield losses in RSD-infected cane ranged from 4.7% for cultivar L 60-25 to 31.7% for CP 70-321 among eight cultivars tested in four to six experiments. Among experiments, yield loss varied, but rankings of cultivars by yield loss tended to be constant.

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INCIDENCE AND SEVERITY OF FUSARIUM WILT OF COTTON AS INFLUENCED BY PLANTING DATE. D. P. Jeffers, Department of Plant Pathology, University of California, Davis, CA 95616.

The Fusarium wilt complex of cotton is caused by Fusarium oxysporum f. sp. vasinfectum (Fov) and Meloidogyne incognita (Mi). The effect of three planting dates (3/23, 4/8, and 4/21) on incidence and severity of Fusarium wilt in cotton cultivars Acala SJ-2 and Acala SJC-1, and nematode resistant breeding lines N6072 and N8577 were examined in 1985 and 1986. Preplant populations of Fov varied from 200 to 1000 propagules/g soil and Mi from 4 to 410 J2 larvae/1000 g soil. Disease was evaluated by foliar symptom development and was recorded periodically throughout the season. Fov was isolated from serial sections of stem and leaf tissue during times of boll stress to determine the extent of plant colonization. Among the cotton selections plant death and yield loss were significantly higher (P=0.05) only for Acala SJ-2 for the 3/24 planting. The two nematode resistant breeding lines gave significantly higher yields (P=0.05) at each planting date than that of the two Acala cultivars.

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COMPARISON OF BROWN STEM ROT INCIDENCE AND SOYBEAN YIELDS IN IOWA FROM 1930 TO 1986. H. Tachibana, and K. G. Bidne, USDA-ARS, Department of Plant Pathology, Iowa State University, Ames, Iowa 50011.

When soybean yield curve for Iowa was studied for changes from 1930 to 1986, a leveling off of yields was observed for recent years. Based upon many surveys for brown stem rot (BSR), nature of the pathogen, and soybean production practices at the time, it was hypothesized in 1979 that the disease incidence in Iowa will be 100% of fields infested by 1986. Previously, BSR was considered a major disease of soybeans that can reduce yield as much as 44%. Thus, the recent leveling off of soybean yields is attributed to BSR. Statistical analysis of the curve by partitioning into four 14-yrs periods provided b-values for each period which were 0.38, 0.38, 0.63, and 0.49 for 1930-44, 1945-58, 1959-72, and 1973-1986, respectively. This study confirms a leveling of soybean production efficiency in Iowa in recent years and is attributed to the disease.

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IMAGE 1 -- IMAGE ANALYSIS SYSTEM FOR DISEASE MEASUREMENTS IN TURFGRASS PLOTS. W. W. Shane and E. B. Lowney, Dept. of Plant Pathology, Ohio State University, Columbus, OH 43210.

A new computer program, IMAGE 1, was developed to increase the speed and accuracy of disease severity assessments on turfgrass. The program utilizes the Oculus 200 digitizer board and Gray Library subroutines (Coreco Inc, Longueuil, Quebec, Canada J4H 3A7). A handheld video camera is used to record images of the turfgrass plots on tape. The video tape is replayed into the digitizer board within an IBM PC. Calculation and recording of plot severity information (% area affected) is very rapid (less than 3 seconds per plot). Advantages of IMAGE 1 versus Apple II computer-based systems are that the relatively faster speed and larger memory of the IBM allow a more comprehensive and user-friendly program. Also, the program is written in the easily-modified higher language C which compiles into compact, rapidly executable computer code.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES (MAB) TO PEANUT STRIPE VIRUS (PStV). J. N. Culver[1], J. L. Sherwood[1], and M. R. Sanborn[2], Dept. of Plant Pathology[1] and Dept. of Botany and Microbiology[2], Oklahoma State University, Stillwater, OK 74078-0285.

MAB to PStV were obtained by fusing spleen cells from BALB/c mice immunized with purified PStV to mouse myeloma cell line p3x63Ag8.653. MAB against PStV from two cell lines, 3AB5 and 7C14, were characterized for antibody isotype, antigenic specificity, and reactivity to other plant viruses. Both cell lines were shown to produce MAB of the IgG_{2A} subclass. A double antibody binding ELISA (Friguet, et al, J. Immunol. Methods 60:351) indicated that MAB produced by cell lines 3AB5 and 7C14 bound to different antigenic sites on PStV. In an ELISA against several other plant viruses, MAB from cell line 3AB5 reacted only to PStV while MAB from cell line 7C14 reacted to PStV and slightly to watermelon mosaic virus-1 and potato virus Y. Neither MAB reacted to four other potyviruses tested or to healthy host tissue.

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CHARACTERIZATION OF POTATO VIRUS S (PVS) GENOMIC RNA. J. Monis, S. Daniels, G. A. deZoeten and S. A. Slack. Department of Plant Pathology. University of Wisconsin-Madison, WI 53706.

PVS-RNA was isolated from purified virus. Under denaturing conditions (formaldehyde and formamide), the Mr of PVS-RNA was determined to be 2.39×10^6. Oligo-dt Cellulose affinity chromatography and 3' end (^{32}P-ATP) RNA sequencing demonstrated that PVS-RNA is polyadenylated. In vitro translation in rabbit reticulocyte lysates indicated that four major protein products of 124K, 112K, 98K, and 36K are produced by PVS-RNA. Antisera specific to the Andean and Type strains of PVS immunoprecipitated a major protein product of 36K. This molecular weight corresponds to that estimated for the coat protein (cp) subunit of other carlaviruses. In pulse chase experiments, the same molecular weight proteins (124K, 112K, 98K and 36K) were observed and cp could be immunoprecipitated by PVS antisera as early as 10^{11} into the experiment. Studies are in progress to determine the replication strategy of PVS-RNA.

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Effects of resistance in muskmelon to watermelon mosaic virus 2 pathogenesis. Gray, S.M. and Moyer, J.W. Department of Plant Pathology, North Carolina State University, Raleigh, NC.

The mechanisms of a resistance identified in muskmelon to watermelon mosaic virus 2 (WMV 2) were investigated in synchronously-infected plants. Symptoms induced by WMV 2 in resistant tissue are suggestive of a localizing effect by the plant on virus within leaves. Systemic movement of the virus among leaves is not inhibited. The resistance significantly reduced levels of infectious virus and capsid protein (CP) in greenhouse grown plants. In synchronously-infected plant tissue the temporal levels of CP in the resistant plants are increased to levels found in susceptable plants. Levels of infectious virus remained 2-4 times lower in the resistant plants. Temporal relationships between CP and cylindrical inclusion protein (CIP) were similar in susceptable plants. The CIP (MW 68 Kd) was inconsistently found in the resistant plants, although CIP breakdown products (MW 64-66 Kd) were extracted from the resistant tissue in varialble amounts over time. The resistance appears to affect cell-to-cell movement and virion production.

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CHARACTERIZATION OF A MECHANICALLY-TRANSMISSIBLE WHEAT SOILBORNE MOSAIC-LIKE VIRUS. T.L. Kendall[1], S.A. Lommel[1], and W.G. Langenberg[2], Depts. of Plant Pathol., Kans. St. Univ.[1], Manhattan, KS 66506 & Univ. of Nebr.[2], Lincoln, NE 68583.

A virus with properties similar to wheat soilborne mosaic virus (WSBMV) has been isolated. The virus can be inoculated to corn, sorghum, and Chenopodium species but not to wheat. The virus has a bipartite RNA genome separately encapsidated in rod-shaped virions composed of a single capsid protein of 20.5 kd and two (+) sense RNAs (6.5 kb and 3.5 kb). Relative concentrations of virions 1 and 2 are opposite those observed for WSBMV. Virions were dissociated by WSBMV antiserum suggesting a serological relationship, although Western blots were negative. Northern blot hybridization indicates no homology between the new virus and WSBMV. Translation products of the RNAs are similar but distinct from those of WSBMV. The virus is distinct from WSBMV and should be considered a new member of the proposed furovirus group.

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CITRUS TRISTEZA VIRUS PARTIAL MOLECULAR CHARACTERIZATION WITH COMPLEMENTARY DNA CLONES. L. A. Calvert, (1), R. F. Lee (2), E. Hiebert (1). 1. Dept. of Plant Pathology, U. of Florida, Gainesville, FL 32611. 2. U. of Florida, Citrus Res. and Education Ctr., 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, was used to prepare complementary DNA libraries. A clone from the T-30 library, designated 30F6, was partially sequenced and an open reading frame was identified. There were similarities between the amino acid composition of the CTV capsid protein and the predicted protein encoded by the clone 30F6. Two clones from T-36 cDNA library, designated P6A6 and P5B8 (7350 and 8500 bases in length, respectively) shared homology for approximately 2200 bases and represented nearly 70% of the CTV genome. Portions of the clone P6A6, which were proximal to the 3' end of the CRV-RNA, were sequenced. An open reading frame coding for at least 109 amino acids and terminating with a TAA stop codon was resolved.

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COMPARISON OF RELATIVE TITERS OF MILD AND SEVERE CITRUS VIROIDS BY INFECTIVITY AND HYBRIDIZATION. R. F. Lee, Univ. of Florida, Citrus Research and Education Ctr., 700 Expt. Sta. Rd., Lake Alfred, FL 33850 and S. M. Garnsey, USDA Horticultural Research Laboratory, 2120 Camden Rd., Orlando, FL 32803

The relative titers of citrus viroids (CV) which produce mild and severe symptoms in two citron (Citrus medica L.) seedling clones of differing reactivity were compared by Raymer and Diener's infectivity index (Virology 37:343) and by determination of the dilution endpoint of extracts in spot hybridization assays using purified severe CV isolate as a probe. The titer of severe CV isolates was higher than titer of mild isolates in comparable hosts. The titers of comparable CV isolates were consistently higher in the more reactive citron host. Relative titers as determined by hybridization assays, which could be done in days, were in agreement with the infectivity indices which required several months. In a separate test, ten CV isolates of variable severity were all recognized by spot hybridization assays with the labeled severe CV probe.

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FIRST REPORT OF MURINE MONOCLONAL ANTIBODIES SPECIFIC FOR ASPARAGUS VIRUS-II. M.S. Montasser and R.F. Davis, Dept. Plant Pathol., Cook College, N.J. Agric. Exp. Sta., Rutgers Univ., New Brunswick, NJ 08903.

Hybridoma technology was applied to produce monoclonal antibodies against asparagus virus II (AV-II). Spleen cells from BALB-c mice immunized with 125 ug of purified AV-II stabilized with 1% formaldehye, were fused with NS-1 myeloma cells. Twenty-seven out of eighty hybridomas secreted antibodies specific for AV-II. Hybridoma line 3-3C11 was cloned to single cells by limiting dilution. One of the clones produced monoclonal antibodies highly specific in AV-II detection by ELISA. Ascitic fluid was produced by injecting this clone into pristane-primed BALB/c mice. Isotyping of monoclonal antibodies using immunoglobulin subclass specific antisera revealed them to be of IgM type. Titer of the monoclonal antibodies in indirect ELISA using AV-II infected asparagus tissues was 102,400. Other hybridoma cell lines are under evaluation for ability to secrete antibodies specific against AV-II.

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PREPARATION OF MONOCLONAL ANTIBODY SPECIFIC FOR DOUBLE-STRANDED RNAS. C. A. Powell, PA Dept. of Agriculture, Harrisburg, PA 17110 and F. E. Gildow, Pennsylvania State University, Dept. of Plant Pathology, University Park, PA 16802.

Approximately 5,000 independently derived hybridoma clones of spleen cells from BALB/c mice, which had been immunized with poly A · poly U and poly I · poly C, fused with NS-1 mouse myeloma cells were isolated by limiting dilution. One hundred and three of the lines secreted antibody which reacted with a mixture of the above double-stranded (ds) RNAs. Antibody from only two of the lines competed favorably with rabbit polyclonal antibody to poly A · poly U for sites on this synthetic RNA. Both of the monoclonal antibodies detected 3 ng/ml of poly A · poly U and 10 ng/ml of poly I · poly C by ELISA. Neither monoclonal antibody reacted with calf thymus DNA or yeast RNA. The two monoclonal antibodies detected similar concentrations of partially purified Rhopalosiphum padi virus, tobacco mosaic virus, cucumber mosaic virus, and brome mosaic virus ds RNAs.

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SPECIFICITY IN TRANSMISSION OF LEAFHOPPER-BORNE MAIZE CHLOROTIC DWARF VIRUS. L. R. Nault, OARDC, The Ohio State University, Wooster, OH 44691.

Laboratory studies indicated semipersistently transmitted maize chlorotic dwarf virus (MCDV) has vector species clustered among grass-feeding leafhoppers in the tribe Deltocephalini, subfamily Deltocephalinae. The greater the phylogenetic distance from the principal MCDV vector, *Graminella nigrifrons*, the less likely a leafhopper species transmitted MCDV. Following are 21 Deltocephalinae leafhoppers tested with the estimated proportion of transmitters: Tribe Deltocephalini, *G. nigrifrons* (.280), *G. sonora* (.130), *Amblysellus grex* (.248), *Stirellus bicolor* (.137) *Endria inimica* (.014); Tribe Eucelini, *Exitianus exitiosis* (.126), *Ollarianus strictus* (0), *Psammotettix lividellus* (0), *Eucelidius variegatus* (0); Tribe Macrostelini, *Macrosteles severini* (.017), *M. fascifrons* (0), *Baldulus tripsaci* (0), and nine *Dalbulus* species (0).

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EVIDENCE FOR INFECTIVITY OF MAIZE CHLOROTIC DWARF VIRUS AND A HELPER COMPONENT FOR ITS LEAFHOPPER TRANSMISSION. R. E. Hunt L. R. Nault, and R. E. Gingery, OARDC, The Ohio State University, Wooster, OH 44691.

The infectivity of maize chlorotic dwarf virus (MCDV) was demonstrated indirectly by *in vivo* neutralization of infectivity in its leafhopper vector, *Graminella nigrifrons*, and directly by leafhopper transmission of purified virus acquired through parafilm membranes. In neutralization of infectivity tests, transmission of MCDV by viruliferous leafhoppers was significantly reduced if they were allowed to feed through parafilm membranes on a suspension containing MCDV antiserum or its IgG fraction compared to control leafhoppers fed on preimmune serum. Transmission of a purified isolate (MCDV-white stripe) from membranes occurred if leafhoppers had previously fed on plants infected with another isolate (MCDV-mild), but not if they had first fed on healthy plants or plants infected with maize dwarf mosaic virus or if subsequently fed on plants infected with MCDV-mild. These experiments suggest that a helper component is necessary for the transmission of MCDV by leafhoppers.

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ULTRASTRUCTURE OF MAIZE CHLOROTIC DWARF VIRUS INFECTED MAIZE AND VIRULIFEROUS LEAFHOPPER VECTORS. E. D. Ammar, D. T. Gordon, and L. R. Nault, The Ohio State Univ., Wooster 44691.

Cytopathology of leaf cells of maize infected with the mild (M) or the white stripe (WS) isolates of maize chlorotic dwarf virus (MCDV) was studied by light and electron microscopy. Both isolates induced two types of intracytoplasmic inclusions, primarily in cells of the phloem and vascular parenchyma: (1) elongate, fibrous inclusions, and (2) electron dense, usually quasi-spherical, granular or finely fibrillar inclusions. The latter contained numerous viruslike particles (VLP) for WS-infected leaves and were mostly devoid of VLP for M-infected leaves. Extensive deformation of chloroplasts was observed for WS-infected, but not M-infected, leaves. Adult Graminella nigrifrons exposed to MCDV-diseased plants for 3 days and then confirmed as vectors by test feeding on healthy plants for 4-5 hr, contained VLP embedded in dense material overlaying a less dense matrix lining the cuticle of the pharynx. VLP were also found in the cibarial pump and esophagous.

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SENSITIVITY OF LATEX AND ELISA TESTS FOR DETECTING POTATO VIRUS Y (PVY) AND POTATO LEAF ROLL VIRUS (PLRV). J. Nakashima, International Potato Center, P.O. Box 5969, Lima, Peru, and C. E. Fribourg, Universidad Nacional Agraria, La Molina, P.O. Box 456, Lima, Peru.

The latex test using $F(ab')_2$ fragments for latex sensitization was about 7 times more sensitive than the test using latex sensitized with entire immunoglobulins (IgG) for the detection of PVY in both purified virus or infected leaf sap extracts. PLRV was detected only in purified virus dilutions by both types of latex tests. Indirect ELISA with $F(ab')_2$ pre-coated plates was as sensitive as direct double antibody sandwich ELISA for the detection of PVY and PLRV. Both detected less than 1 ng/ml of purified virus diluted in extraction buffer or in healthy leaf sap. For the detection of PVY, the agglutination tests using Staphylococcus aureus bacterial cells or industrial grade polystyrene latex as carriers were as sensitive and virus-specific as the traditional latex test using reagent grade polystyrene latex. Bacterial cells are inexpensive and industrial latex is about 40 times cheaper than reagent grade and easy to obtain

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EFFECT OF COMBINING AMMONIA-BASED FERTILIZERS WITH SOIL SOLARIZATION ON PATHOGEN CONTROL AND PLANT GROWTH. J. J. Stapleton, J. E. DeVay, and B. Lear, Department of Plant Pathology, University of California, Davis, CA 95616.

Pre-plant treatment of two field soils with 305 kg N/ha aqua ammonia, urea (U), ammonium sulfate (AS), ammonium phosphate (AP) and/or 3.7 wk of soil solarization (SS) was evaluated with respect to control of Verticillium dahliae and Pythium ultimum. Effect of treatments on growth and yield of fresh-market tomato (Lycopersicon esculentum cv. 'Early-Pak 7') also was studied. Propagules of V. dahliae were reduced to undetectable levels ($P<0.05$) to 46 cm depth in one or both soil types by SS and combinations of SS with AP, AS, or U. Numbers of P. ultimum were reduced 69-100% by SS, and combinations of SS with each fertilizer in at least one soil. Reduction of propagules by fertilizers alone sometimes was found. Increased number of green fruit/plant, fruit fresh wt./plant, and vegetative fresh wt./plant were found in one of the soils treated by SS + AP or SS + AS. Increased vegetative growth was found after treatment with AP alone.

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ROOT ROT OF SNAP BEAN CAUSED BY RHIZOCTONIA SOLANI AG-2 TYPE 2 AND AG-4 IN CONSERVATION TILLAGE. H. H. Win and D. R. Sumner, Coastal Plain Experiment Station, Tifton, GA 31793.

For three successive years, field plots were infested or noninfested with Rhizoctonia solani AG-2 type 2 in March and rototilled 5 cm deep. Field corn was planted, then following corn harvest stalks were chopped with a flail mower, and snap bean was notill planted adjacent to the corn rows. In the first year, yields of green pods were significantly greater in noninfested plots than in infested plots (3185 vs 1056 kg/ha). R. solani AG-2 type 2 was isolated from 6 and 51% of the seedlings and post-emergence damping-off was 6 and 15% in noninfested and infested plots, respectively. In the last two years, R. solani AG-2 type 2 was isolated infrequently, but yield was low in all plots. In two experiments following sweet corn, plots were infested or noninfested with R. solani AG-4, disk-harrowed, and snap bean planted six days later. Yield was significantly greater in noninfested than in infested plots (1769 vs 1293 kg/ha).

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SIMULATED SAMPLING TO DETERMINE PROPAGULE DENSITY AND SPATIAL INDICES FOR MACROPHOMINA PHASEOLINA. C. Lee Campbell, Dept. of Plant Pathology, North Carolina State University, Raleigh 27695.

Sample path and sample number are key factors in determining inoculum density (ID) and spatial pattern of root pathogens. To determine reliability of sample paths (diamond, W, transect, random) and sample sizes (n, n/2, n/4 where n=34, 38, 40, or 40, respectively) for estimating ID and spatial indices of Macrophomina phaseolina, simulated samples were drawn from 10 (150 quadrat) field maps. A 10 g soil sample was assayed from each 6.1x6.1 m quadrat. Mean ID ranged from 3.3-77.8 propagules/10 g soil. Lloyd's index of patchiness (LIP) ranged from 1.17 to 3.09. With n samples, no one path was better in giving estimates within 10 or 20% of the mean ID; with a decrease to n/4, the random path was least reliable. Thus, if n is large, choice of path is not as critical as when n is small. The diamond and W were best for estimating s^2/x with n samples; no path was acceptable for estimating s^2/x at n/4. For LIP estimates, the diamond and W were more reliable than transect or random; LIP was more robust than s^2/x.

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IN SITU OBSERVATIONS OF STRAND DEVELOPMENT BY *PHYMATOTRICHUM OMNIVORUM*. C. M. Kenerley[1], T. J. Gerik[2], and T. L. White[3], Tex. Agri. Exp. Sta., Dept. Plant Path. & Micro., College Station, TX 77843[1,3], and Blackland Res. Ctr., Temple, TX 76503[2].

Strand development of *Phymatotrichum omnivorum* was recorded with a micro-video system (48mm diam color Microsaticon camera, color monitor, and beta video recorder) by inserting the camera into mini-rhizotrons (acrylic plastic cylinders) positioned in PVC containers of nonsterile Houston black clay (HBC). Soil water potential was monitored with tensiometers and soil psychrometers. Initial strand development from sclerotia buried at 30 cm adjacent to tap roots of 8-wk-old-cotton plants in HBC at water potentials of 0 to -1 bar was oriented toward the soil surface. After 4 wk of growth, no difference was detected in strand orientation in HBC. Comparison of strand development in treatments where soil water potential was maintained between 0 and -1 bar or where a monotonic drought was imposed revealed a decline in growth and subsequent apparent death at soil water potentials between -2 and -4 bars. Experiments using the micro-video systems to monitor sclerotium formation following infection of cotton plants are being conducted.

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DISPERSAL OF PHYTOPHTHORA CRYPTOGEA ZOOSPORES IN SOILS AND GLASS MICROBEADS BY WATER FLOW. J. M. Duniway and C. D. McKeen, Department of Plant Pathology, University of California, Davis, CA 95616.

Zoospore infiltration into reconstituted columns of soils and glass microbeads was measured quantitatively. When columns were saturated fully and water flowed down through them, dispersal of encysted zoospores from the surface into soils was confined largely to the top 4 cm. In contrast, as many as 40% of the flagellated or motile zoospores added at the tops of 20 cm columns were dispersed through soils and were collected in fractions of effluent. Active zoospore movement into soils in the absence of water flow was limited to 4 cm depth. Flagellated zoospores were readily dispersed by water movement through columns of 100-um-diam. microbeads, but only 6% were dispersed through columns of 50-um microbeads. Zoospore dispersal in 30-um microbeads was less than 2 cm. The results show that flagellar activity and water movement greatly increase zoospore dispersal through soil.

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INFLUENCE OF SUBSTRATE pH AND TEMPERATURE ON SPORULATION OF CEPHALOSPORIUM GRAMINEUM IN VITRO AND ON COLONIZED OAT KERNELS ON SOIL. T.D. Murray and C. Campbell, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Sporulation of C. gramineum at 20 C was about 10-fold less at pH 7.5 than at pH 4.5 or 5.5 on mineral salts agar (MSA) with phosphate buffer (PB), and about a million-fold less at pH 7.5 than at pH 5.5 on MSA with citrate-phosphate (CB) buffer. Water potential of MSA was slightly higher at lower pH values, but differed by less than 2.5 bars from pH 4.5 to 7.5; it only partially explains the differences in sporulation. On MSA-PB sporulation was greatest at 20 C, but on MSA-CB, sporulation was the same at 15 and 20 C, except at pH 7.5 where it was greater at 15 C than at 20 C. Isolates varied greatly in sporulation but responded similarly to pH. Sporulation of C. gramineum on colonized oat kernels placed on soil was similar from pH 4.5 to 6.5, but was almost 50% less at pH 7.5. Increased sporulation in vitro and on colonized oat kernels at lower pH may help to explain increased disease at low soil pH.

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INFLUENCE OF IRRIGATION SCHEDULE AND PHYTOPHTHORA ROOT ROT ON GROWTH, PHENOLOGY AND YIELD OF PROCESSING TOMATOES. Jean Beagle-Ristaino, J. M. Duniway, and J. J. Marois. Department of Plant Pathology, University of California, Davis, CA 95616.

Processing tomatoes were grown in field plots with soil either infested or free of Phytophthora parasitica. Subplots received either normal (4-8 hr once every 14 days), prolonged (alternately 4-8 or 24 hr every 14 days), or less frequent (4-8 hr every 28 days) furrow irrigation. Root rot developed more rapidly and caused greatest reduction in total plant, leaf, and fruit dry matter accumulation in infected plants receiving the prolonged irrigation. In infested plots, yield was higher on the 28-day irrigation schedule while in noninfested plots yield was higher on the 14-day schedule. Leaf water potential of diseased plants was correlated with foliar symptom severity and final yield. Number of flowers and fruit were reduced most by disease. A delay in disease onset caused by less frequent irrigation of infested plots reduced the impact of disease on numbers of flowers and fruit and on harvestable yield.

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THE IMPACT OF PHYTOPHTHORA ROOT ROT ON WATER EXTRACTION FROM SOIL BY FIELD-GROWN PROCESSING TOMATOES. Jean Beagle-Ristaino and J. M. Duniway. Department of Plant Pathology, University of California, Davis, California 95616.

Plots infested or not infested with Phytophthora parasitica were given normal (4-8 hr once every 14 days), prolonged (alternately 4-8 or 24 hr every 14 days) or less frequent (4-8 hr every 28 days) furrow irrigations. Volumetric soil water content was measured weekly with a neutron probe at 30 cm increments to 150 cm depths. Early in the season, less water was extracted by roots of diseased than of healthy plants in both prolonged and normal irrigation treatments, while plants irrigated less frequently were less affected by disease and extracted the greatest amounts of water at 120 and 150 cm depths. Later in the season, disease reduced rates of water extraction at more shallow depths in the less frequently irrigated treatment, while water extraction by diseased plants in the prolonged irrigation treatment increased in greater soil depths. The depths at which water extraction was altered varied with both irrigation schedule and disease development.

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CORRELATIONS OF PRE-EMERGENCE DAMPING-OFF WITH SOYBEAN SEED LOT QUALITY. R. S. Ferriss, and J. M. Baker. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Fourteen soybean seed lots were evaluated in greenhouse experiments and standardized seed quality tests. Seeds were planted in untreated soybean soil, pasteurized soil, or pasteurized soil infested with Pythium ultimum, and then incubated for 3 days at -0.01 bar soil water potential or under flooded conditions, followed by conditions near optimum for emergence. Among the seed lots, standard germination results (SG) were correlated with incidence of Phomopsis longicolla. Results of artificial aging (AA) and cold tests were highly correlated with each other. Emergence in -0.01 bar pasteurized soil was correlated with SG, but not with the AA or cold tests. Emergence in -0.01 bar untreated or infested soil was correlated with the AA and cold tests, but not with SG. Emergence after flooding was not significantly correlated with results of any seed quality tests. Seed viability and resistance to microbial stress appear to vary independently among seed lots.

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NUTRIENT INTERACTIONS WITH STALK ROT OF MAIZE, H.L. Warren, D.M. Huber, and C.Y. Tsai, Botany and Plant Pathology Department, Purdue University, W. Lafayette, IN 47907.

Stalk rot, generally associated with environmental stress or senescence, is a serious deterrent to improved production efficiency. The general observation that high yielding hybrids are more susceptible to stalk rot than low yielding ones presents a challenge for maximum economic yield programs. Based on biochemical mechanisms determining nitrogen efficiency and yield potential of maize, a consistent relationship exists between the nitrogen response of a hybrid and its susceptibility. All hybrids are more susceptible to stalk rot when deficient in N than when adequate N is provided throughout the growing season. Although excess N may predispose plants to stalk rot, stalk rot is lowest when the level of N is adequate to reach a hybrids yield potential.

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PREVALENCE OF SEEDLING ROOT DISEASES ON WINTER WHEAT GROWN IN THE PANHANDLE REGION OF TEXAS. L. P. Specht and C. M. Rush. Texas Agricultural Experiment Station, Texas A&M University, Bushland, TX 79012.

A root-disease survey of seedling winter wheat grown in the Texas Panhandle was conducted in the fall and winter of 1986-1987. Fifty seven fields in 25 counties were surveyed. Plants from 9, 21, 22 and 5 fields showed 0%, trace, slight to moderate, and severe levels of root rot, respectively. Disease ratings on subcrown internodes (SCI's) of plants from 23, 14 and 5 fields averaged slight, moderate, and severe, respectively. Plants from 12 fields had no lesions on SCI's. The most commonly isolated fungi were Fusarium spp. (50 fields) and Bipolaris sorokiniana (41 fields). Of 536 isolates obtained from rotted roots, 92% were Fusarium spp., while of 319 isolates obtained from SCI's, 65% were B. sorokiniana. All of the Bipolaris and the majority of the Fusarium spp. were pathogenic to wheat. Other fungi isolated from wheat seedlings included Rhizoctonia spp. (15 fields) and Pythium spp. (14 fields).

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CHANGES IN WATER UPTAKE AND ROOT DISTRIBUTION OCCURRING DURING THE DEVELOPMENT OF PHYTOPHTHORA ROOT ROT IN SAFFLOWER. C. M. Liddell and J. M. Duniway, Department of Plant Pathology, University of California, Davis, 95616.

Safflower plants were grown in columns of soil (10 cm diam. x 50 cm) divided horizontally into equal halves by a wax layer. Roots penetrated the wax readily and either the upper or lower half was inoculated with zoospores of Phytophthora cryptogea. Water uptake was measured after inoculation using gamma ray attenuation methods to monitor soil moisture nondestructively. Rates of water uptake were correlated with total root length in each half column. Early stages of infection at 5-7 days after inoculation reduced the length of fine roots but had only slight effects on water uptake. Increased root growth in uninoculated half columns and increased uptake by remaining roots in inoculated halves tended to compensate for the reduction in root length caused by disease. However, lesions that developed on the stem base or tap root reduced water uptake greatly.

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PRODUCTION OF DIFFERENT TOXIC METABOLITES BY TWO PYTHIUM SPECIES. H. Mojdehi and L. L. Singleton, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285.

Two pathogenic Pythium species, isolated from wheat roots, were identified as P. arrhenomanes Drechs. (PA) (isolates #320 and #323), and P. irregulare Buis. (PI). Cell-free culture filtrates of both species grown in a glucose glutamic acid medium (pH 6.0) caused severe inhibition of root elongation and significant reductions in fresh and dry root weights of wheat seedlings (cv. TAM W-101) after 3d of exposure. PA filtrates inhibited root hair formation and caused browning of root tissue, but did not affect shoot elongation. However, PI filtrates stimulated root hair formation near the root tip without tissue browning, but significantly inhibited shoot elongation. Efficacy of PA filtrates was lost after autoclaving, whereas PI filtrates retained efficacy after autoclaving. This suggests that the metabolites produced by the two species were different.

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PRODUCTION OF A MULTICOMPONENT TOXIN BY PYTHIUM ARRHENOMANES. H. Mojdehi and L. L. Singleton, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078-0285.

Pythium arrhenomanes Drechs. (PA.) isolates from wheat roots were grown in a glucose glutamic acid medium (pH 6.0) for 20d. Cell-free culture filtrates caused significant inhibition of root elongation, root hair formation, and severe browning of the roots of two day old wheat seedlings (cv. TAM-101) after 48h of exposure. Autoclaving reduced the efficacy of the culture filtrates, while freezing had no such effect. The culture filtrate was separated into 5000+ and 5000- M.W. fractions by ultrafiltration; both were toxic. Both inhibited root hair formation with slight reduction in root elongation, but neither fraction caused any observable browning of the tissue compared to the complete filtrate. The reconstituted form, containing both 5000+ and 5000- M.W. components, gave results similar to the original, complete filtrate. Autoclaving the 5000- fraction removed its inhibitory effects, but did not so affect the 5000+ fraction.

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GERMINATION OF PHYMATOTRICHUM OMNIVORUM SCLEROTIA IN HOUSTON BLACK CLAY SOIL. T. L. White and C. M. Kenerley, Department of Plant Pathology and Microbiology, Texas A&M University, Texas Agricultural Experiment Station, College Station 77843.

Phymatotrichum omnivorum sclerotia were germinated and rated into classes in non-sterile Houston black clay soil adjusted to different water potentials (-0.1, -0.5, -1.0, -2.5, -5.0, and -10.0 bars) and temperatures (17, 26, 35°C). Temperature and water potential had a significant effect on type of germination. Hyphal germination occurred at all water potentials tested. At -0.1 and -10.0 bars, 95-100% of the sclerotia observed either did not germinate or produced less than 10 hyphae per sclerotium. Sclerotial strand production was observed in soil adjusted to water potentials of -0.5, -1.0, -2.5, and -5.0 bars, but not at -0.1 or -10.0 bars. In another study 41, 34, 25, and 15 week-old sclerotia were germinated at water potentials of -0.5, -2.5, and -10.0 bars. Sclerotia held in culture for 34 weeks or more lost the ability to form strands.

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DETECTION AND QUANTIFICATION OF BLUEBERRY SHOESTRING VIRUS IN ILLINOIA PEPPERI VIA DOT-ELISA AND DOT-BLOT HYBRIDIZATION. B.T. Terhune and D.C. Ramsdell, Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824

Late instars of Illinoia pepperi, the aphid vector of blueberry shoestring virus (BBSSV), were assayed via dot-ELISA and dot-blot hybridization for viral antigen and RNA levels, respectively. Aphids were fed on Parafilm sachets, containing purified BBSSV 50 µg/ml, for various acquisition access periods, homogenized, and spotted on either nitrocellulose or nylon membranes. Dot-ELISA blots were reproduced 1:1 on Kodak EPD120, color transparency film. Transparency and autoradiograph spot intensities were measured as percent transmittance at 539 nm on a Gilford Response scanning spectrophotometer. Linear responses were observed over a logarithmic concentration gradient of purified BBSSV (dot-ELISA R squared = .75). Virus concentrations as low as 1.3 ng per viruliferous aphid, as interpolated from prediction models, were observed after a 48 hr acquisition access period.

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TEPARY BEAN COTYLEDON BIOASSAY FOR THE DIFFERENTIATION OF RACES IN XANTHOMONAS CAMPESTRIS PV. PHASEOLI. Mildred Zapata and Anne K. Vidaver. Depart. of Plant Path., Univ. of Nebraska, Lincoln, NE 68583-0722.

A fast and reliable method of cotyledon inoculation was developed to differentiate races of X. campestris pv. phaseoli in the tepary bean, Phaseolus acutifolius. The inner surface of the cotyledons were inoculated by scratching the surface with two needles and deposition of 10^8 bacterial concentration on the wound. The incompatible reaction, hypersensitivity, was characterized by a dark discoloration within 48 hr. The compatible reaction was expressed as a watersoaked reaction or as callous tissue development in the presence of bacterial exudate. A significant interaction between the tepary lines and the bacterial strains was determined under controlled conditions. The bacterial strains were separated at the level of pathogenic races by their phenotypic reaction (qualitative) on the tepary bean cotyledons. Thus, the tepary bean was useful for the differentiation of the pathogen, due to the specific resistance of some lines. The technique can be used to identify races and potential resistance genes in tepary and in hybrid populations derived from interspecific crosses between tepary and P. vulgaris.

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USE OF TISSUE CULTURE FOR EVALUATING SOYBEANS FOR RESISTANCE TO PHIALOPHORA GREGATA. L. E. Gray, Y. Q. Guan, and J. Widholm. USDA, ARS, and Department of Agronomy, University of Illinois, Urbana, IL.

Tissue culture techniques were developed for evaluating soybeans for resistance to Phialophora gregata (cause of soybean brown stem rot). Culture filtrates from pathogenic and non-pathogenic isolates of the fungus were evaluated for their effect on the growth and viability of soybean callus. Callus of susceptible soybean lines was sensitive to culture filtrates of pathogenic isolates of the fungus. Callus growth and viability of resistant genotypes, PI 437833 and 84946-2, was not affected by the fungus culture filtrates. Culture filtrates from non-pathogenic isolates of the fungus did not affect callus growth or viability of either susceptible or resistant soybean genotypes. The fungus culture filtrates did not affect the growth or viability of tobacco, garden bean, or Datura spp. callus. Factors which affect the callus assay procedures will be discussed.

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THE USE OF FLUORESCEIN DIACETATE AND CALCOFLUOR FOR DETERMINING THE VIABILITY OF VENTURIA INAEQUALIS. C. M. Becker, and T. J. Burr, NYS Agricultural Experiment Station, Cornell University, Geneva, NY 14456

A combination of fluorescein diacetate (FDA) and Calcofluor (CCF) stains were used with epi-fluorescent microscopy to differentiate viable from dead conidia, germlings, and mycelia of Venturia inaequalis. The stain consisted of FDA (10 ug/ml) and CCF (1 mg/ml) in a 100 mM phosphate buffer (pH 7.2). All live and dead propagules of V. inaequalis fluoresced blue when stained with CCF. Similar propagules stained with FDA fluoresced bright green only if cell membranes were intact, indicating the cells had esterase activity. Subcuticular mycelium fluoresced yellow-green when stained. The combination of FDA and CCF has been used to identify environmental conditions during dry intervals of various time intervals that affect propagule survival of V. inaequalis.

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COMPARISON OF TWO SCREENING METHODS FOR DETECTION OF SUNFLOWER RESISTANCE TO PHOMA MACDONALDII. P. Donald, C. Hartman and G. Secor. Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105.

Two methods of screening sunflowers for resistance to Phoma macdonaldii, the causal agent of Phoma black stem and sometimes premature death of sunflower plants in North Dakota, was studied using seedlings and callus tissue. Susceptible and resistant sunflower seedlings, commercial hybrids 'Sigco 432' and 'Sigco 475' respectively, were inoculated at the four leaf growth stage with an atomized conidial suspension and examined one week after inoculation for presence of leaf and stem lesions. Sunflower calli produced from the same hybrids were grown on media containing various levels of fungal culture filtrate and examined for calli weight difference and color reaction. Differential reactions of calli were compared to the results of the seedling test. Both systems were capable of identifying susceptible and resistant hybrids.

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INTERACTION OF LEVELS OF MOISTURE AND OF DISEASE IN BROWN STEM ROT OF SOYBEANS. H. Tachibana, and K. G. Bidne. USDA-ARS, Dept. of Plant Pathology, Iowa State University, Ames IA 50011.

When moisture was controlled under low and high levels of brown stem rot (BSR) disease of soybeans in the field, low levels of moisture produced significantly low BSR incidence and severity; whereas, high moisture levels produced significantly higher levels of the disease. The magnitude and difference in BSR severity within each of the two treatments (moisture and disease) were similar for both treatments. Usual differences in relative severity between susceptible (Hardin) and resistant (BSR 101) cultivars were detected at all moisture and disease levels. The severity of BSR increased significantly with moisture applied beginning at the R6 stage, which seems to be the beginning of the critical period for BSR, moisture stress and soybean yield interactions. These results indicate moisture has a simultaneous effect on both BSR levels and in the interaction of BSR, moisture and yield of soybeans.

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NEW METHOD FOR SCREENING MICROORGANISMS DELETERIOUS TO NEMATODES. J. O. Becker, M. N. Schroth, J. G. Hancock, A. R. Weinhold, and S. Van Gundy, Dept. of Nematology, Univ. of California, Riverside, CA 92521 and Dept. of Plant Pathology, Univ. of California, Berkeley, CA 94720.

More than 4000 rhizobacteria strains were tested in vitro for their ability to affect vitality of Meloidogyne incognita. Agar plugs from the edges of 4 day-old bacterial colonies grown on 10% tryptic soy agar were cut out and transferred into wells of microtiter plates. Buffered water agar (10 mM Hepes, pH 7.2) with rifampicin (0.1 mg/ml) was added and about 20 surface sterilized (5 mg chlorhexidine and 0.1 mg rifampicin/ml water for 30 min) root-knot larvae were applied into each well. Mobility of the nematodes was examined at daily intervals. About 50 bacterial strains caused a partial to complete inhibition of movement. Selected bacterial strains when drenched into soil infested with M. incognita reduced galling of clover (Trifolium repens) up to 30%.

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PURIFICATION OF PHYTOFERRITIN FROM SOYBEAN NODULES. D. W. Laird, J. S. Huang, and K. R. Barker, North Carolina State University, Raleigh, N. C., 27616.

Increases in phytoferritin protein levels in soybean nodules are induced by the nematode Heterodera glycines and by non-nitrogen fixing symbiotic mutants of Bradyrhizobium japonicum(Bj). The role of phytoferritin in nitrogen fixation is unknown. Phytoferritin was isolated by preparative scale electrophoresis, followed by analytical scale electrophoresis (DISC-PAGE). Purified phytoferritin migrated as a single band as detected by coomassie blue staining or iron-specific staining upon subsequent electrophoresis. Efficient recovery of phytoferritin from gels was acheived by electroelution. Yields on a fresh weight basis average 10ug/g nodule for mutant HS145 nodules and 2.5ug/g nodule for H. glycines stressed (nitrogen fixing Bj) soybean. This procedure allows for isolation of pure phytoferritin in quantities sufficient for characterization and antibody production.

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DETECTION OF PEANUT STRIPE VIRUS BY THREE ELISA TECHNIQUES AND A DOT-BLOT IMMMUNOBINDING ASSAY. V.M. Aquino, J. W. Moyer and M.K. Beute, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Three variants of ELISA, the direct double antibody sandwich (DAS-ELISA), Protein-A ELISA, and an indirect ELISA and a dot-blot immunobinding assay on nitrocellulose membrane using $F(ab')_2$ fragments were evaluated for detecting peanut stripe virus in crude and clarified sap preparations from peanut leaves. Whole IgG was used with both DAS-ELISA and Protein-A ELISA. The indirect ELISA and the dot-blot assay were carried out using fragments. Alkaline phosphatase-Protein A conjugate was used for the Protein A and indirect ELISA, while alkaline phosphatase-goat anti-rabbit conjugate was utilized for the dot-blot assay. The indirect ELISA gave significantly lower healthy control absorbance values than the other two variants. However, there were no significant differences among levels from infected leaves. The dot-blot assay was 10-100 times more sensitive than the three ELISA methods.

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TOXIN PRODUCTION BY MYCOSPAERELLA FIJIENSIS VAR DIFFORMIS. Gloria C. Molina and J.P. Krausz. FHIA, Apartado 2067. San Pedro Sula, Honduras.

It is believed that toxin is involved in the pathogenesis of Black Sigatoka disease of banana caused by Mycosphaerella fijiensis var difformis. Repeated trials using crude extracts from 28-day-old M. fijiensis var difformis cultures from both coconut and soybean broths were demonstrated to cause necrosis on inoculated plants. The "toxin" was extracted using organic solvents, and activity was evaluated by dipping cut leaf tips of banana developed from meristem cultures in the "toxin" for 1 min. Death of tissues from the point of inoculation was evident after 24 hours. Activity was recorded up to 1:10 dilution with distilled water. Extracts from uninoculated broths showed no necrosis.

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INOCULATION STUDIES ON THREE EAR ROT DISEASES OF TROPICAL MAIZE. J. M. Fajemisin, J. A. Durojaiye, Y. Efron and S. K. Kim. International Institute of Tropical Agriculture, Ibadan, Nigeria.

The mode of inoculum dispensation (toothpick, spore, suspension) and point of inoculation (shoot tip, middle of the ear, shank, ear-axil) were evaluated for three ear rot pathogens. (Botryodiplodia theobromae, Fusarium moniliforme, Macrophomina phaseoli) at Ibadan, Nigeria. Two susceptible inbreds (5012, 9043) were used. It was demonstrated that each of the fungi can incite rot when introduced through any of the inoculation points tested. Inoculation through the middle portion of the ear gave the highest incidence and severity of rot followed by inoculation through the tip. Infection was lowest for ear-axil and shank inoculations. There were no significant differences between inoculations with infested toothpick or with spore suspension. Covering of the ears with tassel bags immediately after inoculation did not significantly increase the efficiency of ear rot inoculation.

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ISOLATION OF PEPPER (CAPSICUM ANNUUM) PROTOPLASTS. R.P. Niedz, C.T. Stephens and H.H. Murakishi. Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan, 48824-1312.

A new procedure to isolate protoplasts in large numbers (10^6/gm fresh wt), of high viability (73%) and with a rapid cell colony growth rate (1.5-2.0 mm in 24d) was developed. Cotyledons of 21d old seedlings grown in a modified Murashige-Skoog (MS) medium (KNO_3 substituted for NH_4NO_3) were peeled and placed in an isolation solution containing (w/v): Cellulysin (1%), Driselase (0.5%), Macerase (0.1%), $CaCl_2 \cdot 2H_2O$ (10mM), sorbitol (0.4M) at pH 5.8. Digestion was complete in 4 hours. Protoplasts were washed and cultured in KM-T tomato medium (Niedz, R.P. 1987. Ph.D. Dissertation, MSU). First division occurred in 5 days and 30d old micro-calli were transferred to MS medium containing IAA (1 mg/L) and BAP (0.5 mg/L) to induce chlorophyll synthesis. Protoplasts were tested and found suitable for poly-L-ornithine and polyethelene glycol-mediated virus uptake procedures and somatic hybridization studies.

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EVALUATION OF ELITE TROPICAL MAIZE INBREDS FOR RESISTANCE TO THREE EAR ROT PATHOGENS. J. M. Fajemisin; J. A. Durojaiye; S. K. Kim and Y. Efron. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Twenty-eight inbred lines developed at IITA were evaluated for resistance to Botryodiplodia theobromae, Fusarium moniliforme, and Macrophomina phaseoli. The plants were inoculated 10-14 days after silking with infested toothpicks at the middle point of the ear through the husk. There were significant differences among the lines in reaction to each of the rot pathogens. Botryodiplodia was the most severe and for which there was the least genetic variability. Although inbred 1394 gave low rot scores for the three pathogens, there is no strong indication of correlation among the pathogens. Inbred 1368, 4001 and 4008 were appreciably resistant to Fusarium and Macrophomina but susceptible to Botryodiplodia. Inbred 9006 was very susceptible to the three pathogens.

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CRINIPELLIS PERNICIOSA, THE CACAO WITCHES' BROOM FUNGUS: INOCULUM PRODUCTION AND STORAGE. E. R. Dickstein, L. H. Purdy, and G. A. Frias. Dept. Plant Pathology, University of Florida, Gainesville, 32611.

Vegetative mycelium of C. perniciosa grows readily on a variety of media, but sporulating basidiocarps are difficult to produce. The fungus formed a mycelial mat in clarified V-8 juice medium. After 4-6 weeks mats were removed from the culture flasks, placed on a support, and hung in a mist chamber. Initially, mats were attached to glass rods, but basidiocarps produced were small and the number of spores obtained was inadequate for mass inoculation of plants. Woods of several species were evaluated as substrates to improve basidiocarp production. Mycelial mats attached to autoclaved witches brooms from cacao produced the greatest numbers of large (>1 cm) basidiocarps. Different isolates of C. perniciosa varied in their abilities to produce basidiocarps in this way. Basidiocarps have also been obtained from several single basidiospore isolates. Spores were collected and stored in liquid nitrogen for more than a month with minimal loss of viability and pathogenicity.

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EVALUATION OF A SEEDLING SCREENING TEST FOR EARLY BLIGHT (ALTERNARIA SOLANI) RESISTANCE IN POTATOES. C. Martin, H. Mendoza, and H. Torres. The International Potato Center (CIP), Apartado 5969, Lima, Peru.

During 1985 and 1986 two sets of progenies from crosses involving parents with different levels of resistance were evaluated to early blight at seedling and adult plant stage. Seedlings 35-40 days old were inoculated by spraying them with a spore suspension of 2500-3000 spores/ml at CIP-Lima and CIP-San Ramon (eastern slopes of the Andes, 800 m elevation) conditions. After inoculation seedlings were covered with a plastic sheet for four days during which temperature ranged from 20-26°C for Lima and 24-35°C for San Ramon. Under field conditions at San Ramon plants were artificially inoculated at 40 days after transplanting seedlings with the same inoculum concentration. Overhead irrigation was used constantly. Infection and disease development occurred in all cases but was lower in the seedlings at San Ramon. Spearman's correlation coefficients among early blight scores indicated a significant correlation (r=.445) between field evaluation and the seedling test carried out in Lima for the first set of 27 families. For the second set of 22 families the same correlation was positive but not significant (r=.213); however, the correlation coefficient was higher (r=.322) when the field evaluation was compared with the seedling test at San Ramon. Improvements on the tests are being evaluated.

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RESISTANCE OF ALTERNARIA PANAX TO IPRODIONE UNDER FIELD CONDITIONS. M.K. Rahimian, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Iprodione was applied on ginseng plants in three continuous growing seasons following planting to control alternaria leaf blight caused by Alternaria panax. In the first and second growing seasons, plots treated with iprodione at 1134 g/ha had 50% less disease than non-treated plots. In the third season, disease severity in the iprodione-treated plots was not significantly different than in the non-treated plots. Maneb at 1814 g/ha reduced disease severity by about 50% during all three seasons. All 30 isolates of the fungus obtained from the iprodione-treated plots during the third growing season were able to grow on potato-dextrose agar amended with 50 µg/ml iprodione. All ten isolates obtained during the first and second season were sensitive to this treatment. Pathogenicity tests showed that the tolerant isolates of the fungus were as pathogenic to ginseng plants as the non-tolerant isolates. The results indicate that a mutant of A. panax resistant to iprodione developed under the field conditions.

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INHIBITION OF GROWTH OF ALGAE AND WATER MOLDS BY MIXTURES OF METHIONINE AND RIBOFLAVIN IN LIGHT. J. E. DeVay, R. J. Wakeman, and D. D. Tzeng, Department of Plant Pathology, University of California, Davis, CA 95616.

Irradiated solutions of DL-methionine (M) + riboflavin (R) are biocidal to a wide spectrum of plant pathogens (Physiol. Plant. 62:545-552). The photodynamic effect involves a breakdown of methionine to ethylene and formation of several toxic substances, including oxygen radicals. The biocidal activity of M + R has potential use in controlling algae and watermolds in hydroponic plant culture. Cultures of Chlamydomonas eugametos (Ce), C. reinhardtii (Cr), Chlorella pyrenoidosa (Cp), and C. vulgaris (Cv) were grown in Hoagland's solution under fluorescent light with additions of M and/or R. M (> 1 mM) was biocidal to Ce and Cp, but Cr and Cv could tolerate up to 5 mM. However, M (> 0.5 mM)+ R (1.33 uM) was biocidal to Cp and Cv and M (> 1.0 mM) + R (1.33 uM) was biocidal to Ce and Cr. In a synthetic mineral nutrient solution, growth of Pythium ultimum and Phytophthora infestans was completely inhibited by irradiated M (1 mM) + R (1.33 uM).

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APPLICATIONS OF SYSTEMIC FUNGICIDES THROUGH SUBSURFACE DRIP IRRIGATION FOR CONTROL OF PHYMATOTRICHUM ROOT ROT OF COTTON. M. W. Olsen, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721 and S. George, 104 Life Sciences East, Oklahoma State University, Stillwater, OK 74078

The systemic fungicides propiconazole and triadimenol are very active against Phymatotrichum omnivorum, the fungus that causes cotton root rot. Although these fungicides move upward in the plant readily, their downward translocation and mobility in the soil are minimal. To test their efficacy when placed directly in the root zone, the fungicides were applied through subsurface drip irrigation. Applications of both fungicides at 0.5 lb. a.i./acre in 1985 and 0.75 lb. a.i./acre in 1986 (applied as split applications of 0.25 lb. a.i./acre) resulted in a significant reduction (P = .05) in the number of dead plants both years. The percent reduction in the number of dead plants in propiconazole treatments were 72% in 1985 and 66% in 1986 and in triadimenol treatments were 90% in 1985 and 70% in 1986.

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EFFECT OF FIELD APPLICATIONS OF IPRODIONE ON SCLEROTINIA BLIGHT OF PEANUT AND THE CAUSAL FUNGUS, SCLEROTINIA MINOR. F. D. Smith, P. M. Phipps and R. J. Stipes. Tidewater Agr. Exp. Sta., Suffolk, VA 23437; and Dept. Plant Pathol., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Iprodione was evaluated for control of Sclerotinia blight of Florigiant peanut at three locations in 1986. Treated and untreated plots were replicated four times at each location. Iprodione (1.12 kg/ha) was applied three times according to label instructions, and gave 32% suppression of disease according to counts of foci at harvest. Yields were increased 48%, and averaged 2138 and 3167 kg/ha in untreated and treated plots, respectively. Sclerotia (15/replicate) were collected from plant stems in disease foci at each location, and plated on glucose-yeast extract agar (GYEA) with and without iprodione (2 µg/ml). Growth by S. minor on amended GYEA was strongly inhibited, indicating no resistance to iprodione. On non-amended GYEA, S. minor was cultured from 25 and 26% of the sclerotia from untreated and treated plots, respectively.

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HEXACONAZOLE : BROAD SPECTRUM DISEASE CONTROL

Hexaconazole is a triazole fungicide synthesised by the Plant Protection Division of ICI. It has low mammalian toxicity, low environmental impact and an exceptionally broad spectrum of antifungal activity (Shephard et al, 1986). Field testing in the USA and elsewhere has demonstrated excellent control of dollar spot and brown patch of turf, peanut leafspots, powdery mildew of vines, and scab, mildew and rust of apples. Hexaconazole treatment considerably increased peanut yields. Hexaconazole also provided good control of peach brown rot, banana sigatoka, pecan scab, coffee rust and several diseases of vegetables and ornamentals. Glasshouse results demonstrate the strong curative and translaminar activity of hexaconazole. The data indicate that hexaconazole should make a valuable contribution to the control of many of the most significant fungal diseases found in the USA.

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INFLUENCE OF COMMON MAIZE RUST ON YIELD COMPONENTS OF PROCESSING SWEET CORN. H. R. Dillard and R. C. Seem, Dept. of Plant Pathology, N.Y.S. Agr. Expt. Station, Cornell Univ., Geneva, NY 14456.

Disease progress curves of common maize rust (Puccinia sorghi) and yields were evaluated in the presence or absence of fungicides in three commercial fields of processing sweet corn (cv. Jubilee) in western New York. Individual leaves were evaluated for rust severity using Horsfall-Barratt ratings and pustule counts. Rust was detectable by the mid-whorl growth stage, and disease severity increased rapidly by the silking stage. Severity at silk and mature growth stages was negatively correlated to yield. Dithane M-45 (mancozeb) applied by air three times (late whorl, tassel, silk growth stages) at a rate of 1.68 kg/ha (product) significantly reduced foliar disease severity and the area under the disease progress curve. The fungicide applications significantly increased yield components measured as the number of harvestable ears, green weight, and husked weight in two of the treated fields.

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EVALUATION OF FUNGICIDES FOR CONTROL OF DOWNY MILDEW ON BROCCOLI. M.E. Matheron and J.C. Matejka. Department of Plant Pathology, University of Arizona, Yuma Mesa Agric. Center, Yuma, AZ 85364

Downy mildew of broccoli, caused by Peronospora parasitica, is a perennial disease problem affecting winter broccoli production in Arizona. During 1985-87, new fungicides were evaluated in the field for disease control. In the 1985-86 season, a period of high disease incidence, lesion development was significantly reduced by fosetyl-Al-44%, H_3PO_3-42%, oxadixyl/mancozeb-35%, chlorothalonil-33%, metalaxyl/mancozeb-30% and metalaxyl/chlorothalonil-28%. During the 1986-87 growing season, disease incidence was low and lesion development was reduced by H_3PO_3-82%, fosetyl-Al or oxadixyl/chlorothalonil-77%, metalaxyl/chlorothalonil-68% and chlorothalonil-54%. Fosetyl-Al, H_3PO_3 and oxadixyl were as efficacious as currently available materials (chlorothalonil and metalaxyl) for control of downy mildew on broccoli in Arizona.

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EFFECT OF DINITROANILINES AND AMIDES ON ZOOSPORE MOTILITY, MYCELIAL GROWTH AND PATHOGENICITY OF PHYTOPHTHORA CAPSICI. S. A. Johnston and B. A. Majek. Rutgers Research & Development Center, R.D. #5, Box 232, Bridgeton, NJ 08302.

Selected dinitroanilines and amides were evaluated for activity against zoospore motility and mycelial growth of Phytophthora capsici at 0.1, 1.0 and 10 ppm active ingredient. Zoospore motility was inhibited by trifluralin at 1.0 and 10 ppm, oryzalin at 10 ppm and napropamide at 10 ppm. The fungicide, metalaxyl, was noninhibitory to zoospore motility at 0.1, 1.0 or 10 ppm. Metalaxyl resulted in 18, 49 and 80% inhibition of mycelial growth, respectively; while, oryzalin resulted in 10, 29 and 42% inhibition. All other herbicides tested resulted in no inhibition at 0.1 ppm and 10% or less inhibition at 1.0 and 10 ppm. None of the herbicides provided control of Phytophthora blight of peppers in P. capsici infested soil in the greenhouse at 1.0 and 10 ppm active ingredient, while metalaxyl resulted in 40 and 100% control at 1.0 and 10 ppm active ingredient.

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STABILITY AND CONTROL OF BENOMYL-RESISTANT POPULATIONS OF MONILINIA SPECIES IN THE ABSENCE OR PRESENCE OF BENZIMIDAZOLES. J. E. Adaskaveg, B. T. Manji, and J. M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, CA 95616

Population levels of Monilinia fructicola resistant to 1 ug/ml benomyl in a peach orchard remained stable at 35% after 8 yr in the absence of benzimidazoles. In a nectarine orchard, levels changed from 20% to 55% in 7 yr when benomyl was used once during pink bud stage of bloom and once 3-4 wk before harvest each year (triforine was used at full bloom and 1 wk preharvest). Populations of isolates of M. laxa resistant to benomyl in an almond orchard were initially 75% and after 3 yr 82% when only iprodione and triforine were used. In this orchard when low shoot blight incidence occurred, results of one pink bud spray using commercial rates of several fungicides were: control (C) 60.7±28.6 blighted shoots/tree; iprodione (I), 14.0±12.2; benomyl (B), 11.5±6.7; and carbendazim (CA), 13.8±8.1. In an apricot orchard with no benomyl resistant isolates of M. laxa and a high incidence of shoot blight, results of a similar spray program were: C, 74.0±5.9 infected shoots/200 shoots; I, 15.0±6.2; and CA, 4.3±4.7.

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CONTROL OF THREE LEAFSPOT DISEASES IN ORCHARDGRASS GROWN FOR SEED BY FUNGICIDES. R. E. Welty, USDA ARS, Oregon State Univ., Corvallis 97331.

Diseases of orchardgrass (Dactylis glomerata) caused by Rhynchosporium orthosporum (scald), Scolecotrichum graminis (grey streak), and Mastigosporium rubricosum (eyespot) can be severe in cool, wet springs. During 1984-1986, orchardgrass cv. 'Potomac' was sprayed with 1, 2, or 3 applications of either chlorothalonil (1754 g/ha), captafol (1345 g/ha) or propiconazole (252 g/ha) at boot, heading, or flowering to control these and other leaf diseases. In 1984 and 1986 when diseases were most severe, 1 application of chlorothalonil at boot or heading significantly ($P=0.05$) increased seed yield. In 1984, chlorothalonil applied at boot and heading significantly increased yields over 1 spray at either stage alone. Two or 3 sprays of captafol or propiconazole significantly ($P=0.05$) increased seed yields in 1984, but not in 1985 or 1986. In 1985, disease severity was low and seed yields were not different among treatments.

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Potential of using methionine-riboflavin mixture in the control of plant diseases. D. D. Tzeng, M. H. Lee, and H. L. Tzeng. Dept. of Plant Pathology, Natl. Chung Hsing Univ., and Dept. of Plant Pathology & Entomology, Tobacco Res. Inst., Taiwan, ROC.

Phytodynamic reaction of methionine-riboflavin mixture (MR) was biocidal to various microorganisms including plant pathogenic fungi and bacteria. The likely photooxidation products responsible for the killing reaction appeared to be methional, methanethiol, dimethyl disulfide and various oxygen derivatives. Blue rich plant growth light served as a good light source for initiating the biocidal reaction. Incandescent light was less effective. Presence of certain free radical scavengers and antioxidants were inhibitory to the biocidal activity. Whereas the addition of various bivalent cations and detergents enhanced its effectivity greatly. The efficacy of MR in the control of fungal diseases was evaluated on several crops by greenhouse and field trials. By spraying MR solution onto the powdery mildew infected plant parts, the mildew-lawn was killed and the continued infection was discouraged. Other applications of MR in plant protection will be discussed.

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TOP-AGAR OVERLAY (TAO) METHOD FOR SYNCHRONIZED CULTURE OF FILAMENTOUS FUNGI. C. D. Franklin and R. M. Hanau, Dept. of Botany and Plant Path., Purdue Univ., West Lafayette, IN 47907

We have devised a novel method for culturing fungi that yields gram quantities of developmentally synchronized mycelia. Medium containing 1.5% agar is poured into a petri plate and allowed to solidify. Inoculum (spores, mycelia, etc.) is mixed in medium containing 0.8% agar at 48° C and applied as a top-agar overlay (TAO). After the TAO solidifies, a circular sheet of Miracloth is applied over the surface. Aerial mycelia grow through the Miracloth layer and are easily harvested by scraping with a spatula. The homogenous distribution of inoculum across the plate eliminates physiological and developmental gradients associated with point-inoculated cultures and mycelia harvested in this way are free of contaminating agar medium. We have used this technique to study developmental stages associated with conidiogenesis in Helminthosporium carbonum and to compare growth yields and protein profiles from cultures of other fungi.

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A FUNGICIDE SCREEN FOR FUSARIUM WILT OF CHRYSANTHEMUM. A. W. Engelhard and S. S. Woltz, IFAS, Univ. of Fla. Gulf Coast Research and Educ. Center, 5007 60th St. E., Bradenton, FL 34203

Screening results using F. oxysporum f. sp. chrysanthemi vary greatly because N source, soil pH and minor elements affect disease level. A model system is a mix of 5:3:3:1 parts peat, sand, vermiculite and perlite. To 45 liters of mix add 424 g dolomite, 42 g $Ca(OH)_2$, 424 g $CaCO_3$, 106 g superphosphate and 63.5 g Micromax^R. One Royal Trophy chrysanthemum cutting is planted per 10 cm pot. Drench fungicides at 50 ml/pot 3 and 10 days after planting and fertilizer solution (50 ml/pot 6.6 g/l $Ca(NO_3)_2$ + 1.5 g/l KCl) twice weekly beginning immediately. Inoculate plants at 11 days by drenching with 50 ml (10^7 conidia) per pot. Make parallel cuts in the medium 2.5 cm away from the stem to injure roots before inoculation. After 9 wks benomyl at 0.6 and 0.15 g/l gave a rating of 0.4 and 2.3 (0-5, 5 = dead plant); thiophanate M at 0.6 g/l gave 1.0; diniconazole at 0.15 g/l gave 1.5; triflumizole at 1.2 g/l gave 2.1 and the inoculated check was 4.4.

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DEVELOPMENT OF AN INDIRECT FLUORESCENT ANTIBODY STAIN FOR DETECTION OF PYRENOPHORA TRITICI-REPENTIS IN HOST LEAF TISSUE. S. R. Robertson, W. F. Pfender, and S. L. Wootke, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Pyrenophora tritici-repentis, causal agent of wheat tan spot, commonly occurs as part of a fungal leaf-spotting complex. Common histological stains cannot differentiate among these fungi, so an antiserum specific to P. tritici-repentis was developed for use in an indirect fluorescent antibody stain. The fungus was grown in liquid culture, was then collected by filtering, air-dried, ground to a fine powder in liquid nitrogen, and stored frozen. The mycelium was homogenized and centrifuged to collect cell walls, and the supernatant lyophilized for a soluble fraction. Rabbits were injected with the walls or soluble fraction. Infected host tissue was fixed in 95% EtOH, cleared in 6% NaOH, reacted with diluted antisera, and stained with FITC-conjugated goat anti-rabbit antiserum. Antiserum obtained from cell walls resulted in more intense staining than that from the soluble fraction.

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DEVELOPMENT OF A DISEASE SCREEN FOR EARLY BLIGHT RESISTANCE IN SOLANUM SPP. M. J. Bussey and W. R. Stevenson, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

Resistance to early blight, caused by Alternari solani, was examined using leaf tissue from ten cultivars of Solanum tuberosum, grown in a growth chamber or greenhouse. Leaf discs (14 mm) were cut from five leaves of equivalent maturity per cultivar, 10 to 80 days post-emergence. Tissue was sprayed with a suspension of A. solani (3,000 conidia/ml), floated on glass distilled water or a phytohormone solution in 100mm petri dishes and incubated at 22°C in the dark for three days. The effects of cytokinin, abscissic acid, auxin and distilled water upon lesion development were compared. Symptom expression was rated (0-9) based on % infection of individual leaf discs. More reproducible estimates of susceptibility, which were correlated (r=.75) with observations of early blight in Wisconsin field trials, were obtained with NAA than with other tested materials.

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SUBSPECIFIC CLASSIFICATION OF ISOLATES OF VERTICILLIUM DAHLIAE BASED ON VEGETATIVE COMPATIBILITY. T. R. Joaquim*, R. C. Rowe*, and J. E. Puhalla**. *Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691, and **P. O. Box 7583, Berkeley, CA 94707.

Nineteen isolates of Verticillium dahliae (Vd) collected from potato plants or soil in Ohio, and two plant isolates from Wisconsin were assigned to vegetative compatibility groups (VCG) based on pairings of mutants unable to use nitrate (Nit). Nit mutants generated from each isolate on cornmeal-dextrose agar amended with $KClO_3$ (15 g/L) were paired with two complementary Nit mutants obtained from tester isolates representing VCG#1,2,3,4, and 5 (Phytopathology 73:1305). Nine Ohio isolates belonged to VCG#3 while the rest were VCG#4. All isolates belonging to VCG#3 were also compatible with a tester isolate (PG) of VCG#5, suggesting that with this technique, PG would have to be reassigned to VCG#3. Eight of nine isolates in VCG#3 were collected from Wayne County while nine of 10 in VCG#4 were from Portage, Columbiana, and Sandusky counties, thus supporting the concept of localized Vd populations.

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USE OF CLUSTERING METHODS WITH MONOCLONAL ANTIBODIES FOR TAXONOMIC ANALYSIS OF PHYTOPHTHORA AND FOR SCREENING AND CLUSTERING ANTIBODIES. M. W. Ferguson, A. R. Ayers and K. L. Wykoff, South Dakota State University, Brookings, SD 57007; Cedar Crest College, Allentown, PA 18100; Harvard University, Cambridge, MA 02138.

Monoclonal antibody (Mabs) clones raised against Phytophthora megasperma f.sp. glycinea, exhibited a wide range of specificities (using ELISA) to other Phytophthora species, which raised the possibility of use as a taxonomic aid. However, the data set for the various Mab-fungal combinations necessitated finding a method to simplify and draw relationships between the numerical data and the relative serological (and possibly taxonomic) positions of the fungi tested. It was determined that cluster analysis can be used to describe the relationship between a fungal epitope and other fungi with or without this epitope. In addition the antibodies can also be grouped by a simple inversion of the data. Further, this analysis can be used for coded data.

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NONDESTRUCTIVE CHLOROPHYLL DETERMINATION AS A MEASURE OF SENESCENCE IN MAIZE. A. Issoufou Kollo, P. C. Lyons, and R. L. Nicholson, Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, 47907.

Susceptibility to fungal pathogens often increases with leaf senescence. Chlorophyll content decreases with the onset of leaf senescence and can be used as a measure of leaf age and susceptibility. Visual estimates of chlorosis occurring during senescence are subjective while accurate chloropyll determination by conventional methods are destructive. We have used a hand-held spectrochlorophyllometer (Hardacre, R. K. et al 1984, New Zealand J. Exp. Agric. 12:357) to accurately and nondestructively determine chlorophyll content of maize leaves throughout their ontogeny. The meter was calibrated against known concentrations of chlorophyll determined by tissue extraction. With this technique we have accurately quantitated leaf maturation and senescence, and have correlated such measurements with susceptibility to Colletotrichum graminicola.

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A NEW SUGARCANE SMUT INOCULATION PROCEDURE. Stephen A. Ferreira, Dept. of Genetics & Pathology, Hawaiian Sugar Planters' Association, P. O. Box 1057, Aiea, Hawaii 96701.

A new inoculation procedure for sugarcane smut (Ustilago scitaminea Syd.) was developed and is described. Single node vegetative cuttings were germinated in a peat-vermiculite potting mix for 5-7 days or until the developing shoot was about 1 cm tall. Inoculation was accomplished by dipping a dissecting needle into a 10^6 teliospore/ml suspension and puncturing the shoot along its axis, thereby introducing no more than 1000 teliospores into the meristem region located at its base. Although meristem tissue was punctured, less than 1% of the seedlings were killed. Twenty-three varieties, varying in resistance were evaluated using this new inoculation procedure and the standard dip method. The varietal reactions were compared using linear regression analysis. The correlation coefficient for the comparison was 0.91 and the relationship between the two methods can be described with the following equation: $y = 0.140x + 0.015$ with y being the maximum infection level in percent, x being the smut grade (0-9 scale) determined using the standard dip method. Comparison of coefficients of variation for the two methods suggests that the new method is more reliable.

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BACTERIAL HYPERSENSITIVE REACTION IN SUSPENSION CULTURE CELLS OF COTTON (GOSSYPIUM HIRSUTUM). S. Ben-Gweirif and A. Novacky, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Suspension cells were cultured from Immune 216 and Acala 44, cotton resistant and susceptible, respectively, to Xanthomonas campestris pv. malvacearum Race 1. Fifty percent of Acala 44 cells treated with 10^8 cells/ml X. malvacearum were dead 24 hrs after inoculation. Fifteen percent of Immune 216 cells inoculated with the same bacteria were dead 24 hrs following inoculation. Both Acala 44 and Immune 216 cell cultures inoculated with 10^7 incompatible Pseudomonas syringae pv. pisi had 50% dead cells 12 hrs after inoculation. Cells from Acala 44 inoculated with X. malvacearum exhibited a higher K^+ efflux than those from the resistant line. Resistant and susceptible cells inoculated with 10^7 P. pisi had more K^+ efflux than those inoculated with 10^8 X. malvacearum. The efflux of K^+ in both lines was accompanied by a pH increase during the first 6 hrs after treatment. Increase of electrolyte leakage indicates that there is an alteration in plasma membrane function which leads to a K^+/H^+ exchange, and is related to the cell death in the incompatible combination.

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FORMATION OF A STABLE BACTERIAL PRODUCT DURING THE INTERACTION OF PSEUDOMONAS SYRINGAE WITH TOBACCO SUSPENSION CULTURE CELLS. I. Yucel and S.W. Hutcheson, Dept. of Botany, University of Maryland, College Park, MD 20742.

Induction of the ionic response (IR; K^+ efflux/H^+ influx) of tobacco suspension culture cells by incompatible Pseudomonas syringae pathovars requires an induction stage, defined by a 2-hr period of sensitivity to inhibitors of bacterial RNA and protein synthesis. In an effort to elucidate whether a stable gene product accumulates in the bacteria during the induction stage, P. syringae pvs. pisi and syringae were preincubated with tobacco suspension culture cells under IR assay conditions. The bacteria were then reisolated and used to inoculate fresh tobacco cells. IR induction by either pathovar pretreated for longer than 3 hr was insensitive to rifampicin, tetracycline and streptomycin (up to 200 µg/ml). These results indicate that a stable bacterial product is formed during IR induction, possibly the result of a gene induction event.

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EXAMINATION OF HYDATHODE WATER PORE PLUGGING IN RICE EXPOSED TO COMPATIBLE AND INCOMPATIBLE Xanthomonas campestris pv. oryzae RACES. A. Guo and J. E. Leach. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

In some interactions between Xanthomonas campestris pv. oryzae (Xco) and rice, it has been shown using scanning electron microscopy (SEM) that incompatible bacteria are entrapped in an exudate which emanates from hydathode water pores of the host (Phytopathology 74:635). To test if this response is a general resistance mechanism, leaves of four rice differential cultivars, spray-inoculated with incompatible or compatible Xco isolates or water, were sampled for SEM examination at 24, 48, and 72 h postinoculation. More than 50 water pores were examined from each treatment. All treatments (compatible or incompatible bacteria or water) induced plugs. No difference in the percentage of plugs induced by any treatment (over untreated tissues) was observed, even when different environmental regimes were tested. Therefore, water pore plugging is not specifically induced in incompatible Xco/rice interactions.

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CHANGES IN METHANOL-SOLUBLE AND WALL-BOUND PHENYLPROPANOIDS IN MAIZE IN RESPONSE TO INFECTION. P. C. Lyons, J. D. Hipskind, and R. L. Nicholson. Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.

Infection of several maize cultivars with either Colletotrichum graminicola (C.g.) or Helminthosporium maydis race O (H.m.) resulted in the accumulation of specific methanol-soluble and wall-esterified phenylpropanoids. The two major methanol-soluble compounds were identified as caffeoyl-glucose esters by TLC, GC and mass spectral analysis. These compounds were typically minor constituents of uninfected controls; however, inoculation with H.m. and C.g. increased their levels to as much as 500 ug/gfw or more within 48 and 88 hr., respectively. Analysis of base hydrolysed cell wall residues showed that concentrations of wall bound p-coumaroyl and feruoyl esters also increased after infection. Significant increases in the methanol-soluble caffeoyl esters and the wall-bound hydroxycinnamoyl esters at infection sites were observed within 6 to 8 hr. of penetration of the maize cv. B73 inoculated with H.m.

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ACCUMULATION OF MEDICARPIN IN RESISTANT AND SUSCEPTIBLE ALFALFA SEEDLINGS AND FUNGITOXICITY TO COLLETOTRICHUM TRIFOLII. N. R. O'Neill, C. J. Baker, and T. A. Campbell. USDA, Agricultural Research Service, Beltsville, MD 20705.

A method has been developed for growing alfalfa plants suitable for phytoalexin accumulation studies. Two-week-old seedlings resistant or susceptible to anthracnose were inoculated with 2 x 10^6 spores/ml C. trifolii and analyzed by TLC and HPLC for accumulation of phytoalexins. Major differences in accumulation rates of medicarpin and 6 other fungitoxic compounds were observed in time-course experiments. Maximum accumulation of medicarpin (29 µg/g fresh wt.) in the incompatible reaction occurred after 60 hours while susceptible tissue accumulated 1.5 µg/g. Gene-specific resistance was demonstrated in root, shoot, and cotyledon tissues, the differential being greatest in shoots and smallest in cotyledons. In in-vitro tests, C. trifolii race 2 isolates were generally less sensitive to medicarpin than race 1 isolates.

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INDUCTION OF SYSTEMIC RESISTANCE TO ANTHRACNOSE IN CUCUMBER BY OXALATE AND EXTRACTS FROM SPINACH AND RHUBARB LEAVES. N. S. Doubrava, R. A. Dean, and J. Kuć, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

An extract from spinach or rhubarb leaves sprayed on the undersides of leaf one (first true leaf) and two of cucumber plants induced systemic protection against disease caused by Colletotrichum lagenarium. Oxalate was identified as the active component of both extracts using solvent fractionation, ultrafiltration, precipitation, ion exchange chromatography, TLC, and HPLC. Germination and growth of the fungus were not inhibited in vitro by oxalate or the extracts at concentrations equal to those applied to leaves. Dimethyl and diethyl esters of oxalic acid, malonate, formate, glycolate, acetate, phthalate, fumarate, and maleate were inactive, whereas ascorbate and glyoxylate were less active than oxalate. Protection was evident systemically 20-36 h after spraying with the spinach extract.

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NEEDLE-INDUCED WOUND PLUGS, FUNGAL-INDUCED PAPILLAE AND CYTOPLASMIC AGGREGATES IN BARLEY CELLS. V. Russo and W. Bushnell. USDA/ARS, Cereal Rust Lab., Univ. of Minn., St. Paul, MN 55108.

Cytoplasmic aggregates and papillae induced in epidermal cells of Hordeum vulgare coleoptiles beneath appressoria of Erysiphe graminis f. sp. hordei were compared to aggregates and wound plugs induced by incision with glass needles. Epidermal tissues were placed on 0.34 osm sucrose to reduce turgor 12 hr after inoculation or 2 hr before incision. Cytoplasmic aggregates induced 13-16 hr after inoculation were similar to aggregates induced 1-3 min after incision except that aggregates were present only intermittently if needles were removed from cells. The fungus induced aggregates both in infected cells and in cells adjacent, but needles never induced aggregates in adjacent cells. Wound plugs contained pectin and had more cellulose, but were otherwise similar in composition to papillae. Wound plugs often extended through the cell wall as extrusions on the cell surface and held against full turgor when tissues were placed in water 5 min after wounding.

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ULTRASTRUCTURE OF PSEUDOPERONOSPORA CUBENSIS IN MUSKMELONS SUCEPTIBLE AND RESISTANT TO DOWNY MILDEW. Yigal Cohen, Helena Eyal, Judith Hanania and Zvi Malik, Dept. of Life Sciences, Bar-Ilan University, Ramat-Gan, 52100, Israel.

Fungal development was microscopically examined in Cucumis melo Ananas-Yokneam (AY, susceptible) and PI 124111 (PI, resistant). Fungal penetration occurred through stomatal openings in both cultivars. Total mycelial length per inoculated site was similar in AY and PI but host area occupied was x100 in AY than in PI. Mycelia were intercellular, multinucleate and rich in lamellar structures. Haustoria were lobbed ("cauliflower" shape), had a collar, were surrounded with matrix whose size increased with age. Their appearance was similar in both cultivars. Advanced nerosis of host cells was noticed in PI at 100 hrs but not in AY. Invaded and adjacent PI cells had occasionally much thicker walls than normal. The relationship of wall thickness to resistance was not established.
Research supported by BARD.

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MEMBRANE POTENTIAL IN VIRUS-INFECTED CELLS. F. Beleid (El-Moshaty), A. Novacky, & O. Sehgal, Dept. of Plant Path., Univ. of MO, Columbia, MO 65211.

Cells in green tissues monitored with microelectrodes exhibit transient changes in membrane potential (Em) as a response to light and dark. Em of cells in primary leaves of cowpea (Vigna sinensis) infected with tobacco ringspot virus (TRSV) which induces hypersensitive reaction and southern bean mosaic virus, cowpea strain (SBMV-cp) which causes systemic infection were measured under light, dark, and anoxic conditions. In light Em in SBMV and TRSV infected cells was initially at the control level (-170 mV). After lesion development in TRSV infection cells the Em decreased to -137 mV. In dark both control and systemically infected cells transiently hyperpolarized and depolarized. In the long dark treatment Em of systemically infected cells decreased to -100 mV which in dark +anoxia reached the diffusion potential (-92 mV). Em in dark within the lesion of TRSV remained hyperpolarized by 10-15 mV for 7-10 min, followed by a sharp depolarization close to the diffusion potential. Changing the external pH from 5.7 to 4.5 enhanced the dark-induced hyperpolarization by Δ35 mV. At pH 7.5 cells within the lesion behaved similarly to the control. A possible alteration of intracellular pH regulation during viral hypersensitivity will be discussed.

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AUTOFLUORESCENCE OF SOYBEAN PLANTS TREATED WITH TOXIN PRODUCED BY Diaporthe phaseolorum var caulivora. B. Lalitha, J. P. Snow and G. T. Berggren. Dept. of Plant Path. and Crop Phys. Louisiana State Univ. Agric. Center, Baton Rouge, LA 70803.

Diaporthe phaseolorum var caulivora produces a toxin which may be involved in the stem canker disease of soybeans. Soybean plants treated with cell-free culture filtrate, partially purified toxin preparations or purified toxin preparations showed greenish yellow fluorescence of vascular bundles particularly in the sclerenchymatous sheath of the bundle. Excised trifoliates and intact plants were treated with the toxin and observed after 24 hours. Sections of stems, petioles and midribs which showed symptoms were observed using a fluorescece microscope with a H2 filter. In all cases fluorescence of vascular bundles of treated plants was observed. A reddish discoloration seen externally was always associated with the fluorescence. The toxin did not fluoresce and the autofluorescence seen in the treated plants is thought to be a response of the host to the toxin treatment.

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NONRANDOM PATTERNS OF BACTERIAL BROWN SPOT IN FIVE TO 12 METER ROW SEGMENTS OF SNAP BEANS. B. D. Hudelson[1], M. K. Clayton[1], K. P. Smith, D. I. Rouse, and C. D. Upper[2]. Departments of Plant Pathology and Statistics[1], and ARS, USDA[2], University of Wisconsin-Madison, Madison, WI 53706.

Each leaflet on every plant in five to 12 m row segments in commercial fields of snap beans (Phaseolus vulgaris L.) was rated for bacterial brown spot, caused by Pseudomonas syringae pv. syringae Van Hall. Graphs of proportion of diseased leaflets per plant vs. plant location in the row are jagged, suggesting negative correlation. In contrast, runs analysis about the mean indicates fewer runs than expected for a random disease pattern, suggesting positive correlation. ARIMA models, used in time series modeling to describe and quantify autocorrelation in serially ordered data, suggest autocorrelation at two levels: negative autocorrelation associated with interactions between adjacent plants superimposed on a larger scale, positively correlated process. Implications of these autocorrelations for sampling will be discussed.

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EFFECT OF CAPTAFOL ON PHYTOPHTHORA BLIGHT OF PEPPER. J. H. Bowers, D. J. Mitchell, and R. M. Sonoda. Plant Pathology Dept., Univ. of Florida, Gainesville, 32611 and ARC, Ft. Pierce, FL 33454.

Field tests were established in Delray Beach, FL in the spring of 1985 and the fall of 1986 to evaluate the reduction of initial inoculum of Phytophthora capsici on the rate of disease progress. Diseased plants were transplanted into the centers of eight plots to serve as point sources of inoculum. Plots were sprayed weekly for the first 5 weeks of 1985 and 6 weeks of 1986 with 1680 g a.i. of captafol/hectare. Control plots were not sprayed. Disease incidence (characterized by wilt and the presence of lesions) was assessed weekly. Final disease incidences in 1985 in control and treated plots were 78 and 9%, respectively, with average mortality rates as determined by the logistic transformation of .08 and .03/unit/day. Final disease incidences in 1986 in control and treated plots were 100 and 11%, respectively, with average mortality rates of .16 and .02/unit/day. Within each year, the regression lines were significantly different at P=.01.

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EPIDEMIOLOGY OF RHIZOCTONIA AERIAL BLIGHT OF SOYBEAN. X.B. Yang, G. T. Berggren, and J. P. Snow. Dept. of Plant Pathology and Crop Physiology, Louisiana State Univ. Agricultural Center, Baton Rouge, LA 70803.

Components of Rhizoctonia aerial blight (RAB) epidemics of soybean were studied. These components include: distribution of primary disease foci in the field, distribution of primary symptoms during the growing season, development of disease foci, and dispersal of the pathogen. A correlation was developed between Leaf Area Index (LAI) and primary foci and subsequent disease spread through the canopy. The development of disease foci was influenced by LAI and rainfall patterns. The importance of airborne spread of inoculum in disease development was shown. Yield loss was correlated with disease severity at the R3 growth stage (r^2=0.51, n=36). The number of pods was correlated with remaining leaves at the R6 growth stage (r^2=0.70, n=32). These data are being used to aid in developing a disease model for RAB.

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EVIDENCE FOR AN EXOGENOUS SOURCE OF LEAF RUST INOCULUM FOR WINTER WHEAT IN LOUISIANA. K.V. Subba Rao, X.B. Yang, and L. Anzalone, Jr., Dept. of Pl. Path. & Crop Phys., La. Agric. Exp. Sta., La. State Univ. Ag. Center, Baton Rouge, La 70803.

The primary sources of winter wheat (Triticum aestivum L.) leaf rust (Puccinia recondita Rob. ex Desm. f. sp. tritici) inoculum have not been determined in Louisiana. Surveys were conducted during mid-Dec., 1986, at Alexandria (Alex.), and Baton Rouge (BR), in La. The growth stage of the wheat cultivar, McNair 1003 was GS 3 (Feeke's) at BR and GS 4 at Alex. Leaf rust incidence was 71% at BR and 98% at Alex. and was uniform throughout the plots. Seventy-five to 90% of the leaves on each plant exposed at the time of urediniospore arrival were infected. The date of spore arrival was calculated by the published method of Eversmeyer et al, and is based on the accumulated effective temperature. The daily rainfall, temperature, and weather maps indicate the possibility of rain-washed urediniospores from a large exogenous source. The pustule size and absence of secondary pustules suggest that the infection at both locations occured at the same time.

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WHEAT TAN SPOT LOSSES AS INFLUENCED BY TIME OF EPIDEMIC TERMINATION. A. Shabeer and W. W. Bockus. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Field fungicide spray programs on winter wheat were initiated at four different times during the season and compared with a nonsprayed check. Growth stages at initiation of the spray programs were: i) beginning of pseudostem erection (March 15), ii) pseudostem fully erect (April 15), iii) awns just showing (May 11), and iv) watery ripe (May 22). Treatments were replicated five times and the epidemic started with oat kernel inoculum. At spray initiation, Manzate 200 (2.3 kg + 187 L water/ha) was applied with subsequent sprays every 4-7 days until maturity. Approximately half of the total yield loss caused by tan spot occurred before the boot stage. Furthermore, about 10% yield loss occurred from early season (March 15 to April 15) disease. Thus, to achieve adequate control, fungicide sprays for tan spot control may have to be applied earlier than for the rusts.

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EFFECT OF WETNESS DURATION AND TEMPERATURE ON INFECTION OF STRAWBERRY FRUIT BY COLLETOTRICHUM ACUTATUM. L. L. Wilson, M. A. Ellis, and L. V. Madden, Dept of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691

Attached immature (green) strawberry fruit were inoculated with a $25x10^3$ conidia/ml suspension of Colletotrichum acutatum and placed under various wetness (free water) durations ranging from 1 to 48 hr at constant temperatures of 5,10,15,20,25,30, and 35 C. Inoculated plants were then moved to a greenhouse where incidence of fruit infection was recorded daily for 8 days. Symptoms rarely developed on any fruit until they reached maturity (turned red). At temperatures of 25 and 30 C, 12 hr of wetness resulted in >75% fruit infection. More hours of wetness were required for infection at all other temperatures observed. At 10 and 35 C, no symptoms were observed with <24 hr of wetness. No infection was observed at 5 C with up to 48 hr wetness. Inoculations of mature (red) fruit resulted in 100% fruit infection after 12 hr wetness at 25 C.

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A LEAF WETNESS SENSOR FOR MOISTURE DETECTION RELATING TO DISEASE PREDICTION MODELING. Hulse, M.M. and W.H. Shaffer. Electronic Instrument Lab and Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

A "solid-state" device was developed in response to a need for a leaf wetness sensor for the Automatic Reporting Agricultural Weather System (ARAX). It consists of a triangular shaped base and a 3-sided pyramid top approximately 6.35 cm on each side. A gap between the base and pyramid top is made using plastic spacers of various thicknesses. By varying the thickness of the spacers, the sensitivity of the device can be changed to monitor moisture formed by dew or rainfall. An electric current between base and top is measured. If moisture is present, current increases and the ARAX computer records the increased value. The "pyramid" gives similar results to actual wetness on twigs and leaves. Disease prediction programs for apple scab, cedar-apple rust, fireblight of apple, and black rot of grape have been written. The Leaf Wetness Sensor may be used with other data recorders.

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CORYNEBACTERIUM SEPEDONICUM FROM SUGAR BEET SEED. W. M. Bugbee and N. C. Gudmestad, USDA-ARS and Department of Plant Pathology, North Dakota State University, Fargo, North Dakota

Strains of Corynebacterium sepedonicum, the cause of potato ring rot, were recovered from five of six cultivars that were produced in Oregon in 1984. The bacterium was recovered directly from culture plates of diluted seed extracts and from extracts of inoculated eggplants. All strains were pathogenic on potato and eggplant. Over 80% of the seeds from one cultivar were infected. Indirect immunofluorescent monoclonal antibody staining detected the bacterium in seed from one of seven U.S. cultivars produced in 1985 and in two of 26 European seedlots, but the bacterium could not be recovered from dilution plates or inoculated eggplants. Antagonism by other bacteria on dilution plates was evident and might have accounted for the failure to recover C. sepedonicum from the 1986 U.S. and European seedlots.

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A MORPHOLOGICALLY BASED MODEL OF DISEASE PROGRESS FOR POWDERY MILDEWS. J.A. Quinn and T.T. Fujimoto, Rohm and Haas Co., Spring House PA, 19477.

A model has been devised that theoretically relates effects on fungal morphology (e.g germination, hyphal growth, branching and conidial production) in petri plates and growth chambers to disease progress in the field. The number of colonies in the first generation is given by the number of primary infection units times the infection frequency. Daily sporulation of these colonies can be calculated (Ann. Appl. Biol. 107: 163-178) and after a latent period this conidial number is multipled by the infection frequency to give rise to the number of colonies in the second generation. The third generation colony number is calculated from sporulation in the second generation and so on to the nth generation. Effects of environment can be incorporated into the model using multiple regression equations. Percent crop infection can be estimated by keeping track of the age of each colony (which is correlated to colony size). Effects of density on infection frequency are also modelled.

527. Withdrawn

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A MATHEMATICAL MODEL TO DESCRIBE THE MOVEMENT OF PHYTOPHTHORA ZOOSPORES THROUGH SOIL. D. M. Ferrin, Department of Plant Pathology, University of California, Riverside, CA 92521.

A state variables model was developed to describe the movement of zoospores of Phytophthora spp. through water-saturated soil. Both active and passive movement are considered and are represented as transport processes. Active movement encompasses spatially-directed responses to environmental stimuli and nonspatially-directed responses resulting from their random movement. Vertical displacement results from negative geotaxis in response to a gravitational gradient. Horizontal displacement results from dispersal from areas of high zoospore density to surrounding areas of lower density. Both horizontal and vertical displacement result from chemotaxis in response to a chemical gradient. Passive movement results from their convection in moving water.

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RELATIONSHIP BETWEEN GLYCEOLLIN ACCUMULATION AND NITROGEN FIXATION CAPACITY OF NODULES FORMED BY BRADYRHIZOBIUM JAPONICUM STRAINS. Karr, D.B., D.W. Emerich, and A.L. Karr, Biochemistry and Plant Pathology, University of Missouri, Columbia, MO 65211.

Glyceollin accumulates in both effective (strain 143;EC) and ineffective (mutant 122 L3;IC) combinations of Bradyrhizobium japonicum and soybean (cv. Williams 82). The pattern of accumulation is characterized by the appearance of isomer I, followed by the appearance of isomers II and III. Plots of I/II+III vs time show a positive inflection when accumulation begins. From such plots, it is clear that glyceollin accumulation in EC begins at a time just preceding the decrease in nitrogen-fixation and bacteroid viability (days 21-24). IC is characterized by the accumulation of more than 4 times as much glyceollin in the nodules and significant accumulation in the adjoining non-nodulated root system. Glyceollin levels are sufficiently high to account for the decrease in nitrogen-fixing capacity of EC (143;ED_{50} = 50 µg/ml) and the in planta death of mutant 122 L3 in IC (ED_{50} = <20 µg/ml). In addition, at least two unidentified, toxic isoflavones accumulate at the same time as glyceollin.

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MONOCLONAL ANTIBODIES TO PECTATE LYASE FROM ERWINIA CAROTOVORA SUBSP. CAROTOVORA. L.J. Ward and S.H. De Boer, Agriculture Canada, 6660 N.W. Marine Dr., Vancouver, B.C., Canada, V6T 1X2

The 41kd and 44kd forms of pectate lyase were purified from the supernatant of Erwinia carotovora subsp. carotovora strain 567 using substrate affinity chromatography. These proteins were used to generate specific monoclonal antibodies against pectate lyase. Western blot analysis of concentrated Erwinia culture supernatants showed that the monoclonals reacted with three general classes of Erwinia supernatant proteins. In addition to the 41 and 44kd proteins, some monoclonals reacted with very large (>250kd) and small (18-20kd) molecular weight proteins. Several possible explanations for these findings are currently under investigation.

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GENETIC ANALYSIS OF THE ROLE OF SYRINGOMYCIN, PRODUCED BY PSEUDOMONAS SYRINGAE PV. SYRINGAE, IN DISEASE DEVELOPMENT. G.-W. Xu and D. C. Gross, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Genes encoding syringomycin production in Pseudomonas syringae pv. syringae B301D were identified from an EcoRI-pLAFR3 cosmid library and used to assess the role of the phytotoxin in disease development. Two cosmids, pGX56 and pGX183, functionally complemented nontoxigenic Tn5 mutants W4S770 ($Path^-$, HR^+) and W4S2545 ($Path^-$, HR^+), respectively. Results summarizing the salient genetic and biological characteristics of the cosmid-cloned syringomycin genes will be presented. Nontoxigenic strain W4S770 attained a population comparable to that of wild-type strain B301D in inoculated sweet cherry fruits; however, lesion diameters for strain W4S770 were markedly less (average = 1.4 mm) as compared to strain B301D (average = 4.3 mm) 3 days after inoculation. These studies indicate that syringomycin is not required for pathogenicity but does significantly contribute to virulence.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF A XYLANASE FROM ERWINIA CHRYSANTHEMI. E. J. Braun. Dept. of Plant Pathology, Iowa State University, Ames IA 50011-1020.

An extracellular endo-β-1,4 xylanase was partially purified from culture filtrates of a corn stalk rot strain of Erwinia chrysanthemi. The xylanase was precipitated in ammonium sulfate (60-90%), desalted, and bound to CM-Sephadex at pH 5.5. Following elution on a salt gradient the enzyme was dialyzed, concentrated by ultrafiltration and applied to a gel filtration column (Sephadex G-75). The partially purified enzyme had a pH optimum of 5.5 and a temperature optimum of 55 C in a 10 min assay. The xylanase preparation lost 25% of its activity following 1 hr at 40 C. The molecular weight (via gel filtration) was approximately 23,000. The enzyme was shown to cleave internal bonds in xylan polymers. The xylanase was produced consistitutively and appears to be subject to catabolite repression. Studies are underway to assess the role played by this enzyme in the maceration of grass tissues.

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MOBILIZATION OF COSMID DNA INTO Xanthomonas campestris pv. oryzae. S. Kelemu and J. E. Leach. Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A major constraint to genetic analysis of Xanthomonas campestris pv. oryzae (Xco) has been the inability to stably introduce extrachromosomal DNA into the pathogen. Therefore, conditions for introduction of various cosmid vectors into Xco (representing six races) using liquid triparental matings were determined. Initially, when Xco was maintained on nutrient media, only pLAFR5 could be introduced into only one isolate of race 2 at a low efficiency (two transconjugants per 10^7 recipients). Subsequently, we found that isolates of races 1, 2, 5, and 6, when cycled three to five times on a minimal medium, were efficient recipients of pSA747 and pSF6 (2×10^{-5}). pLAFR5 could be introduced only into race 2 (1×10^{-6}) and race 5 isolates (2×10^{-4}). The cosmids are stably maintained in Xco and their presence has no apparent effect on growth or pathogenicity on rice differential cultivars.

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TOBACCO RESPONSES TO COMPATIBLE AND SAPROPHYTIC PSEUDOMONAS STRAINS CARRYING A CLONED P. SYRINGAE PV. SYRINGAE GENE CLUSTER. H.-C. Huang, I. Yucel, A. Collmer, and S.W. Hutcheson, Dept. of Botany, Univ. of Maryland, College Park, MD 20742.

We have previously reported the molecular cloning of P. syringae pv. syringae (Pss) genes that complement Tn5 mutations affecting the elicitation of the hypersensitive response (HR) in tobacco leaves and the ionic response (IR; K^+ efflux/H^+ influx) of cultured tobacco cells. Cosmids pHIR10 and pHIR11 contain 15 and 27 kb, respectively, of Pss DNA in pLAFR3 and complement three and six, respectively, of the six Pss Tn5 mutations studied. In an effort to elucidate whether these cosmids would affect the phenotype of the compatible pathogen, P. syringae pv. tabaci (Pst), and the saprophyte, P. putida, transconjugants were generated by triparental matings. Transconjugant Pst and P. putida cells containing pHIR11, but not pHIR10, elicited typical HR symptoms in tobacco leaves when injected at a concentration of 1×10^8 cells/ml. Pss and Pst carrying pHIR11 also stimulated the IR in suspension cultured tobacco cells more rapidly than Pss and Pst carrying pHIR10.

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INFECTION OF CANADA THISTLE BY UREDINIOSPORES, AECIOSPORES, AND TELIOSPORES OF PUCCINIA PUNCTIFORMIS. R. C. French, S. K. Turner, and A. R. Lightfield, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701

Potted Canada thistle plants inoculated 9/86 with urediniospores produced urediniospores in 4 wk, and aecial stages were produced within 8-24 wk in 10 of 20 plants. Plants inoculated with aeciospores produced urediniospores in 10 of 10 plants. Thistle root extract (Turner, Kwiatkowski and Fay; Phytopathology 72:711, '82) stimulated teliospore germination (optimum 7 da at 15-18 C). Teliospores exposed for 1 hr to volatiles from a steam-distilled thistle root extract germinated at the maximum rate (50%) at 7 days, 18 C. Root cuttings dipped in teliospores floated on stimulator extract for 6 days produced aecial stages only in the secondary shoots after 5 wk. Secondary shoots produced systemic, orange spermagonia, mostly on the abaxial leaf surfaces. These rapidly turned black as aeciospores were produced. These results suggest a potential use of this fungus as a biocontrol agent.

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4-HYDROXYCINNAMATE: CoA LIGASE AND LIGNIN FORMATION IN MAIZE. J. R. Vincent, P. C. Lyons, and R. L. Nicholson. Dept. Botany & Plant Pathology, Purdue University, W. Lafayette, IN 47907.

4-Hydroxycinnamate: CoA ligase (CoA ligase), a key enzyme of phenylpropanoid metabolism, forms CoA thioesters of 4-hydroxycinnamic acids, the precursors of lignin and flavonoids. Thus CoA ligase is central to synthesis of phenols frequently associated with disease resistance. In lignin formation, p-coumaroyl, feruloyl, and sinapoyl CoA thioesters are reduced to alcohols which polymerize to form lignin. When maize leaf cell wall residues were subjected to cupric oxide oxidation and HPLC analysis, the aldehyde products corresponding to these three cinnamyl alcohols were all present in significant quantity. Whereas CoA ligase partially purified from Helminthosporium maydis race O-infected maize leaves exhibited substantial activity using p-coumaric and ferulic acids as substrates, no activity was detected using sinapic acid. Apparently the sinapyl alcohol residues from maize lignin are not generated via CoA ligase mediated sinapoyl: CoA ester formation.

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SUGAR PARTITIONING ALTERATIONS ASSOCIATED WITH AAL-TOXIN-INDUCED CELL DEATH IN Lycopersicon esculentum. **V.V. Moussatos and D.G. Gilchrist, Dept. of Plant Pathology, University of California, Davis, 95616.**

AAL-toxin, secreted by the tomato pathogen Alternaria alternata f. sp. lycopersici, has been demonstrated to be a host-specific pathogenicity factor in the Alternaria stem canker disease of tomato. Interveinal necrotic disease symptoms on leaflet laminae were reproduced 30-40 hours after excised leaflets were exposed to AAL-toxin. The necrotic response to both the pathogen and the AAL-toxin in tomato has been associated with the asc locus. Use of F_9 lines, near-isogenic for the asc locus, indicated a positive correlation between the reduction in sucrose accumulation and the subsequent amount of toxin-induced necrosis observed in the leaflet lamina areas. Within one hour, a significant (α=0.03) decrease in ^{14}C accumulation (applied as ^{14}C-sucrose) was observed in the interveinal areas of the leaflet lamina when AAL-toxin was taken-up through the petiole. Sucrose accumulation also differed in the resistant and susceptible lines in the absence of AAL-toxin, possibly suggesting that the product of asc may physiologically influence sugar partitioning.

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PATHOGENIC FITNESS OF TRANSFORMED COCHLIOBOLUS HETEROSTROPHUS. N. P. Keller, O. C. Yoder, and G. C. Bergstrom, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

It has not been determined whether or not the presence of foreign DNA in the genome of a genetically engineered fungus alters the ability of the fungus to cause disease. To test this, the southern corn leaf blight fungus, C. heterostrophus race O, was transformed with a plasmid containing the Escherichia coli hygromycin B phosphotransferase gene (hygB) as a selectable marker. Conidia of transformants and their corresponding nontransformed progenitors were inoculated in 1:1 mixtures onto maize leaves in some mixtures. The frequency of transformant phenotype decreased through successive generations on host plants. This may be due to reduced ability of some transformants to sporulate. Altered virulence of transformants may be conditioned by vector insert position, copy number, or size. Any of these may affect the field survival or virulence of genetically engineered organisms.

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ORNITHINE DECARBOXYLASE "SUCIDE" INHIBITOR PREVENTS GROWTH OF WOOD DECAY FUNGUS, POSTIA PLACENTA. B. L. Illman, USDA, FS, Forest Products Laboratory, Madison, Wis 53705-2398.

DL-α-Difluoromethylornithine (DFMO), a "sucide" inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) effectively inhibited in vitro growth of the economically important brown-rot wood decay fungus, Postia placenta. DFMO (0.1 mM, 1.0 mM, or 5.0 mM) was added to a basal agar medium amended with or without nitrogen. A 10 mm diameter innoculum of P. placenta mycelium was placed in the center of each culture plate and incubated in the dark at 27° C. Diameters of colonies were measured at intervals during a 5-week period and hyphae observed using fluorescent and transmitted light microscopy. Inhibition of the rate of mycelial growth was linear with DFMO concentration. DFMO-containing cultures were characterized by sparse mycelial growth and smaller hyphae. Because ODC is believed to be the sole fungal pathway for putrescine biosynthesis, DFMO has promise as a growth retardant of wood decay fungi. Studies are currently being conducted on its ability to inhibit wood decay.

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EFFECT OF PEAT ON THE PRODUCTION OF HYDROLYTIC ENZYMES IN PHANEROCHAETE CHRYSOSPORIUM. M. Nawaz, T. Sambandam, and M. Gunasekaran. Dept. of Biology, Fisk University, Nashville, TN 37203.

This study reports on the effects of peat, a partially decomposed plant material abundant in minerals, lignocellulose used as a substrate for the growth and production of hydrolytic enzymes in Phanerochaete chrysosporium. Three types of peat extracts viz: cold, hot water and autoclaved, were prepared and tested. The mycelium was harvested, washed thoroughly, homogenized with acid washed sand and Tris HCL 0.05M buffer (pH 7.0), centrifuged, and the resulting supernatant was used as crude intracellular enzyme source. The three hydrolytic enzymes viz: cellulase, cellobiase and xylanase, were assayed with CMC cellulose, salicin and xylan as substrates. The reducing sugar released was determined with dinitrosalicyclic acid reagent. Among them, autoclaved extract supported the maximum mycelial growth. The intracellular enzyme activities peaked on the fifth day after incubation.

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ISOLATION AND CHARACTERIZATION OF THE GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) GENE IN COCHLIOBOLUS HETEROSTROPHUS. **S. L. Van Wert and O. C. Yoder. Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908.**

One approach to defining the roles of genes during disease development is to study altered expression of a particular gene by placing it under control of a strong constitutive promoter. In many species the GAPDH gene is known to have such a promoter. We have isolated the GAPDH gene from a C. heterostrophus EMBL4 lambda library by heterologous hybridization using an Aspergillus nidulans GAPDH gene as probe. A 2200bp fragment, which includes the gene, was subcloned into pUC18, restriction mapped and sequenced. The sequence was compared with GAPDH sequences from other species. A 1300nt GAPDH transcript was identified by Northern analysis. The 5' and 3' borders of the GAPDH gene were mapped by S1 nuclease protection. We are currently analyzing the DNA sequences required for transcription initiation.

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DETECTION OF CERCOSPORIN IN GREY LEAF SPOT-INFECTED MAIZE LEAF TISSUE. Jon Duvick, Pioneer Hi-Bred, Box 38, Johnston, IA 50131

Cercospora zeae-maydis (Tehon & Daniel), the causal agent of Grey Leaf Spot of maize (GLS), produces in culture a nonspecific toxin, cercosporin, whose role in the disease is uncertain. As an initial step towards determining cercosporin's role in GLS, we developed methods for extracting and quantifying cercosporin in infected maize leaf tissue. Acetone extracts of infected tissue were dried, dissolved in chloroform, and partitioned with dilute NaOH. Upon lowering the pH of the aqueous phase, cercosporin precipitated and was recovered by partitioning again into chloroform. The chloroform extract was concentrated and analyzed for cercosporin by reverse-phase HPLC (phenyl-substituted silica column, methanol:water: trifluoroacetic acid, 70:30:0.2), monitored at 473 nm. Recovery of cercosporin from spiked extracts of control tissue was approx. 50%. Cercosporin was not detected in uninfected leaf tissue, while significant amounts (>1 microgram per gram fresh weight of lesion tissue) were detected in tissue with young, watersoaked lesions as well as mature, necrotic lesions. Comparable amounts of cercosporin produced necrosis when applied to maize leaves in a suitable solvent. Although the localized concentration of cercosporin in lesions cannot be determined by these methods, the data are consistent with a role for the toxin in GLS symptom development.

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STIMULATION OF GERMINATION OF OOSPORES OF PHYTOPHTHORA INFESTANS BY LIGHT AND VOLATILE FLAVOR COMPOUNDS. R.C. French and P. W. Tooley, USDA-ARS, Frederick, MD 21701

Oospores produced on V8 agar were concentrated via passage through water snails. Oospores on 1% agar in plastic petri plates exposed to fluorescent light through double sheets of colored cellophane germinated 24% in blue, 1% in red and far red, and 2% in darkness. Sporangial formation from oospores was the most common type of germination observed. Spores producing only a germ tube, spores with multiple germ tubes, and multiple sporangia also were observed. Optimum germination temperature under continuous blue light was 25 C, with 46% germination at 10 days. Chemically treated oospores were exposed to blue light 10 hr, darkness 14 hr at 21 C. Salicylaldehyde treatment at 50 ppm resulted in 62% germination (controls 39%) at 13 days. Benzaldehyde treatment at 25 ppm resulted in 50% germination (controls 14%) at 10 days.

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ISOLATION OF THE β-TUBULIN GENE FROM THE OBLIGATE PLANT PATHOGEN, ERYSIPHE GRAMINIS. John E. Sherwood and Shauna C. Somerville, DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

Genomic DNA isolated from conidia of Erysiphe graminis f.sp. hordei race CR3 was probed with a cloned β-tubulin gene from Neurospora crassa. This probe hybridized to a single restriction band when the E. graminis DNA was cut with Bam H1, suggesting that there is only one copy of this gene per genome. A genomic library was constructed in the vector lambda 2001 using partially digested and sized E. graminis DNA. Twenty nine out of 75,000 plaques screened cross-hybridized with the N. crassa β-tubulin gene. The inserts in these phage are currently being characterized.

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PRESENCE OF PLASMID IN ENDOTHIA PARASITICA. N. Mahanti, D. W. Fulbright. Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312

Hypovirulent strains of Endothia parasitica have been found in Europe and North America where the chestnut trees survive. Characterized hypovirulent strains of E. parasitica are infected with double stranded RNA (dsRNA) molecules which appear to be correlated with changes in virulence, growth rate and culture morphology. One strain of E. parasitica (CL25) from Michigan has all the characteristics of dsRNA associated hypovirulent strains but harbors no detectable dsRNA. Genetic studies of CL25 show that this "new" hypovirulent phenotype is carried in 20% of the conidia, is maternally inherited and is cytoplasmically transferred via hyphal anastomosis. These results suggest that there is a cyptoplasmic agent other than dsRNA that might be involved in the hypovirulent phenotype. CL25 contains a small DNA plasmid about 4 kilobase pairs in size. At this time, the relationship of the plasmid to the hypovirulent phenotype is under study.

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LACK OF HOMOLOGY BETWEEN GENES ENCODING PISATIN DEMETHYLATING ACTIVITY IN TWO FUNGAL PATHOGENS OF PEA (PISUM SATIVUM). L. M. Delserone and H. D. VanEtten, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Previous studies have established that the ability to rapidly demethylate (detoxify) the phytoalexin pisatin is required by Nectria haematococca for high virulence on pea. Preliminary studies suggest that this is also true for Mycosphaerella pinodes. Thus, both fungi may have evolved similar means to neutralize the phytoalexin-based resistance mechanism of pea. Recently a gene from N. haematococca encoding pisatin demethylating activity (pda) has been cloned. To determine if there is a relationship between genes encoding pda in both fungi, genomic DNA of M. pinodes was probed with the cloned gene from N. haematococca. Southern hybridizations of the DNA from several isolates, conducted at several stringencies, indicated no homology. Our data suggest that different structural genes encode pda in M. pinodes and N. haematococca.

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DNA-MEDIATED TRANSFORMATION OF COLLETOTRICHUM GRAMINICOLA. **D. G. Panaccione, R. M. Hanau, and M. McKiernan. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907**

A procedure has been developed for the transformation of Colletotrichum graminicola with homologous and heterologous fungal DNAs. In this procedure, a cloned benomyl resistance gene (BmlR), from either a Neurospora crassa or C. graminicola benomyl resistant mutant, is used as a selectable marker. Osmotically stable protoplasts and plasmid DNA are incubated in 10 mM $CaCl_2$ and 54% polyethylene glycol. After incubation, protoplasts are washed and plated on regeneration medium containing 500 ng/ml benomyl. Benomyl resistant transformants appear in 10-14 days. Transformation efficiencies and molecular rearrangements resulting from chromosomal integration of the N. crassa and C. graminicola BmlR genes have been compared.

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LIGHT-INDUCED CONIDIATION AND GENE EXPRESSION IN HELMINTHOSPORIUM CARBONUM. C. D. Franklin and R. M. Hanau. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

We are studying molecular and genetic factors responsible for differential gene expression during conidiogenesis in Helminthosporium carbonum. In these studies we have shown that conidiogenesis in light-induced cultures can be highly synchronized. Comparable cultures grown in the dark fail to differentiate within the same time period. The uniform nature of the light-induced cultures has enabled us to harvest gram quanities of fungal tissue at specific stages of conidial development. A comparison of protein profiles from conidiating and nonconidiating cultures demonstrates that there are numerous changes in gene expression that precede the formation of conidia. In addition, in vitro translations of poly-A^+ RNA isolated from conidiating and nonconidiating cultures has shown that there is differential gene expression regulated at the level of translatable RNA.

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GLYCEOLLIN PRODUCTION IN SOYBEAN IN RESPONSE TO METALAXYL RESISTANT MUTANTS OF PHYTOPHTHORA MEGASPERMA f.sp. GLYCINEA. D.M. Cahill and E.W.B. Ward, Research Centre, Agriculture Canada, University Sub-Post Office, London, Ontario N6A 5B7 Canada.
Metalaxyl tolerant mutants of Phytophthora megasperma f.sp. glycinea (Pmg) races 1, 4 and 6 were obtained from UV-irradiated colonies. The mutants were very stable and growth rates of single zoospore isolates on metalaxyl amended medium were uniform. Inoculation of soybean hypocotyls demonstrated that the mutants varied in virulence. They also varied in sensitivity to metalaxyl both in vivo and in vitro. After inoculation, synthesis of the phytoalexin, glyceollin, was enhanced at low concentrations of metalaxyl but suppressed at concentrations greater than 1 mg/ml. Similar results were obtained following treatment with the abiotic elicitor, silver nitrate. Preliminary results suggest that in both parent and mutant isolates of Pmg release of elicitors into culture fluids is enhanced in cultures treated with metalaxyl.

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PHENOL OXIDASE PRODUCTION IN VIRULENT AND HYPOVIRULENT STRAINS OF ENDOTHIA PARASITICA. Rigling Daniel and Heiniger Ursula, Swiss Fed.Inst.For.Res., CH-8903 Birmensdorf, Switzerland.

Virulent (dsRNA-free) and hypovirulent (dsRNA-containing) strains of E.parasitica isolated in Switzerland were compared for phenol oxidase production using the Bavendamm test. In contrast to the hypovirulent strains that showed weak or no activity, all virulent strains produced a strong reaction zone around the mycelium on malzagar with tannic acid. Three dsRNA-containing strains representing different levels of hypovirulence were used to convert a dsRNA-free virulent strain. Transfer of dsRNA resulted in transfer of the level of hypovirulence and loss of positive phenoloxidase reaction. Attempts to correlate the Bavendamm reaction with specific enzyme activities are in progress.

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INDUCTION OF KIEVITONE HYDRATASE AND DIFFERENTIAL mRNA TRANSCRIPTION IN FUSARIUM SOLANI F. SP. PHASEOLI BY ISOFLAVONOIDS. G. H. Choi, C. L. Schardl, and D. A. Smith, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Kievitone hydratase (KHase) catalyses detoxification of the bean phytoalexin, kievitone, to kievitone hydrate. Four bean phytoalexins, kievitone, phaseollin, phaseollinisoflavan, and phaseollidin, as well as two other isoflavonoids, biochanin-A and rotenone, were tested for their abilities to induce KHase in a virulent isolate of the bean pathogen, Fusarium solani f. sp. phaseoli. Measurement of KHase activity in cell-free culture filtrates and mycelial sonicates indicated that biochanin-A was the most effective KHase inducer, causing 13- and 76-fold increases, respectively. The enzyme's substrate, kievitone, caused only 2- and 5-fold increases, respectively. Electrophoresis revealed changes in the extracellular protein profiles and in in vitro translation products of poly(A)$^+$ mRNA upon biochanin-A treatment.

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A CELLULAR SYSTEM FOR BIOCHEMICAL STUDIES ON SOYBEAN-PHYTOPHTHORA INTERACTIONS. P. E. Pierson and T. L. Graham, Department of Plant Pathology, The Ohio State University, Columbus, OH 43210.

The examination of discrete biochemical events in host-pathogen biochemistry is complicated by the need to deal with two separate living systems co-developing during the infection process. Tissue culture is one attractive way to circumvent some of these problems. However, normal tissue culture procedures (callus, cell suspension, etc.) often are plagued by micro-heterogeneity of cell type, stressful culture conditions and the loss of tissue specific gene expression. We report here the modification of a cell culture technique (Schwenk, F. W., Plant Sci. Lett. 17 437, 1980) and its characterization. Mechanically isolated cells, grown on polysulfone membranes supported on a solid hormone containing medium, are homogeneous, rapidly dividing and display tissue specific gene expression. The cells are isolated and used on an as needed basis. Responses to Phytophthora and its elicitors will be described.

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MITOCHONDRIAL DNA OF THE BIOCONTROL AGENT TALAROMYCES FLAVUS. Sue Mischke and G. C. Papavizas, Soilborne Diseases Laboratory, Plant Protection Institute, USDA-ARS, Beltsville, MD 20705.

DNA was isolated from a strain of Talaromyces flavus (anamorph: Penicillium dangeardii Pitt, usually reported as P. vermiculatum Dangeard) that effectively antagonizes Verticillium dahliae, the soilborne pathogen responsible for Verticillium wilt of eggplant, potato and other plants. Fractions containing mitochondrial and chromosomal DNAs were identified. Restriction endonuclease analysis of mitochondrial DNA suggested that the size of the mitochondrial DNA is 29-36 kilobases. Preliminary Southern hybridization experiments using a heterologous biotinylated DNA probe located the cytochrome oxidase subunit III gene on a 9.4-9.9 kb EcoRI fragment. Probe detection was by streptavidin-alkaline phosphatase conjugation followed by dyes for colorimetric visualization. This is the first report of mitochondrial DNA analysis of a biocontrol fungus. The technique may be useful for strain identification in field experiments.

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DETECTION OF ISOZYMES OF ALCOHOL DEHYDROGENASE FROM ARMILLARIA MELLEA DURING RHIZOMORPH DEVELOPMENT. S. D. Cohen and J. J. Motta, Department of Botany, University of Maryland, College Park, Maryland, 20742.

Levels of activity of alcohol dehydrogenase (ADH) in Armillaria mellea increase during rhizomorph differentiation. Activity staining of proteins obtained from A. mellea and resolved by non-denaturing polyacrylamide gel electrophoresis revealed multiple isozyme bands at different stages of rhizomorph differentiation. The presence of ADH was further tested by immuno-dot blots with polyclonal antisera raised against yeast ADH. The polyclonal antisera recognized 50 ng of yeast ADH at 10^{-5} dilution. SDS extracts of undifferentiated and differentiated rhizomorph stages of A. mellea reacted at 1/50 and 1/100 dilutions, respectively. This antisera has proven useful for detection of yeast ADH with Western blotting and is being used to further characterize ADH isozymes of A. mellea during rhizomorph differentiation.

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ANALYSIS OF A RIBOSOMAL RNA GENE PROMOTER FROM A VIROID HOST. K. L. Perry and P. Palukaitis, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853-5908.

Our objective is to identify the plant host enzyme that mediates viroid replication. One candidate enzyme is DNA-dependent RNA polymerase I (RNAPI). We are analyzing sequences of the promoter region which this enzyme and/or associated transcription factors recognize. If viroid RNAs are templates for RNAPI, they should have sequences in common with the normal substrate for this enzyme, a ribosomal RNA gene (rDNA). The rDNA from a viroid host, Lycopersicon esculentum, has been cloned and 2000 base pairs (bp) upstream of the structural 18S RNA have been sequenced. An 11 bp homology was observed between rDNA sequences and those of the central conserved region found in a number of viroids. These L. esculentum sequences lie upstream of the assumed region of transcription initiation. Experiments are underway to precisely identify the transcription start site and surrounding promoter sequences.

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LABORATORY STUDIES ON ANTAGONISM OF TRICHODERMA SPPS. AND GLIOCLADIUM VIRENS AGAINST DECAY FUNGI, T. L. Highley and J. Ricard, Forest Products Laboratory, Madison, Wis. 53705-2398

The antagonistic abilities of Trichoderma spp. and Gliocladium virens against white- and brown-rot wood decay fungi were evaluated by testing their ability to (1) inhibit decay fungi on malt-agar and, (2) prevent and arrest decay in wood. G. virens and the Trichoderma spp. overgrew the decay fungi, and usually killed them. SEM observation of the interaction of Trichoderma spp. and the decay fungi showed Trichoderma attached to the hyphae of decay fungi. Hyphae of Trichoderma appeared to be present within the mycelium of the decay fungi even though perforation was not evident. The hyphae of decay fungi were void of cytoplasmic contents whether or not contacted by Trichoderma. Pretreatment of pine with G. virens prevented brown-rot but was ineffective against the white-rotters. Pretreatment with Trichoderma spp. generally prevented decay by the brown-rotters except for Gloeophyllum trabeum. G. virens was evaluated for its ability to stop the progress of decay in wood and was effective only against Poria carbonica.

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A CANKER DISEASE OF AUTUMN OLIVE CAUSED BY NECTRIA CINNABARINA. Kenneth J. Kessler, Jr., USDA Forest Service, North Central Forest Experiment Station, Forestry Sciences Laboratory, SIU, Carbondale, IL 62901.

Nectria cinnabarina was repeatedly isolated from recently killed or dying stems of autumn olive, Elaeagnus umbellata, in plantations in Illinois. Affected stems bore basal cankers that ultimately girdled the stems. Girdled stems either failed to leaf out or developed small chlorotic leaves that wilted by midsummer. The anamorphic sporodochial state of the fungus (Tubercularia ulmaea) was frequently present, particularly at canker margins. The perithecial state was not found. Three months after field inoculations of 60 healthy stems, 82% of them had developed cankers averaging 16 cm in length. N. cinnabarina was reisolated from all cankers. This is the first report of N. cinnabarina's pathogenicity to autumn olive.

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DISSEMINATION OF VIRULENT AND HYPOVIRULENT STRAINS OF THE CHESTNUT BLIGHT FUNGUS, ENDOTHIA PARASITICA, IN WEST VIRGINIA. S. Jakobi and W.L. MacDonald. Dept. Plant Pathol., West Virginia Univ., Morgantown, WV 26506-6057.

Each of 216 sapling-sized American chestnut stems in a 7-year-old clearcut received 4 punch, and 1 branch wounds 4 times during a 1-year period. A brown virulent (v) , and 2 brown dsRNA-containing hypovirulent (hv) isolates of the fungus were placed on 1.6 m tall inoculum sticks in the centers of three 0.01 ha plots, respectively. The 344 E. parasitica cankers that developed on 159 trees were sampled for the presence of wild-type orange-pigmented v isolates, the introduced brown v or hv marker, or the presence of dsRNA. Only 4 of the cankers yielded brown isolates that originated 3.4-4.6 m from the central inoculum sources. Few punch or branch wounds were infected initially, but after 12 and 16 mo, 114 and 71 additional punch wounds, respectively, developed cankers. While 118 of the 185 (63 %) punch wound cankers were found in wounds less than 4-mo-old, 67 (37 %) were in wounds made 6-16 mo earlier.

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ENZYME VARIABILITY IN ENDOCRONARTIUM HARKNESSII FROM THREE DISTINCT SITES IN NORTH DAKOTA. G. A. Tuskan and J. A. Walla, North Dakota State University, Fargo 58105.

Spores were collected from individual E. harknessii galls on various host genotypes in each of 3 widely separate stands of Pinus sylvestris and/or P. ponderosa. The 3 stands consisted of a natural pine forest with an "endemic" rust population, a 45-yr-old shelterbelt which likely had a limited inoculum source introduced during nursery production, and a Christmas tree planting which has had multiple establishment dates and likely multiple rust introductions. The objectives of this study were to determine if isozymes in E. harknessii varied among isolates from 1) distinct stands and 2) within stands among host genotypes. Horizontal starch gel electrophoresis was used to examine spore enzyme phenotypes. Twenty-one enzymes were examined representing 33 putative loci. Of the 33 loci, 12 were polymorphic across sites, with no heterozygosity present. Allelic frequencies varied among sites. Contingency chi-square was used to determine if there were differences among sites and among host genotypes.

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ANATOMY OF FIELD-RESISTANT SHORTLEAF PINES INOCULATED WITH CRONARTIUM QUERCUUM F. SP. FUSIFORME. C. H. Walkinshaw, Southern Forest Experiment Station, Gulfport, MS 39505.

Control-pollinated and open-pollinated shortleaf pine (Pinus echinata Mill). families were inoculated with 23 spores per mm^2 of a composite of three rust isolates from slash pine in Florida and Louisiana. Seed was from the Ouachita Seed Orchard at Mount Ida, Arkansas. Twenty-four specimens were fixed 15 days after inoculation and paraffin sections, 12-15 µm were stained with a mixture of acridine red, fast green, and rose bengal. Examination of fixed stems revealed an infection rate of 0 to 100% and fungal hyphae were routinely observed in the cambium and cortex. Haustoria were in low numbers. Swelling of cortical parenchyma resulted in a 40% increase in stem diameter. Other inoculated seedlings of these same families formed fusiform galls (0 to 72% range) in the greenhouse. Galls appeared inactive after 90 days. Thus, resistance to fusiform rust in shortleaf pines appears to occur after initial penetration and growth of the fungus in the cortex and cambium.

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RESISTANT REACTIONS IN FOUR PINE SPECIES INOCULATED WITH CRONARTIUM QUERCUUM F. SP. FUSIFORME. C. H. Walkinshaw, and T. A. Roland, So. Forest Exp. Sta., Gulfport, MS 39505.

Loblolly (Pinus taeda L.), longleaf (P. palustris Mill.), shortleaf (P. echinata Mill.), and slash (P. elliottii var. elliottii Engelm.) pine seedlings were examined histologically at 15 days and 1 to 6 months after inoculation with the rust fungus. Isolation of invading hyphae within small lesions (a resistant response) occurred for a percentage of seedlings in three of the pine species but was not seen in longleaf pines. In slash pines at 15 days, except for the resistant lesions, susceptible and resistant reactions were similar. By 30 days, the rust fungus and surrounding cells died in shortleaf pines without marked cambial proliferation. The other species exhibited wound responses and proliferation of cambial derivatives. The resulting cellular disorganization was most obvious in longleaf pines. Resistant loblolly and slash pines often exhibited death of their pith and inner ray cells. Tannin accumulated in large amounts in these areas.

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VARIATION IN VIRULENCE WITHIN HOST SPECIFIC VARIANTS OF VERTICICLADIELLA WAGENERI. W. J. Otrosina, USDA Forest Service, 1960 Addison Street, Berkeley, CA, F. W. Cobb, Jr., and T. Popenuck, University of California, Department of Plant Pathology, Berkeley, CA 94701.

One-year-old, greenhouse grown Douglas-fir (DF) and ponderosa pine (PP) were inoculated at the root collar to determine variability in virulence among 12 isolates of host specific variants of Verticicladiella wageneri from DF and PP on their respective hosts. The length of the characteristic black stain at time of seedling death averaged 80% to 90% of total tap root length, while the length of stain in the main stem averaged less than 30% of the stem length in both tree species. Mortality in DF seedlings was nearly twice that of PP seedlings, which may be attributed to high greenhouse temperatures early in the study. Mortality differed among seedlings of both tree species inoculated with different isolates indicating possible variation in virulence.

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DEVELOPMENT OF HYPOXYLON ATROPUNCTATUM IN INOCULATED OAK SEEDLINGS. Patrick Fenn. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Pathogenicity of H. atropunctatum was examined by mycelial or ascospore inoculations of 2-yr-old, greenhouse-grown, black and Shumard oaks. Inoculum was placed in stem cuts, applied to the stem surface after cuticle abrasion, or applied to unwounded stems. Inoculation sites were moistened and wrapped with plastic film for one wk after inoculation. Mycelial inoculations in stem cuts resulted in slow developing, sunker cankers with variable amounts of callus formation. Internal discoloration extended up to 3 cm beyond the external canker margins after 3 mon. Other inoculation treatments appeared no different from uninoculated controls. H. atropunctatum was recovered from 100% of stem cut inoculations, from 60% of abraded sites inoculated with mycelium or ascospores, but not from unwounded sites. Isolations and microscopic examination of leaves inoculated with ascospores indicated that H. atropunctatum penetrated living leaf tissue.

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DISEASES AND ARTHROPOD PESTS OF SPRUCE IN NORTH DAKOTA. J. A. Walla and R. W. Stack, Plant Pathology Dept., North Dakota State Univ. and D. R. Nelson, Survey Entomologist, North Dakota Dept. of Agric., Fargo, 58105.

A total of 8,560 spruce in 138 plantings of various ages, species, and planting densities were examined in 14 counties in eastern and central North Dakota in 1984 and 1986. Diseases identified were Rhizosphaera needlecast, R. kalkhoffii Bub., Lirula needle blight, L. macrospora (Hartig) Darker, and Cytospora canker, C. kunzei Sacc.; each caused severe damage at some sites. Rhizosphaera needlecast was the most widespread, severely damaging disease. Arthropod pests noted were spruce needle miners (Tortricidae), pine needle scale, Chionaspis pinifoliae (Fitch), spider mites (Tetranychidae), a spruce gall midge, Mayetiola piceae (Felt), spruce bud scale, Physokermes piceae (Schrank), and yellowheaded spruce sawfly, Pikonema alaskensis (Rohwer). Spruce needle miners were the most widespread, damaging insects. Pine needle scale, yellowheaded spruce sawfly, and spider mites caused significant damage at some sites.

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RESPONSE OF OAK LEAVES TO TOXINS PRODUCED BY CERATOCYSTIS FAGACEARUM. F. H. Tainter and L. M. Haugen. Department of Forestry, Clemson University, Clemson, SC 29634-1003.

Liquid cultures of Ceratocystis fagacearum were grown in White's medium B (1955 Amer. J. Bot. 42:759-764) in shake culture at 26°C for 22 days. Toxin separation was performed (White 1955), yielding a polysaccharide toxin (PT) and an "unknown" toxin (UT). Young mature leaves of Quercus velutina from greenhouse-grown seedlings were placed with cut petiole ends in each of 1/8, full and 8X toxin concentrations or White's medium B for 12 and 24 hrs. At 12 hr, wilting was associated only with PT at 8X conc and almost no solution was taken up. No wilting was associated with medium B at 8X conc even though almost no solution was taken up. At 24 hr, wilting was associated with PT at 8X and full conc and some wilting with UT at 1/8 and full conc, and medium B at 1/8 conc. Wilting in leaves exposed to both toxins, but especially PT, appeared related to a dense material occluding xylem elements.

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AN AUTOMATED SYSTEM FOR PRODUCING DROUGHT STRESS EFFECTS ON ASPEN, *POPULUS TREMULOIDES*, SAPLINGS. D. Griffin, M. Devit, M. Schaedle and P. Manion. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210.

Following the technique of Boot et al. (J. Ecol. 74:485, 1986) for drought stressing plants growing in nutrient solutions, we have developed an automated system of pumps and valves to move nutrient solutions into and out of a system of culture vessels in a growth chamber to provide periodic stress periods in which the roots are expose to air in the empty vessels. Measurements of height growth rate, internode length, stomatal closure response with a diffusive resistance porometer, and leaf water activity by thermocouple psychrometry will be presented. Physiological adaptation of the aspen trees to repeated daily stress periods over a period of six weeks has been observed. We are developing this system to examine the relationship between clonal response variation to drought stress and susceptibility to cankering by *Hypoxylon mammatum*.

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INCIDENCE OF FUNGI ON SEED COLLECTED FROM CONES AND FROM PLASTIC NETTING IN A LOBLOLLY PINE SEED ORCHARD IN MISSISSIPPI. V. Ammon, Dept. of Plant Pathology & Weed Science, Mississippi State University, Mississippi State, MS 39762.

During a 70-day period from Dec. 1985-Feb. 1986, cone and seed samples were taken 5 times from a seed orchard in southeastern MS. Over 50% of the seed collected initially were infested with fungi. Incidence levels increased to over 60%,80%, and approximately 90% on seed collected at the second, third, and fourth periods respectively before declining to 80% at the last collection period in late Feb. 1986. There were no significant differences in fungal incidence between seed collected directly from cones and those collected from net surfaces. The major fungal genera isolated were Curvularia, Phomopsis, Pestalotia, Aspergillus, and two Fusarium spp. Less than 5% of surface sterilized embryonic tissues separated from their seed coats and placed on PDA contained fungi. Histological studies of paraffin embedded embryonic tissues also documented the sparsity of fungal colonization of newly cast, filled loblolly pine seed.

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ULTRASTRUCTURAL EFFECTS OF OZONE TO FIELD-GROWN LOBLOLLY PINE SEEDLINGS. C. R. Krause[1], S. R. Shafer[2], and A. S. Heagle[2], [1]USDA-ARS, Nursery Crops Research Laboratory, Delaware, OH, 43015, and [2]USDA-ARS, N. C. State Univ. Dept. of Plant Pathology, Raleigh, NC 27695-7616

Seedlings of loblolly pine (Pinus taeda) were planted in 1985 in open top field chambers and exposed daily (12 hr/day) during two growing seasons (May through October, 1985; April through October, 1986) to charcoal-filtered (CF) air, non-filtered (1X/NF) air or non-filtered air supplemented with ozone to achieve a concentration twice that of 1X/NF (2X/NF). Needles formed during 1986 were sampled in October and prepared for analysis using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Needles from plants exposed to CF appeared asymptomatic. Chlorotic mottle was observed in needles exposed to 1X/NF and 2X/NF. SEM and TEM examination of needles exposed to both 1X/NF or 2X/NF revealed injury to epicuticular wax and cytolysis of subsidiary and mesophyll cells.

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RHIZOBIA INHIBIT THE GERMINATION OF MACROPHOMINA PHASEOLINA MICROSCLEROTIA. R.E. Zdor, S.G. Pueppke, and T.D. Wyllie, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Several species of Rhizobium have been reported to be antagonistic to fungal pathogens, including M. phaseolina. We examined the effect of several species of Rhizobium on the germination of microsclerotia of M. phaseolina with an in vitro agar-slide method. Both fast-growing (R. meliloti and R. fredii) and slow-growing (B. japonicum) rhizobia reduced microsclerotial germination, with R. meliloti resulting in the greatest reduction (50% of control). Bacterial cell contact with the agar slide was required for the inhibition. Both rhizobitoxine-producing and non-producing strains of B. japonicum were active.

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PSEUDOMONAS SYRINGAE PV. TABACI IN THE RHIZOPHERE OF TOBACCO AND INOCULATED WHEAT. K. K. Knoche and R. D. Durbin, Dept. of Plant Pathology and USDA, ARS, University of Wisconsin-Madison, Madison, WI 53706.

Rhizosphere soil dilutions from field-grown tobacco were infiltrated into tobacco leaves. Bacteria were isolated from wildfire-type lesions, those which were fluorescent on King's Medium B were characterized by physiological and biochemical tests and pathogenicity assays on tobacco. P. s. pv. tabaci was isolated from the rhizosphere of tobacco in a field with epidemic wildfire and from wildfire-resistant tobacco in a field with no wildfire. Ten days after planting wheat seeds coated with P. s. pv. tabaci Rif^r, 2 cm-segments of the first seminal root were scored for presence of the bacterium. In autoclaved soil the bacterium was present along the entire root. In nontreated soil it was generally recovered from only the top half of the root. This work supports an earlier observation that P. s. pv. tabaci may be found in association with roots.

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THE ROLES OF IRON COMPETITION AND ANTIBIOSIS IN THE INHIBITION OF PYTHIUM ULTIMUM BY PSEUDOMONAS FLUORESCENS BIOVAR IV. G. Yuen, M. Hendson, M. Rella, and M. N. Schroth, Dept. of Plant Pathology, Univ. of California, Berkeley, CA 94720.

P. fluorescens biovar IV strain S9, isolated from endosperm of sugarbeet seed, inhibits mycelial growth of P. ultimum (Pu) in vitro and produces a siderophore and an antibiotic. Siderophore minus (Sid$^-$) and antibiotic minus (Ant$^-$) mutants were obtained by Tn5-751 mutagenesis. Sid$^-$ mutants were as effective as S9 in inhibiting the growth of Pu. Inhibition by Ant$^-$ strains was greatly reduced compared to the wild type strain. S9 and 3 strains of each mutant class were tested for their effect on infection of sugarbeet seed by Pu. Seeds inoculated with bacteria were planted in field soil and cultured for Pu after 24 hr. Treatment with S9 and Sid$^-$ strains reduced the frequency of infection to 50% of the control. Ant$^-$ strains exhibited significantly less or no effect. These results suggest that antibiosis plays a greater role than iron competition in the inhibition of Pu by S9 in soil.

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DECREASED AGGRESSIVENESS OF SCLEROTIUM ROLFSII SCLEROTIA IN RESPONSE TO NUTRIENT STRESS IN SOIL. M. Hyakumachi, H. J. M. Löffler, and J. L. Lockwood. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Endogenous carbon from ^{14}C-labelled Sclerotium rolfsii sclerotia was progressively lost during incubation in a loam soil at -10 mb matric potential. Total ^{14}C loss at 7, 26, and 50 days incubation was 11.2%, 18.1%, and 44.5%, respectively. ^{14}C loss, as percentage of total label, was partitioned as follows: i) dissolved $^{14}CO_2$ = 0.1-0.2%, ii) residual ^{14}C in soil = 2.1-8.7%, and iii) $^{14}CO_2$ evolved from the soil = 8.5-34.8%. Sixty-72% of total $^{14}CO_2$ evolved came from sclerotial respiration, and the remainder from microbial metabolism of sclerotial exudates. Sclerotia incubated in soil became nutrient-dependent for germination and lost viability at ca. 20% and 40% of ^{14}C loss, respectively. Radish seedlings inoculated with sclerotia which had lost 20% of their ^{14}C were significantly less diseased and had longer shoots than seedlings inoculated with nonincubated sclerotia.

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BIPOLARIS SOROKINIANA AND FUSARIUM SPECIES ON ROOTS OF SPRING WHEAT IN DIFFERENT ROOT ROT SEVERITY CLASSES. C. E. Windels and C. Holen, Northwest Expt. Stat., Univ. of Minn., Crookston, MN 56716.

In northwest Minnesota in 1985-86 spring wheat (Triticum aestivum L.) was sampled from 30 fields (early milk to hard dough stage) where the previous crop was wheat. Subcrown internodes (SCI) were rated (0-3 scale) as an indicator of root rot: 0=clean, 1=1-25% discoloration, 2=26-50%, and 3=>50%. Bipolaris sorokiniana and Fusarium spp. were isolated from SCI and crowns from each disease class. When SCI=0, B. sorokiniana was isolated from 27% of SCI; from class 1=52%, 2=71% and 3=70%. When SCI ratings=0, isolation of B. sorokiniana from crowns of the same plants averaged 73%, 1=80%, 2=82% and 3=83%. In 1985, F. graminearum, F. culmorum and F. avenaceum were in <1% of all SCI; in 1986, for SCI rated as 0, the species were recovered from 7%, 1=8%, 2=9% and 3=10%. These Fusarium spp. were isolated from 8% of all crowns in 1985 and 18% in 1986, but frequency of recovery was not related to SCI index values.

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EFFECT OF SOIL TEXTURE AND MATRIC POTENTIAL ON POLYMYXA GRAMINIS ZOOSPORE MOVEMENT. W. Zhang and W. F. Pfender, Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

Influence of soil matric potential (Ψ_m), soil texture, and pore size on active movement of Polymyxa graminis zoospores to wheat roots was investigated by arranging wheat seedlings at various distances from a source of zoospores. Infection of bait seedlings by P. graminis was assessed by observation of cystosori in roots. At Ψ_m=0 and -10 mb, zoospores readily moved 15 mm in a coarse sand, whereas in a fine silt loam or a fine soil mix, zoospore movement was detected only over a distance of 5 mm. Infection of bait seedlings was significantly reduced in the loam soil or the soil mix compared with the sand. At Ψ_m=-20 and -50 mb, no infected bait plants were detected in any of the three soil materials. Influence of pore size on zoospore movement was examined by using glass beads to simulate soil. Zoospores moved 9 mm through beads providing pore necks of 25-35 μ or 230-300 μ, but zoospore movement was restricted in the smaller beads.

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HOST RANGE OF CALIFORNIA ISOLATES OF POLYMYXA BETAE. J. S. Gerik and J. E. Duffus, USDA-ARS-PBA, 1636 E. Alisal St., Salinas, CA 93905

The host range of five isolates of Polymyxa betae Keskin, were investigated. The isolates included three from Beta vulgaris L. (sugarbeet), one from Amaranthus spinosus L. (spiny amaranth) and one from Portulaca oleracea L. (purslane). The tested plants included 24 common crop and weed species in 10 families. The isolates from sugarbeet were found to infect species in the Chenopodiaceae and Amaranthaceae. In addition to their original host, the isolates from A. spinosus and P. oleracea were found to infect sugarbeet 'US H-11'. Previously, isolates from these species from Japan were reported not to infect sugarbeet (Ann. Phytopath. Soc. Japan 52: 235-247). The data indicate, for the first time, weed species outside the Chenopodiaceae may increase inoculum of P. betae in the absence of sugarbeet.

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RESISTANCE TO POLYMYXA BETAE IN SUGARBEET VARIETIES WITH DIFFERENTIAL REACTIONS TO RHIZOMANIA. J. S. Gerik, R. T. Lewellen, and J. E. Duffus, USDA-ARS-PBA, 1636 E. Alisal St. Salinas, CA. 93905

Rhizomania of sugarbeet is caused by beet netrotic yellow vein virus which is transmitted by Polymyxa betae. Five sugarbeet varieties exhibited a differential response to infection by a non-viruliferous isolate of P. betae. The greenhouse test included varieties susceptible, resistant, and tolerant to rhizomania. Measurements were made on inoculated and non-inoculated plants to determine the amount of damage caused by P. betae. Significant interactions between the two factors (variety and P. betae inoculation) were observed for several variables, including infection of P. betae, top weight, root weight, and number of healthy root tips. The data suggest that rhizomania resistant and tolerant sugarbeet varieties are resistant to P. betae when compared to a rhizomania susceptible variety.

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DECLINE OF CEPHALOSPORIUM STRIPE WITH CONTINOUS PLANTING OF MODERATELY RESISTANT WHEAT VARIETIES. **P. A. Shefelbine, and W. W. Bockus, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.**

A multi-year field experiment was established to study carryover of Cephalosporium stripe. Six winter wheat varieties ranging from highly susceptible to moderately resistant were seeded in plots, 2.4 x 7.6 m, arranged in a split-plot design, varieties as main plots, infested or non-infested as sub-plots. First-year inoculum consisted of colonized oat kernels. At harvest, straw from each sub-plot was collected, tilled into an identically sized area and later reseeded with the same variety. In the second and third years, disease declined more for moderately resistant varieties relative to highly susceptible varieties. Thus, moderately resistant varieties, in addition to suffering less disease severity, afffect disease carryover. Therefore, multi-year planting of those varieties should greatly reduce Cephalosporium stripe.

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INCIDENCE OF FUNGI ASSOCIATED WITH ROOTS AND CROWNS OF DECLINING ALFALFA IN NORTH DAKOTA. B. Salas and R. W. Stack. No. Dak. St. Univ., Fargo 58105.

Alfalfa fields in three southeastern North Dakota counties were examined for decline symptoms during 1986. Patches of yellowing, stunted or dying plants were found in five of the fields examined and representative plants were collected for quantitative isolation. There were 100 isolations each from washed and surface disinfested crowns, taproots and lateral roots from each field for a total of 1500 isolations from which 640 fungal colonies resulted. Among known alfalfa pathogens the most frequently recovered fungi were Fusarium (51%), Phoma (8.3%), Plenodomus (2.9%), Rhizoctonia (1.8%), Cylindrocladium (0.9%) and Ascochyta (0.9%). Of the fusaria identified to species, predominant were F. oxysporum (34%) and F. solani (27%) while F. acuminatum, F. culmorum and F. avenaceum together comprised 22%. Incidence of fungi varied among fields and plant part isolated.

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DISTRIBUTION OF SOILS SUPPRESSIVE TO PHYTOPHTHORA CINNAMOMI ON THE ISLAND OF HAWAII. W. H. Ko and S. S. Shiroma, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI 96720

A method was developed to determine which areas are suppressive to Phytophthora cinnamomi and are therefore more suitable for planting of eucalyptus trees. Chlamydospores of P. cinnamomi were suspended in carrot extract for 1 hr before being placed on the surface of soil blocks. After incubation for 5 hr, spores were stained with rose bengal and germination was counted directly on the soil surface with a vertical-illumination microscope. Suppressive soils are widely distributed on the island of Hawaii. About 40% of the 155 soil samples collected from various locations were moderately or strongly suppressive to P. cinnamomi, supporting 50% or less of chlamydospore germination. The pathogen was strongly suppressed in 20 soil samples in which less than 30% of its chlamydospores germinated.

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A METHOD FOR INDUCING HIGH FREQUENCY OF OOSPORE GERMINATION OF PHYTOPHTHORA PARASITICA. P. J. Ann and W. H. Ko, Department of Plant Pathology, University of Hawaii, Beaumont Agricultural Research Center, Hilo, HI 96720.

Oospores from the cross between A^1 and A^2 mating types of P. parasitica were exposed to light after their formation and treated with 0.25% $KMnO_4$ for 20 min before incubation at 24 C under light on a medium consisting of basal salts, lecithin and agar. Under such conditions, germination commenced within 24 hr and exceeded 90% after 10 days. Oospores harvested within 21 days of mating did not germinate under the same conditions. Percentage germination of oospores increased with increasing age of oospores, reaching more than 90% at the age of 56 days. Germination of 56-day-old oospores decreased from 95 to 44% when light was omitted during aging, and to 15% when light was omitted during germination. Oospores did not germinate when light was not provided during aging and germination.

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EFFECT OF SOIL BACTERIA ON GERMINATION AND DEGRADATION OF SCLEROTIA OF SCLEROTINIA SCLEROTIORUM. Hsiaoling Wu and Berlin Nelson, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Sixty strains of soil-borne bacteria were isolated from sclerotia of Sclerotinia sclerotiorum (Lib.) deBary collected from soils in North Dakota. These bacteria were tested in vitro for their effects on germination and degradation of sclerotia. The sclerotia were produced in a cornmeal-vermiculite medium. They were soaked for 30 min in two-day-old bacterial shake cultures then placed on potato dextrose agar in 100 X 20 mm test tubes and incubated at 22-25 C. After 30 days the sclerotia were examined for germination and medulla color and integrity. Eight strains of bacteria prevented germination of 50% or greater of sclerotia. Two strains degraded sclerotia, and ten strains prevented germination and degraded sclerotia.

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SCREENING FOR MYCOPARASITES OF SCLEROTIA OF SCLEROTINIA SCLEROTIORUM. D. L. Duval and B. D. Nelson, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

An in vitro dual culture screening technique for evaluating soil fungi for mycoparasitism on sclerotia of Sclerotinia sclerotiorum (Lib.) deBary was developed. Fungi were isolated from sclerotia sieved from soil of 33 naturally infested fields in North Dakota. From a collection of 275 fungi, 45 fungi were selected and tested for mycoparasitism. Sclerotia were produced in axenic culture on a cornmeal-vermiculite medium. Sclerotia, 3-6 mm in diameter, were placed on actively growing colonies of the fungi on potato dextrose agar in 100X20 mm petri dishes and incubated at 20°C for 30 days in the dark. Mycoparasitism was evaluated using sclerotial germination, medulla color and integrity. Approximately 15 fungi demonstrated evidence of mycoparasitic activity. These isolates included previously reported and unreported mycoparasites, including Coniothyrium minitans, Trichoderma sp., Cladosporium sp. and an unidentified sclerotia producing fungus.

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THE ULTRASTRUCTURE OF HYPHAL ANASTOMOSES BETWEEN VEGETATIVELY COMPATIBLE AND INCOMPATIBLE STRAINS OF ENDOTHIA PARASITICA. J. R. Newhouse and W. L. MacDonald, Dept. of Plant Path. and Ag. Micro., West Virginia University, Morgantown, WV 26506.

European hypovirulent (hv) strain Ep-50 was paired with virulent (v) strains Ep 15-7-7 (compatible) and Ep 7-5-1 (incompatible) on cellophane membranes. When anastomoses were a few hours old, the hyphae were preserved by freeze-substitution. Observations of anastomoses between Ep-50 and Ep 15-7-7 revealed complete cytoplasmic continuity. Aggregates of spherical, membrane-bounded virus-like particles (VLPs) were seen in the Ep-50 hypha, a short anastomosis bridge, and the Ep 15-7-7 hypha. A longer anastomosis bridge between the strains possessed small supporting bridges at both ends. Anastomoses between Ep-50 and Ep 7-5-1 showed marked cellular collapse and cytoplasmic degeneration. The results indicate that passage of VLPs between vegetatively compatible v and hv strains of Endothia parasitica can take place within hours of hyphal fusion. Similar transmission of VLPs between vegetatively incompatible v and hv strains may be severely limited.

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REGENERATION OF PROTOPLASTS FROM AXENIC MYCELIA DERIVED FROM THE WHEAT STEM RUST FUNGUS. [1]D. Huang, [1]R.C. Staples, [2]W.R. Bushnell, [3]D.J. Maclean. [1]Boyce Thompson Institute, Ithaca, NY 14853; [2]ARS USDA Cereal Rust Laboratory, St. Paul, MN 55108, [3]University of Queensland, St. Lucia Q4067, Australia.

Protoplasts were prepared from sterile mycelium of strain V1B of Puccinia graminis f. sp. tritici race 126-ANZ-5,6,7,11. To release protoplasts, confluent mycelium grown in liquid medium was collected and resuspended in 10 ml of an enzyme solution (Novozyme 234 [3 mg/ml], cellulase [5 mg/ml], 0.8 M $MgSO_4$, and 0.5% MES buffer). Protoplasts were shown to be wall-free by Uvitex staining. After incubation (3 h/24°C), the mixture was layered over a sorbitol step-gradient (1.0 M x 2.0 M), and centrifuged (2,000g/10 min). The protoplasts at the bottom of the gradient were resuspended in 5 ml liquid medium supplemented with 0.5 M sorbitol, and incubated without shaking for 2 weeks at 20°C. Hyphal outgrowth from protoplasts was seen microscopically. No growth was observed in media without sorbitol. Abundant mycelial growth was obtained when protoplast-derived cultures were subcultured.

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HETEROKARYON FORMATION IN *PHYTOPHTHORA MEGASPERMA* F.SP. *GLYCINEA* AFTER PROTOPLAST FUSION. Layton, A. C., and Kuhn, D. N. Purdue University, West Lafayette, In 47904.

Phytophthora megasperma f.sp. *glycinea* (Pmg) is used to study the genetics and biochemistry of race-specific resistance in the soybean (*Glycine max*). However, the genetics of race-specific pathogenicity in Pmg is unknown. Classsical genetic approaches are difficult because Pmg is diploid, multinucleate, and self-fertilizing. An alternative approach to Pmg genetics is to produce heterokaryons by fusing protoplasts. We made protoplast fusions between fluorotryptophan and metalaxyl resistant mutants of race 1 and race 3. Heterokaryons were selected by growth on both drugs. The heterokaryons were tested for race-specific pathogenicity. In the heterokaryons nonpathogenicity (race 1 phenotype) was dominant to pathogenicity (race 3 phenotype). In some of the heterokaryons a large number of uninucleate zoospores contained both drug resistance markers suggesting karyogamy occurred. Protoplast fusions may be a useful tool for *Phytophthora* genetics. They can be used to outcross self-fertilizing species, study dominance relationships in heterokaryons, make interspecific crosses, and determine cytoplasmic and nuclear incompatibility.

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EFFECT OF SHADE AND MULCH ON STUNTING OF WHITE SPRUCE SEEDLINGS. M. A. Palmer, USDA Forest Service, St. Paul, MN 55108.

Stunted, mycorrhizal deficient 1st-year white spruce (Picea glauca) are common in forest tree nurseries in the north-central United States. Field studies were begun in 1986 to determine the effect of shade and mulch on the incidence of stunting. Soon after emergence, seedlings in 1 x 1.2 m plots were: 1) mulched with 1 inch of sawdust, 2) shaded with polypropylene shade cloth (47%), 3) shaded and mulched or 4) not protected. At the end of the first growing season, stunting was evident in 41% of the unprotected seedlings, 25% of the shaded seedlings, but only about 11% of the trees that had been mulched or shaded and mulched. These results indicate that although shading provides some protection, mulching alone is the best way to prevent stunting.

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EFFECT OF VESICULAR-ARBUSCULAR MYCORRHIZAE AND PHOSPHORUS FERTILIZATION ON GROWTH AND DISEASE SEVERITY IN ASPARAGUS. T.L. Wacker, G.R. Safir, and C.T. Stephens. Michigan State University, East Lansing, MI 48824.

Field studies, in which asparagus plants inoculated with a vesicular-arbuscular mycorrhizal fungus (VAM) were compared to non-VAM plants, indicate that VAM colonized plants grow significantly larger roots and shoots, and develop more storage roots than non-VAM plants (first season data). Preliminary data also suggest that VAM plants are more efficient at absorbing phosphorus (P); addition of P to the soil (total P: 350 ppm) did not eliminate the VAM growth difference. Root rot symptoms caused by Fusarium oxysporum f.sp. asparagi were increased by P addition in both non-VAM and VAM inoculated plants. The presence of VAM did not appear to directly affect root rot severity; however, apparent differences between non-VAM and VAM roots suggest that the disease rating system used may not have been sensitive to these differences. Increased crown size in VAM plants may indirectly affect survivability of asparagus.

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EFFECTS OF RED PINE MYCORRHIZOSPHERE STREPTOMYCETES ON IN VITRO GROWTH OF ECTOMYCORRHIZAL FUNGI. D.L. Richter, T.R. Zuellig, S.T. Bagley, and J.N. Bruhn. Michigan Technological University, Houghton.

Streptomycete strains isolated from red pine ectomycorrhizae showed either commensalism or antagonism in vitro with specific fungi ectomycorrhizal with this conifer. Such streptomycetes may prove useful to favor root colonization by preferred or co-inoculated mycorrhizal fungi. An enrichment technique was used to isolate streptomycetes from mycorrhizae of red pine seedlings outplanted in cleared hardwood sites in Michigan's Upper Peninsula. This technique involved 7 days incubation of washed mycorrhizae in starch casein broth with fungal inhibitors. One-tenth ml broth was then spread onto 0.45µm-pore-size filters overlying yeast-malt extract agar. Filters were removed after 14 days. Plates were reincubated for streptomycete colony formation. Streptomycete strains were selected for study based on their routine recovery from mycorrhizae and/or demonstration of their differential effects (commensalism/antagonism) on mycorrhizal fungus growth in pilot studies. One isolate each of Laccaria bicolor, L. laccata, and Thelephora terrestris was selected for study due to the frequent occurrence of these species in nurseries, greenhouses, and at outplanting sites. Effects were determined by comparing radial growth of fungus plated on MMN agar 1.0cm away from a centrally positioned streptomycete colony with radial growth of control fungus cultures. Streptomycete strains were found which 1) favor growth of both Laccaria isolates, but inhibit growth of the T. terrestris isolate, 2) favor growth of the L. laccata and T. terrestris isolates, but inhibit the L. bicolor isolate, 3) favor growth of the T. terrestris isolate but inhibit both Laccaria isolates, or 4) prevent growth of all three fungi.

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A PROCEDURE FOR DETERMINING SPORE VIABILITY OF VA MYCORRHIZAL FUNGI. Y. Pérez and N. C. Schenck, Plant Pathology Dept., University of Florida, Gainesville, 32611.

Spores of VA mycorrhizal fungi to be used for inoculation should be verified for viability. A rapid procedure using inexpensive materials was developed to determine spore viability. Spores sieved from soil are placed in deionized water. Membrane filters moistened with deionized water are folded into quarters and opened into a cone. The spore suspension is placed in the cone and the membrane filter is then inserted in a nylon mesh bag. The bags are placed in a shallow tray filled with the same type of soil to be used in the inoculation experiment. The soil is moistened with deionized water, kept covered and maintained in a dark location at constant temperature. On day 7 the spores are stained with Trypan blue while still in the membrane. Germination is determined by dissecting microscope evaluation. Using this procedure, species of Acaulospora, Entrophospora, Gigaspora, Glomus, and Scutellospora were evaluated effectively for spore germination.

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EFFECTS OF ACID SPRAYS ON PEROXIDASE ACTIVITY IN SILVER MAPLE (ACER SACCARINUM L.) LEAVES. R. L. Patton, U.S.D.A. Forest Service, 359 Main Rd., Delaware, OH 43015.

One-year-old dormant seedlings were potted and placed in a greenhouse. After 12 weeks there were 11 to 17 leaf-pairs along the stem of each plant. The plants were divided into 4 groups (8 per group) and each group was sprayed with a different acidified solution (pH 2.6, 3.6, 4.6, 5.6) to determine whether pH had an effect on peroxidase activity (PA) in leaves from any of several nodes. The solutions were prepared by adding a mixture of 1 M H_2SO_4 and 1 M HNO_3 (2:1) to distilled water. About 50 ml were sprayed daily on each plant with a hand-held trigger sprayer for 10 consecutive days. On the day after the final spray, leaves at nodes 3, 5, 7, 9, and 11 were harvested, examined for injury, and lyophilyzed. They were stored at -15°C until assayed for PA. Only leaves sprayed with the pH 2.6 solution showed signs of injury. PA differed among leaves from different nodes but pH had no effect on PA. PA was greatest in leaves from node 11 and in injured leaves.

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EVIDENCE IN SUPPORT OF THE USE OF EDU (ETHYLENEDIUREA) TO ASSESS OZONE-INDUCED PLANT INJURY. B. Greenhalgh, E. Brennan and I. Leone, Dept. Plant Pathology, Rutgers University, NJAES, Cook College, New Brunswick, NJ 08903.

A method for determining the impact of ozone pollution on crops involves a comparison of yields of EDU-treated and non-treated plants. Questions concerning the soundness of the methodology have been answered by experiments with 'Williams' soybean. Exposure of EDU-treated and non-treated plants to an O_3 dose of 0.20 ppm for 6 h on two consecutive days in a controlled chamber caused severe foliar injury and significant chlorophyll reductions in -EDU plants, but not in +EDU plants. EDU-treated and non-treated plants were grown in filtered open-top modules and unfiltered modules where the O_3 exceeded 0.10 ppm for 122 hrs. In filtered chambers +EDU and -EDU plants were similar in plant height, pods/plant, seed yield, chlorophyll content; and neither had foliar injury. In non-filtered chambers foliar injury occurred on -EDU plants but not on +EDU plants. Plant height, pods/plant, and seed yield tended to be greater although not significantly so in +EDU plants.

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SALT SPRAY AS A FACTOR PREDISPOSING WOODY STEMS TO CANKER FUNGI. S. M. Huhndorf and D. Neely, Illinois Natural History Survey, Champaign, IL 61820.

Dormant stems of red-osier dogwood, European mountain ash, white birch, sweetgum, sycamore, and black walnut were wound-inoculated with facultative canker fungi. Twenty plants of each species, maintained in a greenhouse, were sprayed three times a week for 9 weeks during two consecutive winter seasons with de-icing salt at either 2,800, 7,600, or 17,700 ppm. Two weeks after the last salt spray treatment, stems were harvested and serial stem sections were cultured on nutrient media to determine the extent of fungal colonization from inoculation points. Canker fungi could be recovered only from the inoculation point in salted and unsalted dogwood, mountain ash, sweetgum, and birch. In stems of sycamore and black walnut, canker fungi were isolated from stem tissue remote from inoculation points as well as the inoculation points themselves. The salt spray concentrations applied did not appear to predispose stems of any of the six woody species to the canker fungi selected for inoculation.

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EFFECTS OF OZONE AND SULFUR DIOXIDE ON PHYLLOPLANE FUNGI. M.E. Fenn, P.H. Dunn, and D.M. Durall, Pacific Southwest Forest and Range Experiment Station, USDA Forest Service, 4955 Canyon Crest Drive, Riverside, CA 92507.

Mature Valencia orange (Citrus sinensis) trees were fumigated for 4 years with charcoal-filtered air, 50% ambient air plus 50% filtered air, ambient air, or filtered air plus 10 pphm SO_2. Phylloplane fungi were reduced 33% by treatment with ambient air and 75% by SO_2 exposure compared to filtered air treatment. This indicates that chronic exposure to atmospheric pollutants can affect fungal phylloplane communities. In another experiment seedlings of California black oak (Quercus kellogii) and giant sequoia (Sequoia gigantea) were fumigated for 2 months with charcoal-filtered air, ambient air, or ambient air plus 1.5X ambient ozone. Ozone did not have a significant effect on numbers of leaf surface fungi. Seedlings of California black oak and giant sequoia located outside of chambers had higher populations of fungi than seedlings within chambers, thus indicating a significant chamber effect on phylloplane fungi.

595. Withdrawn

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Conidial germination, appressorial formation and infection of *Alternaria porri* on onion leaves. K. L. Everts and M. L. Lacy, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Onion plants were inoculated with dry conidia of *Alternaria porri* in a settling tower and placed in a dew chamber at 24C. Leaves were removed randomly after 3, 6, 12 and 24 hr and examined for conidial germination and penetration under the light microscope. After 3, 6 or 12 hr of dew, 73, 84 and 84% of conidia on leaf surfaces had germinated and 5, 34 and 44% had formed appressoria respectively. After 12 hr dew, 13% of conidia had formed infection hyphae and cell collapse was visible beneath 6% of conidia. After 24 hr dew, 23% of conidia had formed infection hyphae and 68% of those had caused visible lesions. Leaf sections were removed, vapor-fixed with OsO_4, air-dried, and examined under the scanning electron microscope. Conidia germinated from more than one cell and germ tubes grew in any direction across the leaf surface. Infection occurred either directly with well formed appressoria or through stomata.

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EFFECT OF HC TOXIN ON MAIZE PROTOPLASTS. S. Wolf and E. D. Earle, Plant Breeding Dept., Cornell Univ., Ithaca, NY 14853.

Leaf mesophyll protoplasts derived from susceptible and resistant plants were treated with concentrations 0, 1, 5, 10, and 100 µg/ml of a host-specific toxin from Helminthosporium carbonum (Hc toxin). A differential effect of the toxin was seen at 36 hours after treatment, but at 84 hours the effect was most dramatic. Ninety-five percent of the susceptible protoplasts treated with toxin at 1 µg/ml had chloroplasts which were distributed throughout the cytoplasm; the susceptible control (no toxin) had only 21%. In contrast, resistant protoplasts treated with 1 µg/ml or no toxin had 40 and 36%, resp. At 100 µg/ml there was no difference between resistant and susceptible protoplasts. The remaining intact protoplasts contained chloroplasts which were localized to one side. Low levels of toxin stimulated budding of susceptible protoplasts. Resistant protoplasts responded similarly at greater toxin concentrations. Studies are in progress to investigate a possible mechanism for differences in chloroplast distribution.

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ISOZYMES vs. VIRULENCE FOR MEASUREMENT OF PHENOTYPIC DIVERSITY IN BEAN RUST. D. C. Linde, J. V. Groth, and A. P. Roelfs, Dept. of Plant Pathology, Univ. of Minn., and USDA Cereal Rust Laboratory, St. Paul, MN 55108.

Twenty-four isolates of Uromyces appendiculatus were electrophoretically screened for 57 enzymes on three starch gel systems. Over half of the enzymes could be detected, and twelve were found to be suitable as phenotypic markers. All isolates were scored for isozyme patterns and also rated for virulence patterns on 17 differential bean lines. Phenotypic relationships of isolates as measured by isozymes vs. virulence did not closely correspond. However, three of six isolates that apparently lack the ability to produce telia were phenotypically distant from all other isolates with respect to both markers. Understanding diversity in sexual and asexual populations of bean rust is important for devising effective control measures, including disease resistance breeding strategies.

600

THE EFFECT OF PREHARVEST AND POSTHARVEST CALCIUM TREATMENT OF PEACHES ON DECAY CAUSED BY MONILINIA FRUCTICOLA. W. S. Conway, USDA, ARS, Hort. Crops Quality Lab, BARC-West, Beltsville, MD 20705, and G. M. Greene II and K. D. Hickey, The Pennsylvania State University, Fruit Research Lab, Biglerville, PA 17307.

Jerseyland peaches were treated with solutions of $CaCl_2$ either by 10 weekly preharvest sprays at rates of 0, 30, 60, or 90 lb/A or pressure infiltrated (68.95 kPa) at harvest with 0, 2, or 4% solutions of $CaCl_2$. Fruit from all treatments were stored at 0 C for 3 weeks. After removal from storage, the peaches were inoculated with a conidial suspension of Monilinia fructicola. Following 3 days at 20 C, the fruit were rated for decay severity and analyzed for calcium content. Fruit sprayed at the rate of 90 lb/A of $CaCl_2$ had 70% more calcium in the flesh than untreated fruit, but caused no reduction in area of decay. Fruit pressure infiltrated with 2 or 4% solutions of $CaCl_2$ had two and four times as much calcium in the flesh, and 40 and 60% less decay, respectively, than untreated fruit. Pressure infiltration of the solutions, however, resulted in fruit surface injury.

601

RELATIONSHIP OF APPLE CELL WALL CALCIUM TO TISSUE MACERATION BY POLYGALACTURONASE PRODUCED BY PENICILLIUM EXPANSUM. W. S. Conway and K. C. Gross, USDA, ARS, Hort Crops Quality Lab, Beltsville, MD 20705, C. D. Boyer, Penn State Univ., University Park, PA 16802, and C. E. Sams, Univ. of Tennessee, Knoxville, TN 37996

Apples were pressure infiltrated at harvest with solutions of $CaCl_2$ and stored at 0 C. After 6 months, the fruit were removed from storage and the cell walls were extracted and analyzed for calcium content. Polygalacturonase was partially purified from the decayed area of nontreated apples which had been inoculated with Penicillium expansum. The apple cell walls, which had increasingly higher amounts of calcium as the concentration of the solutions with which the fruit were infiltrated increased, were used as the substrate for polygalacturonase. Following incubation of the enzyme-substrate reaction, there was approximately 50% less product formed when high calcium cell walls were the substrate than when low calcium cell walls were used. Since calcium stabilizes the cell wall, decay in apples with high levels of calcium may be decreased because maceration by polygalacturonase is reduced.

602

PAGE ANALYSIS OF TINANGAJA AND BRISTLE TOP OF COCONUT ON GUAM AND CADANG-CADANG IN THE PHILIPPINE ISLANDS. C.B. Carpio, J. Rodriguez, and G.C. Wall, Philippine Coconut Authority, Albay Research Center, Banao, Guinobatan, Albay, and Agricultural Experiment Station, University of Guam, Mangilao, Guam 96923.

Out of 70 coconut trees sampled during a survey throughout Guam and assayed by polyacrylamide gel electrophoresis (PAGE), 33 were found to be infected by the Cadang-cadang Coconut Viroid (CCCV). Samples from diseased trees in the Philippines were used as controls. Using 20% gel, it is possible to separate 4 viroid forms associated with Cadang-cadang in samples from the Philippines. Only 1 of these, CCCV form 247, was found associated with Tinangaja disease of coconuts on Guam. Bristle top symptom, believed to be a separate disease, was found associated with CCCV-infected trees on Guam, particularly dwarf varieties. Results from previous work had already indicated a relationship between Cadang-cadang and Tinangaja; this study produced additional information as to the CCCV form found on Guam. It also revealed that dwarf varieties, regarded as more resisitant in the Philippines, have a high incidence of disease on Guam. No Javanica red dwarves were found infected in this survey, but this may be due to its rare occurrence and not resistance. Tarague and Tanguisson beaches have very low disease incidence and are recommended as sources of seed. There is no apparent relationship between soil type and disease incidence.

603

AN EFFECTIVE SYSTEM FOR DETECTING WHEAT SPINDLE STREAK MOSAIC VIRUS (WSSMV) IN FIELD SAMPLES. D. C. Bays[1], B. M. Cunfer[2] and J. W. Demski[2]. [1]Dept. of Plant Path., Phys. and Weed Science, Va. Polytech. Inst. and State Univ., Blacksburg, VA 24061, and [2]Dept. of Plant Path., Univ. of Georgia, Experiment, GA 30212.

A double-antibody sandwich enzyme-linked immunosorbent assay (das-ELISA) system has been developed for detecting WSSMV in field samples of wheat. Virus for immunization of rabbits was purified from naturally-infected wheat plants collected in Plains, GA, using a modification of a potyvirus purification procedure with extraction in Tris-EDTA buffer, PEG precipitation, and centrifugation through sucrose that yielded up to 13 mg virus per kg. A polyclonal antiserum collected 5 to 6 wks after injection gave IgG fractions reacting specifically to virus when used in das-ELISA at 1 µg/ml. The system has been used to screen small grain breeding material in Georgia for the presence of WSSMV. Under these das-ELISA conditions, there is little or no healthy wheat background reaction and no evidence as yet of reaction with WSSMV.

604

THE REDUCTION IN INCIDENCE OF APHID-BORNE STRAWBERRY VIRUSES IN THE FIELD BY USE OF PYRETHROID INSECTICIDES. R. H. Converse and M. T. AliNiazee, USDA, Agricultural Research Service, and Dept. of Entomology, Oregon State University, Corvallis, OR 97331

The pyrethroids cypermethrin, permethrin, and fenvalerate significantly reduced aphid-borne virus incidence to 44-63% of that in adjoining unsprayed plots at the end of a 2-yr field test on Totem strawberry in western Oregon. By then, 59% of the test population of 150 plants was infected with aphid-borne viruses. The luteovirus, strawberry mild yellow-edge, transmitted by the aphid Chaetosiphon fragaefolii (Ckll.), was present in 97% of these virus-infected plants. Although the pyrethroid bifenthrin was four times more effective in reducing C. fragaefolii incidence than the other pyrethroids, aphid-borne virus incidence in the bifenthrin plots was 85% of that in unsprayed plots, not a significant difference. The incidence of aphid-borne viruses can be cut in half by certain pyrethroids in strawberry plants lacking spatial isolation from viruliferous C. fragaefolii. Pyrethroid effects on incidence of these aphid-borne viruses are poorly predicted by aphid kill.

605

PURIFICATION AND SEROLOGY OF A NEWLY RECOGNIZED VIRUS DISEASE IN SORGHUMxSUDANGRASS HYBRIDS. L. M. Giorda, R. W. Toler, Dept. of Plant Path. & Micro., and G. N. Odvody, Tex. Agr. Exp. Sta., College Station 77843 and Corpus Christi 78410.

Virus was extracted from mature leaves of sweet corn "Silver Queen" in 0.1 M potassium phosphate buffer (PPB), pH 7.0 containing 1% 2-mercaptoethanol, clarified with chloroform-butanol (1:1) and precipitated with 8% PEG (w:v) and 0.2 M NaCl. Preparations were subjected to two cycles of differential centrifugation at 9750x*g* for 10 min and 125000x*g* for 150 min. The resuspended pellets were centrifuged on 10-40% sucrose density gradients. Viral fractions had a 260/280 ratio of 1.53 and yielded 2.5 mg/ml buffer (17 mg/100 g of tissue). Antiserum collected in the 8th week had a titer of 1/2048 when tested against a 1/10 dilution of infected sap using an Ouchterlony gel-diffusion test. Using the same antiserum, protein A sandwich ELISA (PAS-ELISA) detected purified virus at 9.7 ng/ml. The virus was detected in PAS-ELISA when dried or fresh leaf discs (6 mm in diam.) were smashed in a plastic bag with phosphate buffer saline, pH 7.5 in a 1/100 proportion (w:v).

606

PSOROSIS VIRUS OF CITRUS: HOST RANGE AND DSRNA ANALYSIS. L. Levy AND D. J. Gumpf, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

A virus has been implicated as the etiological agent of Psorosis (Ps), a scaly bark disease of citrus. A Psorosis-associated component (PsAC) was mechanically transmitted from citron infected with a California isolate of Ps to Nicotiana benthamiana and Chenopodium quinoa inducing a mild systemic mosaic and necrotic local lesions, respectively. PsAC was transmitted from citron to Capsicum frutescens (c.v. Mexican Chili) by dodder, graft, and mechanical transmission. In all cases, a systemic vein clearing was observed 20-25 days post inoculation. Disease specific dsRNAs have been detected in symptomatic hosts.

607

VALIDATION OF A TEST FOR DETECTION OF SEEDBORNE BACTERIA OF BEANS USING ANTIBIOTIC RESISTANT BACTERIA. J. R. Venette and R. S. Lamppa, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Pathogenic rifampin and streptomycin resistant mutants of bean bacterial pathogens, Pseudomonas syringae pv. syringae, Pseudomonas syringae pv. phaseolicola and Xanthomonas campestris pv. phaseoli, were recovered from bean seedlings growing in high humidity (the dome test) after the mutants had been seeded into infusions used to vacuum infiltrate pregerminated seeds. Mutants were detected in symptomatic and presymptomatic leaves. Pathogenic mutant populations increased in the plants as did populations of saprophytes. Saprophytes were 100-1000 times more numerous than pathogens. Numbers of pathogenic mutant bacteria in leaves with hydrosis were 120-240 times higher than numbers in leaves without hydrosis.

608

SEROLOGICAL VARIATION IN XANTHOMONAS CAMPESTRIS PV. PHASEOLI, PSEUDOMONAS SYRINGAE PV. SYRINGAE, AND PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA FROM PHASEOLUS BEANS IN NORTH DAKOTA. H. M. Khalif and J. R. Venette, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Serological variation was observed within collections of Xanthomonas campestris pv. phaseoli (Xcp), Pseudomonas syringae pv. syringae (Pss) and Pseudomonas syringae pv. phaseolicola (Psp) from Phaseolus beans in North Dakota. When heated antigens were tested against cross-absorbed antisera in Ouchterlony double diffusion tests, 40 strains of Xcp were grouped into 4 different serogroups, 38 strains of Pss were separated into 4 serogroups, and 25 strains of the Psp were grouped into 6 serogroups. Xcp from seeds were serologically distinct from Xcp isolated from leaves. This was not the case with Pss and Psp from seeds which were serologically grouped with other leaf strains.

609

EFFECTS OF INCOMPLETE HYPERSENSITIVITY AND SLOW-LEAF RUSTING IN TRITICALE. J. Wilson and G. Shaner, Dept. Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

The effects of incomplete hypersensitivity and slow-rusting resistance to *Puccinia recondita* were evaluated by separating the genetic factors for low infection type (IT) and long latent period (T50) of the triticale cultivar PI 429155 into different experimental lines, and measuring the components of resistance on adult plants. The derived lines possessed no resistance factors (SUSC), the factors for long T50 but high IT (SR), the factors for low IT and moderately short T50 (HR), or the factors for both forms of resistance (SR+HR). SR, HR, and SR+HR all reduced infection frequency. It was not possible to separate completely the low IT from long T50 in the HR line. The IT, T50, and uredinium areas of SUSC, SR, HR, and SR+HR were IT 4, 3, 2, and 1+; 7.1, 13.0, 9.5, and 14.3 days; and 0.282, 0.124, 0.093, and 0.069 mm^2, respectively. The gamma distribution fit the pattern of spores produced/uredinium/day, measured to 30 days after inoculation. Cumulative spores produced/uredinium on SR, HR, and SR+HR were 27.5, 16.4, and 13.6% of that produced on SUSC. Computer-generated models of rust epidemics and observations of rust increase in hill plots revealed that the long T50 of SR provided greater disease control than the reduced sporulation of HR.

610

DIFFERENCES IN HOST RESPONSE OF NEAR-ISOGENIC WHEAT LINES TO LEAF RUST. Beatriz A. Perez, and A. P. Roelfs, Cereal Rust Laboratory, USDA/ARS and Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Infection severities by Puccinia recondita f. sp. tritici on wheat (Triticum aestivum) lines TcLr12, TcLr13, TcLr34, TcLrT3, TcLr34&T3 and Thatcher were evaluated at four growth stages in the field. TcLrT3 and TcLr12 were similar in response to Thatcher until the early dough stage but terminal severities were 20 to 30% less. TcLr13, TcLr34 and TcLr34&T3 had a lower severity through the season; terminal severities were 50% less than on Thatcher. TcLr13 and TcLr34 were similar in response in a nursery with a low initial inoculum density. Slower rusting was observed with TcLr13, TcLr34 and TcLr34&T3. TcLr34&T3 was more resistant than TcLrT3 but less than TcLr34. Methods are being developed to evaluate adult plant resistance in breeding for durable resistance in wheat.

611

GREENHOUSE SCREENING OF CELERY SOMACLONE PROGENY FOR RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2. K. F. Ireland, and M. L. Lacy. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI. 48824.

Somaclones regenerated from single cells of the celery cultivar Tall Utah 52-70 HK (TU-HK) were significantly more resistant to *F. oxysporum* f. sp. *apii* race 2 than TU-HK plants. Eight of the somaclones were induced to flower and were self-pollinated. The selfed progeny (S_1) were screened for resistance to *F. o.* f. sp. *apii* race 2 in the greenhouse by planting seedlings in muck soil naturally infested with *F. o.* f. sp. *apii* race 2. Disease ratings were based on the degree of vascular discoloration and were: 1= none, 2= trace, 3= moderate, 4= severe, and 5= dead plant. S_1's from 3 somaclones had significantly lower mean disease ratings than TU-HK plants. The mean disease ratings for the S_1's from the other 5 somaclones were not significantly different from TU-HK. These results suggest that the increased resistance was stable genetically because it was passed on to the S_1 progeny.

612. Withdrawn

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SPECIFICITY OF MINOR GENE RESISTANCE OF DIPLOID ALFALFA 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 3% resistant (no conidial production) to P. trifoliorum isolate I-7, were crossed and their F_1s were selected for resistance to I-7. This increased resistance not only to I-7, but also to pathogenically different isolates I-5 and I-8, which suggested that the increased resistance was general in nature. Two F_1 plants, whose S_1 populations were 59% and 37% resistant to I-7, were crossed. Two of their progeny, whose S_1 populations were 73% and 76% resistant to I-7, were crossed. Selection for resistance and susceptibility in progeny of this cross was successful. Segregation in S_1 populations of the resistant plants indicated that the apparent general resistance was due to a collection of isolate-specific interactions. One of these resistant plants produced an S_1 population 73%, 98%, and 58% resistant to I-5, I-7, and I-8, respectively. However, when this plant was backcrossed to P1, progeny were only 9%, 14%, and 0% resistant to I-5, I-7, and I-8, respectively.

614

REACTIONS OF SWEET CORN HYBRIDS TO MULTIPLE DISEASES AND ASSOCIATED YIELD REDUCTIONS. J. K. Pataky and J. M. Headrick. Dept. Plant Pathology, University of Illinois, Urbana 61801.

A total of 146 commercial sweet corn hybrids were evaluated in 1984, 1985, and 1986 for reactions to common rust (Puccinia sorghi), northern leaf blight (Exserohilum turcicum), Stewart's wilt (Erwinia stewartii), and Goss' wilt (Clavibacter michiganense subsp. nebraskense). For each disease in each year, hybrids were categorized into resistant, moderately resistant, moderately susceptible and susceptible classes based on mean seperation tests (BLSD). A hierarchical cluster analysis grouped hybrids into 7 major groups based on reactions to all diseases in all years. Main groupings were defined by reactions to NLB, Stewart's and Goss' wilts. Subgroups were defined by reactions to rust. Correlations between reactions to NLB, Stewart's and Goss' wilt were significant within and between years ranging from r = 0.41 to 0.86. Reaction to rust was not correlated with the other diseases. Yield reductions associated with resistance categories were evaluated by regression analyses.

615

COMPARISON OF IN VIVO PROTEINS OF BIOLOGICALLY DISTINCT SOYBEAN MOSAIC VIRUS STRAINS. S. A. Tolin and D. C. Bays. Dept. of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Soybean mosaic virus isolates that had been differentiated into strain groups by reaction on soybean cultivars were further characterized by their reaction on cowpea (Vigna unguiculata) and by the severity of the necrotic reaction on resistant soybean cultivars. The virus titer as measured by ELISA in soybeans exhibiting mosaic symptoms was relatively equal among cultivars regardless of strain. Viral coat protein from dissociated purified virions was of identical size in SDS polyacrylamide gels, but varied quantitatively between strains. Proteins detected in preparations of inclusions from infected leaves differed both quantitatively and qualitatively between strains. SMV-G6, for example, appeared to accumulate coat protein preferentially to nonstructural inclusion proteins.

616

TRANSLATION OF THE IN VITRO TRANSCRIPT OF DOUBLE STRANDED RNA OF THE 190S VIRUS FROM HELMINTHOSPORIUM VICTORIAE. S. A. Ghabrial and W. M. Havens. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The virion-associated RNA polymerase of H. victoriae 190S virus has been found to catalyze the synthesis and release of full length ssRNA transcripts of the genomic dsRNA. The in vitro transcripts were purified from transcription mixtures by phenol extraction, ethanol precipitation, selective precipitation of ssRNA in 2M LiCl, and Cellex N-1 cellulose chromatography. In a rabbit reticulocyte translation system, the in vitro transcripts directed the synthesis of a single major polypeptide of mol. wt. 88,000. This was identical to the translation product of denatured dsRNA and to the capsid polypeptide p88. The capsids of the 190S virus contain two major proteins p88 and p83. Peptide mapping has shown p83 and p88 to be closely related and suggests that the capsids are encoded by a single gene. The in vitro transcripts thus comprise the mRNA for the major capsid polypeptide p88.

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INDEPENDENT REPLICATION OF RNA-1 OF SWEET CLOVER NECROTIC MOSAIC VIRUS IN COWPEA PROTOPLASTS. H.R. Pappu and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.

Mesophyll protoplasts from cowpea (Vigna unguiculata) were infected with sweet clover necrotic mosaic virus and its bipartite genomic RNA (RNA-1 and RNA-2). With a purified virus preparation, 60% of the protoplasts were infected in the presence of poly-L-ornithine. Polyethylene glycol-mediated infection with total genomic RNA resulted in 40% infection of viable protoplasts. Protoplasts were inoculated separately with fractionated RNA-1 and RNA-2 and the synthesis of each RNA species was followed by Northern hybridization analysis using specific complementary DNA probes. When protoplasts were inoculated with RNA-1 alone, synthesis and accumulation of RNA-1 was detected by Northern blotting 12 hr after inoculation. However, no replication of RNA-2 was detected in protoplasts inoculated with RNA-2 alone.

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STRAIN SPECIFIC PROTEINS ISOLATED FROM MDMV AND SCMV INFECTED PLANTS. S. G. Jensen, N. J. Van Pelt, Agricultural Research Service, U. S. Department of Agriculture and Department of Plant Pathology, University of Nebraska, Lincoln Nebraska 68583-0722.

Cylindrical inclusions were purified from maize tissues infected with 20 strains or isolates of maize dwarf mosaic virus (MDMV) or sugarcane mosaic virus (SCMV). The concentrated proteins were separated by SDS polyacrylamide gel electrophoresis. Silver staining of the gel revealed the major protein for each isolate was the cylindric inclusion subunit of about 66k da. With two isolates, KS-1 and I-188, a second major protein band of about 48k da was observed. The 48k da protein was stable and represented another protein induced by strains of MDMV. Antibody to this protein reacts on Western blots. Surprisingly, the antibody also detected the 48k da protein in many closely related strains where silver staining failed to detect the protein. Characterization and implications of the 48k da protein will be presented.

619

EXPRESSION AND MAPPING OF BEAN GOLDEN MOSAIC VIRUS GENES. J. G. Utermohlen and A. J. Howarth, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

A portion of the coat protein gene of bean golden mosaic virus (BGMV) was cloned in frame into the beta-galactosidase gene cloning region of pTZ18U. Truncated BGMV-coat protein was expressed in transformed Escherichia coli cells after induction with isopropyl-beta-D-thiogalactopyranoside (IPTG). This protein was identified in bacterial extracts by SDS-PAGE using silver staining and by western blot analysis with BGMV antisera. Polysomal RNA extracts from BGMV-infected and non-infected bean plants were analyzed for virus-specific transcripts. Five RNase-sensitive, DNase-resistant, BGMV-specific transcripts were detected by northern hybridization in extracts only from virus-infected plants. The polarities and the 5' termini of these viral transcripts were determined and mapped to the viral genome by primer extension assays using synthetic oligonucleotide primers and reverse transcriptase. These putative viral mRNAs corresponded to 5 of the six open reading frames indicated by the sequence of the viral genome.

620

ORGANIZATION AND *IN VITRO* TRANSLATION OF MAIZE CHLOROTIC MOTTLE VIRUS RNA. Gregory Hobbs and Charles O. Gardner, Jr., Department of Biochemistry, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK 74078.

Maize chlorotic mottle virus sediments as a single species, but extracted RNA sediments as two major species. The faster-sedimenting species corresponds to an RNA of 1.5 x 10^6 MW. The slower-sedimenting species shows a smear upon electrophoresis of about 0.65-0.1 x 10^6 MW. RNA inoculations suggest that the larger RNA must be present for infection to occur. *In vitro* translations in wheat germ extracts show unfractionated MCMV RNA to code for proteins of 12, 16, 18, 29, 36 and 45 kilodaltons. The 1.5 X 10^6 MW RNA alone codes for the same set of proteins, but the smaller RNA(s) are unable to code for the 36 and 45 kilodalton polypeptides. From this we infer that the 36 and 45 KD proteins are involved in viral replication. The 29 KD protein co-migrates with isolated viral coat protein on PAGE, and reacts with MCMV-specific antisera in western blots. We thus conclude that the coat protein is 29 KD.

621

CHARACTERIZATION OF AN RNA POLYMERASE ASSOCIATED WITH dsRNA-CONTAINING VESICLES IN A HYPOVIRULENT STRAIN OF ENDOTHIA PARASITICA. D. R. Hansen, N. K. Van Alfen, Dept. Biology, Utah State University, Logan, UT 84322-4500.

An RNA polymerase can be co-purified with double-stranded (ds)RNA-containing vesicles associated with hypovirulent strains of Endothia parasitica. Enzyme activity was determined by assaying the incorporation of radiolabeled UTP into a TCA-precipitable product. Activity depended on the presence of the other three ribonucleotides and Mg^{++}. Optimal activity was obtained at 5 mM $MgCl_2$. Vesicles isolated from virulent strains showed no incorporation of label. The majority of the products isolated from the reaction mixture were single-stranded (80%), as determined by CF-11 chromatography and RNase digestion experiments. The double-stranded products are the same size as the dsRNA contained in the vesicles. The single-stranded products range from 1.5 to 3 kb in size and are probably subgenomic. The nature of the product depended on the template present in the reaction mixture.

622

MEASURING VIRAL ANTIGEN-MONOCLONAL ANTIBODY AFFINITY BY ENZYME-LINKED IMMUNOSORBENT ASSAYS. H. T. Hsu, J. A. Aebig*, and R. L. Jordan, USDA-ARS, Beltsville, Md. 20705, and *ATCC, Rockville, Md. 20852.

The affinity of a monoclonal antibody for a specific antigen is expressed as the dissociation constant (K_d) of the antigen-antibody complex. The fraction of bound antibody and the concentration of free antigen in solution at equilibrium were calculated using the molecular weight of viral coat protein. Scatchard plots were derived from linear regression analysis and the K_d was expressed as negative of the reciprocal of the slope value from Scatchard plot. The K_d for the three monoclonals, NA70C9, NA49F8 and N63F10, with prunus necrotic ringspot ilarvirus were 3.5 x 10^{-8} M, 15 x 10^{-8} M and 0.07 x 10^{-8} M, respectively; those for A63E10 and NA70C9 with apple mosaic ilarvirus were 2.3 x 10^{-8} M and 17 x 10^{-8} M, respectively. The method provides a simple, sensitive and precise assessment for measuring monoclonal antibody-plant virus interaction.

623

DETECTION OF STRAINS OF POTATO VIRUS X AND OF A BROAD SPECTRUM OF POTATO VIRUS Y ISOLATES BY NUCLEIC ACID SPOT HYBRIDIZATION (NASH). D.C. Baulcombe, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U.K., and E.N. Fernandez-Northcote, International Potato Center, Aptdo. 5969, Lima, Peru.

Cloned complementary DNA hybridization probes for potato virus X (PVX) and potato virus Y (PVY) have been evaluated for the detection of PVX and PVY by NASH. Results with four probes for PVX indicated a genomic difference among two Andean and two non-Andean strains. Three of the four PVX probes tested would be useful for detecting PVX strains. Results with three probes for PVY indicated specificity of the probes to the broad spectrum of 27 PVY isolates from the PVY^O, PVY^N, and PVY^C group of strains obtained from different geographical areas. None of the PVY probes detected genomic differences among the PVY groups of strains. The PVY probes provided conclusive evidence that the other potyviruses tested namely potato viruses A, potato V, Peru tomato, wild potato mosaic and tobacco etch are distinct viruses from PVY. The three PVY probes would be useful for detecting PVY strains.

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A COMPARISON OF ELISA REACTIONS FROM DRY, FROZEN, AND FRESH WHEAT LEAF TISSUE INFECTED WITH WHEAT SOILBORNE MOSAIC VIRUS. C. R. Armitage, R. M. Hunger, and J. L. Sherwood, Dept. of Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078.

Leaves of winter wheat (cv. Sage and Vona) showing symptoms of wheat soilborne mosaic virus (WSBMV) infection were cut in half along the midrib. One half was either refrigerated (referred to as "fresh") or frozen for 3 to 5 days, while the other half was desiccated over CaCl2. Each half was ground in PBS-Tween with 2% PVP (1:100 w/v) and analyzed by ELISA. The mean difference in fresh weight between the two leaf halves was 0.0112 g. Weight loss was a result of desiccation was between 60-75%. Desiccated tissue generally exhibited a higher ELISA absorbance than either fresh or frozen tissue (mean difference is at least 20%) while frozen tissue exhibited a higher ELISA absorbance than fresh tissue (mean difference is at least 11%). These results suggest that desiccated tissue could be used in ELISA analyses of leaf samples that have been collected over time.

625

AN ULTRASTRUCTURAL STUDY OF A CLOSTEROVIRUS INFECTION IN DISEASED GRAPEVINES OCCURRING IN ARKANSAS. K. S. Kim[1], D. Gonsalves[2], D. Teliz[2], and K. W. Lee[1]. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701[1] and Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.[2]

Flexuous, rod-shaped particles were consistently associated with diseased Riesling and Chardonnay grapevines. Early in the season stunting, chlorosis, and cupping of leaves were typical of diseased grapevines. Fall symptoms were typical of grapevine leafroll disease (GLRD). The viruslike particles, approximately 12nm in diameter and of undetermined length, occurred only in phloem cells. In sieve elements, particles were associated with a large number of membranous vesicles containing fibrils characteristic of closterovirus infections. Unlike other closteroviruses, however, the fibril-containing vesicles appeared to have originated from modified mitochondria. Ouchterlony double diffusion and direct ELISA tests indicated that the virus was serologically identical to a New York isolate (NY-1) of a closterovirus associated with GLRD.

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CONVERSION OF COMOVIRUS ELECTROPHORETIC FORMS BY BEETLE REGURGITANT. M.A.C. Langham, R.C. Gergerich, and H.A. Scott, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Regurgitant of leaf-feeding beetles, which is a mixture of nutrients, enzymes, and other components, has been shown to be a determining factor in plant virus transmission. Comoviruses, which are beetle transmissible, exist as two electrophoretic forms (Virology 51:279-286). Purified bean pod mottle, cowpea mosaic and squash mosaic viruses were incubated with equal volumes of Mexican bean beetle regurgitant for 2 hr at 37 C. When the mixtures were separated by agarose gel electrophoresis and stained with ethidium bromide, only the converted form of the virus remained. Analysis of virus in regurgitant obtained from beetles fed on infected bean tissue showed only the converted form in regurgitant. This indicates the presence of proteolytic activity in regurgitant and that only the converted form of comoviruses is naturally transmitted to plants by beetles.

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PARTICLE COMPLEXITY OF TOBACCO RINGSPOT VIRUS. A.M.R. Almeida, R.M. Lister, and C.E. Bracker. Botany & Plant Pathology, Purdue Univ., W. Lafayette, IN 47907.

Preparations of various isolates of tobacco ringspot virus separated on rate-zonal density gradients (made by freezing 20% sucrose solutions in Spinco SW41 tubes) into the 3 major sedimenting species expected - top (T), middle (M), and bottom (B) components. However, the T component, which lacked RNA, subdivided into two closely sedimenting subspecies, T1 and T2. These sedimented in linear log sucrose gradients at 53S (T1) and 63S (T2). Electron microscopy indicated two particle sizes (about 25 and 29 nm) within the T component. Smaller particles were generally better penetrated with uranyl acetate, and were more common in T1 than in T2. In CsCl gradients T component resolved into two subspecies, of density 1.28 and 1.29. Similar observations showed that the M component also contains two particle types differing in density, size, and s value, and that the B component is probably similarly complex.

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ALTERATIONS IN LEAF PROTEINS ACCOMPANYING VIRUS-INDUCED HYPERSENSITIVE REACTION IN PHASEOLUS VULGARIS L. cv. 'PINTO'. F. Mohamed and O.P. Sehgal, Univ. of Missouri, Columbia, MO 65211.

Infection of the primary 'Pinto' leaves by southern bean mosaic virus (SBMV), tobacco ringspot virus, bean pod mottle virus and tobacco mosaic virus, results in the production of local lesions and an enhanced synthesis of two host proteins of mol. wts. 17K and 21K. These proteins are confined largely to the symptom-bearing areas of the leaf but are also present in the intercellular fluid. The 17K and 21K proteins from SBMV-infected tissue have been purified and high-titred antisera against these proteins have been prepared. Data based upon gel diffusion, rocket immunoelectrophoresis, as well as peptide mapping shows that the 17K proteins from 'Pinto' leaves infected with these four viruses are identical. Preliminary data indicate that a similar situation exists for the 21K protein. The possible role of the 17K and 21K proteins in virus localization in vivo is under investigation.

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QUANTIFICATION OF BEAN POD MOTTLE VIRUS IN PLANT TISSUE BY DOT IMMUNOBINDING ASSAY. S. Kartaatmadja and O.P. Sehgal, Univ. of Missouri, Columbia, MO 65211.

A dot immunobinding assay was standardized for quantifying bean pod mottle virus (BPMV) in leaf tissue. Samples (1 µl) of diluted soybean leaf extract from healthy plants containing known amounts of purified virions were spotted on a nitrocellulose membrane and then reacted with rabbit anti-BPMV IgG. Following incubation in an appropriately diluted solution of goat anti-rabbit IgG conjugated with alkaline phosphatase, the membranes were treated with a histochemical substrate for the enzyme, cleared in paraffin oil, and then scanned with a densitometer. A standard curve (peak heights versus concentration) was obtained which proved linear (R^2 = 0.96) within the range of 0.5 to 5.0 ng of BPMV. Quantitative estimation of BPMV in the individual trifoliate leaves of soybean cv. Williams, approximately 7 wks post-infection, gave values of 150 to 400 µg/g tissue. However, the second oldest soybean trifoliate leaf consistently contained less than 100 µg virus/g tissue. Preliminary studies indicate that the overall BPMV concentration in the leaves of garden bean (Phaseolus vulgaris) is comparable to that in soybean, while cowpea (Vigna unguiculata) leaves contained one-tenth as much BPMV.

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CLONING OF cDNA TO SOYBEAN MOSAIC VIRUS RNA. L. M. Mansky, P. J. Berger, R. E. Andrews, J. H. Hill and D. P. Durand. Depts. of Microbiology and Plant Pathology, Iowa State University, Ames, Iowa 50011.

Complementary DNA (cDNA) was synthesized to soybean mosaic virus (SMV-G2) RNA. Using non-denatured SMV RNA in the synthesis reaction, cDNAs approximately 600 bases in length were synthesized. Double stranded cDNA was cloned using pUC 9 as the plasmid vector. The longest cDNA cloned was determined by restriction enzyme analysis to be approximately 710 bases in length. Confirmation that the cDNA was complementary to SMV RNA was determined by Southern blot analysis of cloned cDNA, using single stranded cDNA as a probe. In attempts to synthesize and clone longer cDNAs, SMV RNA was heat denatured prior to reverse transcription and also was added to a first strand synthesis reaction mixture that was 10% DMSO. Heat treated SMV RNA yielded double stranded cDNAs ranging from 600 to 2000 bases; DMSO treated RNA yielded double stranded cDNAs of 600 bases. These cDNAs are being cloned.

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GENERATION OF ANTI-IDIOTYPE HYBRIDOMA ANTIBODIES TO SOYBEAN MOSAIC VIRUS RABBIT POLYCLONAL ANTIBODIES. R. L. Mernaugh, D. P. Durand, and J. H. Hill. Depts. of Microbiology and Plant Pathology, Iowa State University, Ames, Iowa 50011.

Antigen-mimicing anti-idiotype (a-id) antibodies (Abs) to virus-neutralizing monoclonal antibodies (McAbs) have been used to study virus receptor sites in mammalian systems. Since virus receptors and neutralizing McAbs have not been identified in the soybean mosaic virus (SMV) system, a-id hybridoma Abs were shotgun-generated to anti-SMV rabbit polyclonal Abs (RAbs). To limit the number of hybridomas producing nonspecific Abs to common RAb epitopes, mice used for fusions were first made tolerant to another RAb with cyclophosphamide and were then sensitized by SMV RAbs. Four hybridomas produced Abs which reacted in ELISAs to a greater extent with SMV RAbs than with control RAbs or SMV RAbs in the presence of SMV. In preliminary experiments, three of these appeared to inhibit SMV local lesion formation on bean leaves. It has yet to be determined if this is due to Abs mimicing SMV or is a nonspecific phenomenon.

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USE OF AN ANTI-IDIOTYPE MONOCLONAL ANTIBODY IN LIEU OF A VIRUS REFERENCE STANDARD IN ELISAS. R. L. Mernaugh, D. P. Durand and J. H. Hill. Depts. of Microbiology and Plant Pathology, Iowa State University, Ames IA 50011.

Some antibodies produced by different animal species to the same antigen possess cross-reactive idiotopes (CRIs). Based on this observation, four syngeneic IgM K anti-idiotype (a-id) monoclonal antibodies (McAbs) generated to the soybean mosaic virus (SMV) McAb (S1) were assayed for specific activity to protein A-purified SMV rabbit polyclonal antibodies (RAbs). Of the four a-ids, only 1a reacted with RAbs to SMV and not to RAbs to two other plant viruses. S1 and SMV competed with SMV RAbs for bound 1a in ELISAs, indicating that S1 and SMV RAbs have nearly identical CRIs and that 1a identifies an idiotope near the paratope of SMV RAbs. ELISA plates coated with either 1a or SMV could detect as little as 50 ngs of SMV RAbs. Since RAbs are commonly used in ELISAs to detect plant viruses, an a-id McAb to an appropriate virus RAb could be used as a positive control in ELISAs for detection of exotic viruses subject to quarantine restrictions.

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APHID TRANSMISSION OF CLOVER YELLOW VEIN AND BEAN YELLOW MOSAIC VIRUSES. H. A. Hobbs and M. R. McLaughlin, USDA, ARS, Crop Sci. Res. Lab., Forage Res. Unit, Mississippi State, MS 39762

High levels of transmission of clover yellow vein virus (CYVV-Pratt) and bean yellow mosaic virus (BYMV-KY 204-1) were obtained with Aphis craccivora. Using either five or ten aphids per test plant, acquisition access periods of 4-5 min, and test feeding periods of 2-4 hr, transmission levels for both viruses were 60-100% from infected to healthy pea (Pisum sativum) plants. Only sporadic transmissions occurred from pea to bean (Phaseolus vulgaris). Acyrthosiphon pisum rarely transmitted CYVV or BYMV from pea to either pea or bean.

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COMPARISON OF SEVERAL LUTEOVIRUSES BY SEROLOGY, DSRNA AND MOLECULAR HYBRIDIZATION. R. A. Valverde*, S.-H. Liu**, B. W. Falk*, and J. E. Duffus**, Department of Plant Pathology, University of California, Davis 95616* and USDA-ARS, Salinas, CA 93905**.

Different luteoviruses, including several isolates of beet western yellows virus (BWYV) and barley yellow dwarf virus (BYDV) were compared by DAS-ELISA, dsRNA and molecular hybridization using cDNA clones to BWYV and BYDV. All BWYV isolates tested reacted with antiserum to BWYV. All other luteoviruses reacted only with their homologous antisera. Obtained dsRNA profiles were useful to differentiate some but not all luteoviruses analyzed. cDNA clones to BWYV-mild hybridized with all BWYV isolates. Nevertheless a clone specific for BWYV-severe hybridized only with BWYV-severe. A cDNA clone to BYDV-PAV hybridized with NY-PAV and NY-MAV, but not with any of the other luteoviruses. These results reemphasize the biological and molecular diversity among luteoviruses and their isolates.

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THE NUCLEOTIDE SEQUENCE OF THE 3' TERMINUS OF SOYBEAN MOSAIC VIRUS. P. L. Gunyuzlu, S. A. Tolin, and J. L. Johnson. Dept. of Plant Pathology, Physiology and Weed Science, and Dept. of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, VA. 24061.

The nucleotide sequence of the 3' terminus of soybean mosaic virus (SMV) VA/G1 RNA has been determined by dideoxynucleotide sequencing of oligo(dT) primed cDNA cloned into a pUC19 cloning vector via EcoRI linkers. One recombinant plasmid, pSMV49, identified by colony and dot-blot hybridization to ^{125}I-SMV RNA, contained an insert of 1443 nucleotides exclusive of the poly(A) tail and had a single open reading frame of 1119 nucleotides terminating 224 nucleotides from the 3' poly(A) tract. The coat protein cistron identified by a glutamine:serine dipeptide cleavage site 789 nucleotides upstream from the termination sequence could potentially code for a 29.8 kDa protein. The amino acid sequence predicted from the nucleotide sequence of this cistron contains regions identical to other potyvirus coat proteins.

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WHEAT LEAF BLIGHT CAUSED BY PSEUDOMONAS SYRINGAE IN ARGENTINA. E. Teyssandier and D. C. Sands, Cargill, Pergamino, B. A., Argentina and Department of Plant Pathology, Montana State University, Bozeman, MT 59717-0002.

A widespread occurrence of bacterial leaf blight was observed on wheat in Argentina in 1985. Symptoms, initiated during a period of heavy rainfall, progressed from watersoaking at the leaf margins to confluent areas of white necrosis. Pseudomonas syringae was found in high numbers in the symptomatic tissue. These oxidase negative, fluorescent pseudomonads were capable of ice nucleation and induced hypersensitivity in tobacco. Wheat seedlings infiltrated with the bacterium showed limited watersoaking symptoms in 7 days and complete leaf necrosis in 10 days. Inoculated sorghum and corn showed less severe symptoms. In vitro studies showed inhibition of Fusarium moniliforme by these bacteria.

637. Withdrawn

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BURKHOLDERIELLA, AN EARLIER NAME FOR THE PHYTOPATHOGENIC BACTERIAL GENUS CLAVIBACTER. M.J.Thirumalachar, Jeersannidhi Anderson Institute, Walnut Creek, CA 94596.

The amino acid and sugar composition of cell walls used for chemotaxonomy of actinomycetous genera, is now being adopted for classifying phytopathogenic bacteria. The coryneform bacterial species with *L*-4-diaminobutyric acid, fucose and rhamnose in the peptidoglycans of cell walls along with similarity of protein band patterns in polyacrylamide gel electrophoresis was basis for erecting Clavibacter as new genus in 1984. C.michiganense subsp.michiganense was type. New subspecies of C.michiganense, insidiosum, nebraskense, rathayi, sepedonicum, tessellarius and tritici were proposed. Coryneform gram positive bacteria, with often V-shaped cells, inciting wilting of alfalfa was placed in the new genus Burkholderiella by Savulescu in 1947, with B.insidiosa (McCulloch) Savul. as type. The name Clavibacter is pre-empted by Burkholderiella, which becomes valid name with well defined morphological and biochemical characters. The other subspecies formerly placed under C.michiganense, now become subspecies of B.insidiosa.

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SYNONYMY OF PSEUDOMONAS AVENAE MANNS 1905, AND PSEUDOMONAS RUBRILINEANS LEE ET AL 1925. L. E. Claflin and B. A. Ramundo, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Thirteen Pseudomonas rubrilineans isolates and nine P. avenae strains were compared in biochemical, physiological, serological, and host assays. Most strains of P. avenae and P. rubrilineans produced acid from arabinose, fructose, galactose, glucose, glycerol, and sorbitol. Strains of P. avenae did not produce acid from myo-inositol and strains of P. rubrilineans were unable to utilize malonate as a sole carbon source. No apparent differences were observed in their cellular protein bands by SDS-polyacrylamide gel electrophoresis. The P. avenae and P. rubrilineans strains exhibited no differences in dot-immunobinding assays. All isolates except two strains of P. rubrilineans and one strain of P. avenae were pathogenic to corn, sorghum, pearl millet, and sugarcane.

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THE TAXONOMIC POSITION OF XANTHOMONAS CAMPESTRIS PV. HOLCICOLA (ELLIOTT 1930) DYE 1978 AND XANTHOMONAS CAMPESTRIS PV. VASCULORUM (COBB 1893) DYE 1978. M. Qhobela and L. E. Claflin, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Xanthomonas campestris pv. holcicola isolated from sorghum and X. c. pv. vasculorum isolated from maize, sugarcane, royal palm, and tiger grass appeared to be similar pathovars. Biochemical and physiological tests, dot-immunobinding assay, and SDS-polyacrylamide gel electrophoresis of total membrane proteins revealed that strains of X. c. pv. vasculorum from maize and some from sugarcane were indistinguishable from strains of X. c. pv. holcicola. X. c. pv. vasculorum isolates from tiger grass were different from all other strains. X. c. pv. holcicola strains were not pathogenic on sugarcane and none of the X. c. pv. vasculorum strains infected sorghum. Our data indicate that these two pathovars are more closely related to each other than to other pathovars of X. campestris and are separated only by host range.

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PSEUDOMONAS AVENAE, THE CAUSAL AGENT OF BACTERIAL LEAF STRIPE OF PEARL MILLET IN NIGERIA. L. E. Claflin, B. A. Ramundo, J. E. Leach, and I. D. Erinle, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A bacterial disease of pearl millet (Penisetum americanum L.) Leeke was observed in nearly every field surveyed in the Central Plateau, Kaduna, Kano, and Sokoto states of northern Nigeria in 1984. Lesions were usually confined within the leaf veins and ranged in length from several to 25 cm. Watersoaking was generally present at the margins of the lesions. Results of biochemical, physiological, and serological tests showed that the pearl millet pathogen was Pseudomonas avenae. This pathogen produced acid from arabinose, fructose, galactose, glycerol, salicin, and sorbitol. Citrate was utilized as a sole carbon source. Lipase was produced, nitrates were reduced, and the strains were negative for arginine dehydrolase. The tobacco hypersensitivity test was positive. Corn, sorghum, and sugarcane were also susceptible hosts.

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IDENTIFICATION OF THE CAUSAL AGENT OF BACTERIAL LEAF STREAK OF PEARL MILLET (PENNISETUM AMERICANUM (L.) LEEKE). M. Ohobela and L. E. Claflin, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A survey for bacterial diseases of pearl millet was conducted in northern Nigeria during the 1984 growing season. Plants with leaf tissue exhibiting bacterial-like diseases consistently yielded a yellow, mucoid bacterium when plated on yeast-dextrose-calcium carbonate medium. The bacterium was characterized as a pathovar of Xanthomonas campestris from results of biochemical, physiological, and host range tests. Dot-immunobinding assay and SDS-polyacrylamide gel electrophoresis of total membrane proteins revealed that the pearl millet pathogen was different from other pathovars infecting cereal crops, including X. c. pv. graminis, X. c. pv. holcicola, X. c. pv. oryzae, X. c. pv. translucens, and X. c. pv. vasculorum. The pearl millet pathogen was not pathogenic to maize, sorghum, sugarcane, wheat, barley, and oats.

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CHARACTERIZATION OF Tn5-INDUCED PATH$^-$ AND VIR$^-$ MUTANTS OF PSEUDOMONAS SYRINGAE PV. TOMATO. D.A. Cuppels and R.A. Moore, Agriculture Canada, University Sub Post Office, London, Ontario, N6A 5B7, Canada.

Tn5, carried on suicide plasmid vector pGS9, was used to generate Path$^-$ and Vir$^-$ mutants of Pseudomonas syringae pv. tomato DC3000. None of the 5 Path$^-$ mutants elicited disease symptoms on either tomato leaves or fruit. Although all 7 Vir$^-$ mutants caused very weak or atypical symptoms on leaves, 4 produced wild type symptoms on fruit and one strain elicited no fruit symptoms at all. Three of the Vir$^-$Fruit$^+$ mutants were unable to produce the toxin coronatine. Six Vir$^-$ mutants but only one Path$^-$ mutant induced a hypersensitivity reaction on tobacco. Although Vir$^-$ strains were the only mutants to grow on tomato leaves, they grew very slowly. One of the Path$^-$ mutants differed from the wild type in being unable to use Krebs cycle intermediates as sole C sources.

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Differentiation of soft rotting Erwinia species on the basis of cellular fatty acid composition.

H. E. Moline. USDA-ARS, Horticultural Crops Quality Lab, BARC, Beltsville, MD and J. M. Wells, USDA-ARS, Plant Science Lab, ERRC, Philadelphia, PA

Cellular fatty acids of Erwinia carotovora pv. carotovora (Ecc), E. carotovora pv. atroseptica (Eca), E. chrysanthemi (Ech), E. cypripedii (Ecy), and E. rhapontici were analyzed by gas-liquid chromatography with a fused-silica capillary column. Ecc and Eca could be easily distinguished from the remaining 3 soft rotting species, Ech, Ecy, and Erh on the basis of cyclic fatty acid content. Although Ecc and Eca contained only trace levels of cyclic fatty acids the remaining 3 species all contained more than 5%. Ecc and Eca could be differentiated by a significant difference in the content of 18:1 and saturated odd carbon fatty acid chains.

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SURVIVAL OF ERWINIA CAROTOVORA subsp. CAROTOVORA IN BRUISED POTATO TUBERS STORED UNDER COMMERCIAL CONDITIONS. D.A. Galuska, M.J. Klopmeyer, and A. Kelman, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

The relationship between survival of Erwinia carotovora subsp. carotovora (Ecc) in bruises and the soft rot potential of injured tubers was evaluated. Hand-dug potatoes were bruised with a pendulum device, inoculated at bruise sites with 10^4 to 10^8 cfu of a rifampicin-resistant strain of Ecc, and placed in a commercial storage bin. The temperature of the bin was reduced gradually from 12C to a holding temperature of 6C (R.H. 95%). At 2 to 4 wk intervals, tissue from the bruise site (about 4 g) was homogenized and dilution plated. Populations of the Ecc strain increased exponentially to approximately 10^8 to 10^9 cfu/sample in the first 2 to 4 wk of storage, then decreased gradually, but were still detectable for the next 4 mo. Tubers did not decay at bruise sites either in storage or when transferred to a mist chamber where conditions favor soft rot. Soft rot bacteria that survive in the bruise site may be a source of inoculum when tubers are reinjured upon removal from storage.

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CHARACTERIZATION OF A PLASMID OF ERWINIA AMYLOVORA, Ea322. E. M. Steinberger and S. V. Beer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Several strains of E. amylovora harbor two plasmids of ca. 30 kb and ca. 60 kb. Spontaneous loss or curing of the 60 kb plasmid from strain Ea322 did not affect pathogenicity or change the strain's virulence towards apple seedlings or immature pear fruits. But, when a Tn5-labelled derivative of the plasmid (pCPP60.1), was conjugated into strain Ea266, that strain's virulence towards the apple cultivar Novole was eliminated (Norelli, Aldwinckle, Steinberger and Beer; this abstract series). A complete restriction map of pCPP60.1 was constructed using EcoRI, BamHI, SalI, and XbaI. The site of Tn5 insertion and the size of the indigenous plasmid (57 kb) were determined. The mobilization region of pCPP60.1 was cloned in pUC9-Cm and mapped to a 1 kb sequence delineated by two EcoRI sites. Other possible functions of the indigenous plasmid are being investigated with the aid of a complete library of its DNA.

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RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF THE Xanthomonas campestris pv. oryzae GENOME FOR DIFFERENTIATION OF RACES. J. E. Leach, M. L. Rhoads, and F. F. White. Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

Restriction fragment length polymorphism (RFLP) analysis was used to rapidly and reliably identify races of Xanthomonas campestris pv. oryzae (Xco). Total DNA was extracted from isolates representing six Xco races found in the Philippines. Race 5 isolates can be distinguished from other races by an EcoRI fragment pattern containing four distinct bands at approximately 21.0, 11.6, 10.4, and 6.0 kb. To simplify analysis of the pattern, the four differential bands were cloned into pUC8. Probes, constructed from the cloned fragments, are being used in Southern blot analysis of RFLP gels and evaluated for use in race differentiation. Additional restriction endonucleases are being used to develop probes specific for other races.

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EVALUATION OF MACTABLET AS A TOOL FOR CREATING FIELD KEYS AND FOR MEASURING DISEASED LEAF AREA. G. C. Wall and P. L. Wall, Agricultural Experiment Station, University of Guam, Mangilao, GU, 96923.

Severity of bacterial leaf spot of bell pepper, caused by Xanthomonas campestris pv. vesicatoria, and cassava blight (X. campestris pv. manihotis) were determined with the use of a drafting tablet (Summagraphics MacTablet) attached to a Macintosh computer. These were used in conjunction with a drafting program (MacDraft). Diseased bell pepper and cassava leaves were traced 6 times each by 3 individuals, and percent diseased area determined. Accuracy and precision were both dependent on individuals. Geometric figures were also traced. Area readings of these were checked by direct measurement. The drafting tablet and software employed resulted in precise, but inaccurate, readings of certain sizes of figures; only size increments of 5mm were read accurately for rectangles and circles. The smallest area read accurately was that of triangles (3 sq. mm). The drafting tablet was useful in creating field keys of given percent diseased areas. Actual diseased leaves were traced, diseased area determined, and field keys printed to represent given severity levels.

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CHARACTERIZATION OF AGROBACTERIUM TUMEFACIENS STRAINS FROM NATURALLY OCCURRING CHRYSANTHEMUM TUMORS. A.L. Bush and S.G. Pueppke, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211.

Five isolates of A. tumefaciens from chrysanthemum tumors (designated Chry 1, 3, 5, 8, 9) were characterized with regard to biotype, opines, antibiotic resistance, and host range. Tests for 3-ketoglycoside production, growth on 2% NaCl, and growth patterns on Sule's selective medium indicate that the isolates are all biotype I. Preliminary results suggest that the isolates contain nopaline-type plasmids. All five isolates are resistant to nalidixic acid, 3 of 5 are also resistant to vancomycin. Pathogenicity has been demonstrated on kalanchoe and sunflower; further host range tests are in progress. Eighty-four cultivars of chrysanthemum were inoculated with Chry 5 and a standard A. tumefaciens strain B6 (octopine-type). Tumor production was scored four weeks after leaf inoculations - 5% of the cultivars were resistant to both strains, 15% were susceptible to Chry 5 only, and 14% were susceptible to B6 only.

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EFFECT OF NOVEL LYTIC PEPTIDES ON PLANT PATHOGENIC BACTERIA. L.J.C. Destéfano-Beltrán[1], J.M. Jaynes[1]*, and C. Clark[2]. Departments of Biochemistry[1] and Plant Pathology & Crop Physiology[2] Louisiana State University Baton Rouge, LA 70803 *To whom correspondence and inquiries should be addressed.

A number of plant pathogenic bacteria, from many different genera, have been assayed to determine their relative sensi--tivity to lytic peptides produced on a BioSearch peptide synthesizer. All plant pathogenic bacteria tested were sensi--tive and most were lysed over a very narrow range of peptide concentration (about 0.5---1.0 microMolar).A marked synergistic effect was demonstrated between the lytic peptides and lysozyme which increased the activity of the lytic peptides by 2.5-4.0 fold depending upon which plant pathogenic bacteria was tested. All bacteria tested were affected by this synergism. The genes, encoding the lytic peptides and lysozyme, have been inserted into appropriate Agrobacterium plasmid vectors and experiments are underway to determine the efficacy of this novel approach to augment bacterial disease resistance in plants.

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BIOLOGICAL CONTROL OF BACTERIAL BROWN SPOT DISEASE OF BEAN WITH Tn5-INDUCED AVIRULENT MUTANTS OF THE PATHOGEN, S. E. Lindow, D. K. Willis, and N. J. Panopoulos. Dept. of Plant Pathology, Univ. of California, Berkeley, CA 94720

A nonpathogenic strain of Pseudomonas syringae pv. syringae selected following Tn5 mutagenesis produced no visible symptoms when inoculated into bean leaves, exhibited similar growth rates on leaf surfaces, and achieved the same population size on leaves as its parental strain. The population size of mutant NPS3136 inoculated onto greenhouse-grown bean plants increased logarithmically with time for two days to about 10^7 cells/g fr. wt. when incubated in a mist chamber. The population size of parental strain B728a inoculated onto plants treated with NPS3136 48 hr earlier, increased only about 10 fold to about 10^3 cells/g after 72 hr of incubation, but increased to over 10^6 cells/g on plants not pre-treated with NPS3136. The number of brown spot lesions on leaves treated with NPS3136 prior to B728 were reduced 85% compared to control plants not pretreated with the nonpathogenic strain.

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DISTRIBUTION OF XANTHOMONAS CAMPESTRIS PV. PRUNI ON PEACH LEAVES. P. S. Randhawa, Yoder Bros, Inc., P.O. Box 68, Alva, FL 33920; H. S. Sohi, PAU Ludhiana, India and E. L. Civerolo, USDA-ARS, Fruit Laboratory, Beltsville, MD 20705.

Asymptomatic leaves (5th and 8th from shoot apex) of field-grown peaches were detached. Both surfaces of each leaf were pressed for 10 min against a nutrient agar medium, selective for Xanthomonas campestris pv. pruni, in 30 x 15 x 1 cm glass dishes. Leaf shape and location of X. c. pv. pruni colonies were marked on the plate lid and the pattern transferred onto tracing paper of 288 leaves examined, 175 and 200 leaves contained X. c. pv. pruni on upper and lower surfaces, respectively. A mean of 10 cfu per upper surface and 39 cfu per lower surface were recovered. The distal half of each leaf contained 1.2 and 1.5 times more cfu than the proximal half on upper and lower leaf surfaces, respectively. Pathogen occurrence was greater on the 8th leaf. Frequency of pathogen recovery increased as the disease progressed within the tree canopy.

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IRRIGATION WATER AS A SOURCE OF INOCULUM OF ERWINIA CAROTOVORA subsp. CAROTOVORA FOR AERIAL STEM SOFT ROT OF POTATOES. M.R. Cappaert and M.L. Powelson. Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Russet Burbank potatoes from one seedlot were planted at four locations in 1985 and 1986. Prior to planting, seed tubers and field soil were assayed for presence of Erwinia spp. After planting, irrigation water, leaflets and diseased stems were assayed bimonthly for soft rot erwinias. A total of 2681 Erwinia carotovora subsp. carotovora strains were tested serologically against 20 different antisera by Ouchterlony agar double diffusion. In 1985 none of the soil or seed tuber strains were identified serologically. Serogroups IV, V, XVIII, and XXIX were present in irrigation water and were recovered from diseased stems. In 1986, serogroups XXIX, XXXVII and XXXIX were not found on seed or in soil but were present in water, on leaflets, and in diseased stems. One fourth of the strains were identified serologically and of these 15% were common to water, leaves and diseased stems. Contaminated water sources are a potential source of inoculum for plant infection.

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FREQUENCY OF DETECTION OF CLAVIBACTER XYLI SUBSP. XYLI IN SUGARCANE WITH RATOON STUNTING DISEASE BY DIFFERENT DIAGNOSTIC TECHNIQUES. N. A. Harrison and M. J. Davis

Four diagnostic techniques, phase-contrast microscopy (PCM), a fluorescent-antibody staining procedure (FAS), and the enzyme-linked immunosorbent assays, dot-blot (DB) and tissue blot (TB) were used to detect Clavibacter xyli subsp. xyli in sap extracts from stalks of five sugarcane cultivars CP 72-1210, CP 63-588, CP 70-1133, CP 74-2005 and CP 65-357 with ratoon stunting disease. The third and ninth internodes above ground of two stalks from ten plants of each cultivar were examined. The TB, DB and PCM detected 94.7%, 91% and 87.8% respectively, of the samples containing C. x. subsp. xyli as pre-determined by FAS (189 positive of 200 total samples), the most sensitive of the four diagnostic techniques.

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APPLICATION OF A RADIO-IMMUNOASSAY FOR THE DETECTION OF CLAVIBACTER XYLI SUBSP. XYLI IN SUGARCANE SAP. P.W. Reeser[1], M. DeCoste[2], S.J. Kostka[1], K. Hoffman[2] and A.G. Gillaspie Jr.[3]. [1](Crop Genetics Int'l., Hanover, MD 21076), [2](Medical & Scientific Designs, Rockland, MA 02378), [3](USDA, Experiment, GA 30212)

A radio-immunoassay has been developed for detection of Clavibacter xyli subsp. xyli (Cxx). This assay uses a tracer consisting of ^{125}I-labelled, affinity-purified, polyclonal antibodies derived from sheep immunized with a LiCl-extract of Cxx cells. A 50 ul sap sample is incubated with 100 ul of tracer and filtered through a 0.45 u PVDF membrane in a 96-well vacuum manifold. Unbound tracer is rinsed through with buffer. Membrane segments are placed in test tubes and CPM recorded with a gamma counter. Used in a field screen for ratoon stunting disease, the system reliably detects as few as 1 X 10^6 cells of Cxx per ml in sugarcane sap, at a throughput of 500 samples per operator per day. Cross-reactivity occurs with C. xyli subsp. cynodontis, but not with other coryneform species tested.

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SURVIVAL AND GROWTH OF PROTOPLAST-DERIVED POTATO CALLI ON MEDIA CONTAINING CULTURE FILTRATES OF ERWINIA CAROTOVORA PV. CAROTOVORA. R. J. Taylor and G. A. Secor. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Protoplast-derived calli (cv. Crystal) were grown on a callus proliferation medium containing aseptic culture filtrates of Erwinia carotovora pv. carotovora (Ecc). Filtrates were produced from 5, 11, and 18 day (d) old shake cultures of Ecc grown at 24°C in either a minimum salts medium (MinS) or nutrient broth (NB). Growth was inhibited by all six filtrates and calli failed to grow after 28 days exposure to 5, 11 and 18 d MinS filtrates. None of the calli exposed to the 11 d and 18 d MinS filtrates survived after 70 days, while only 5% treated with the 5 d filtrate remained viable. The NB filtrates were less toxic, as 70%, 45% and 85% of the calli exposed to the 5, 11, and 18 d filtrates survived. The pectinase activity of the MinS filtrates was approximately 20X that of the NB filtrates, but mortality and growth inhibition were directly proportional to pectolytic activity only in the NB filtrates.

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EGG YOLK EXTRACT FOR THE GROWTH OF SPIROPLASMAS. C. J. Chang and L. Impson, Department of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

Yolks from unfertilized chicken eggs were suspended in sterile phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}, at pH 7.2 and at a concentration of 20% (w/v). After thorough stirring, the suspension was centrifuged at 15,000Xg for 40 min. The supernatant fluid containing penicillin G at 500 IU/ml was designated EYS1. EYS1 was used to replace horse serum in R-2 medium at 1, 5, 10, 20 and 30% (v/v). Spiroplasma citri, S. floricola, S. melliferum, and S. apis grew in all five EYS1 concentrations. The growth in each of the concentrations was comparable to that in R-2 medium. These data suggested that egg yolk extract can be used as a replacement for serum in R-2 medium for the growth of spiroplasmas. Egg yolk extract may also possibly be used for cultivation of currently noncultivable mycoplasmalike organisms.

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CLONED RIBOPROBE FOR DETECTION OF A MYCOPLASMALIKE ORGANISM (MLO). I.-M. Lee, R.E. Davis, R. Hammond, and B. Kirkpatrick. Department of Botany, University of Maryland, College Park, MD 20742; Microbiology and Plant Pathology Laboratory, Agricultural Research Service, USDA, Beltsville, MD 20705, and Department of Plant Pathology, University of California, Davis CA 95616.

A western X (WX) disease-specific DNA fragment in plasmid pWX3 was subcloned in plasmid vector SP64 and amplified in Escherichia coli strain JM83. High specific activity (^{32}P) single-stranded RNA probe was synthesized in vitro using SP6 RNA polymerase and the linearized plasmid DNA template containing the WX-specific DNA fragment. The labeled probe hybridized with nucleic acid extracted from WX-infected plants of periwinkle (Catharanthus roseus) but not with nucleic acid from healthy plants. Cloned riboprobes offer a sensitive and specific means for detection of MLOs in infected host tissues.

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CLONING OF SPIROPLASMAVIRUS SVTS2 REPLICATIVE FORM. S. L. McCammon, E. L. Dally, R. E. Davis and R. L. Frank, Agricultural Research Service, USDA, Beltsville, MD 20705

A 6.5 kbp circular, double-stranded replicative form (RF) is formed after infection of Spiroplasma citri indicator strain M200H with spiroplasmavirus SVTS2. The RF of SVTS2 was cloned into the cloning vector pUC19 and then amplified in Escherichia coli strain JM109. The restriction maps of SVTS2 RF purified from infected cells and of cloned RF were similar. However, whereas the site for Sau 3A1 was present in both uncloned and cloned RF, the site for its isoschizomer Mbo I was absent in the cloned RF. Normally, in the presence of 43% polyethylene glycol, SVTS2 RF transfects S. citri at efficiencies of 3.3 X 10^{-7} transfectants per DNA molecule in circular form and 4.0 X 10^{-5} transfectants per DNA molecule when linearized with Eco RI. However, cloned SVTS2 RF DNA did not transfect S. citri whether linearized with Eco RI; recircularized; or used as a pUC19::SVTS2 RF cointegrate. The inability of cloned SVTS2 RF to transfect S. citri possibly may be explained by a change in the methylation of nucleotides.

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DNA PROBE FOR DETECTION OF THE PIERCE'S DISEASE BACTERIUM AND OTHER XYLEM-LIMITED BACTERIA. L.G. Jimenez A. and M.J. Davis. University of Florida, IFAS, Ft. Lauderdale, FL. 33314.

Using a recombinant plasmid rpPD31, comprised of the E. coli pUC8 plasmid and a DNA segment of approx. 1 kb of chromosomal DNA from the VT-1 strain of the Pierce's disease (PD) bacterium, a DNA probe for PD and almond leaf scorch (ALS) bacteria was developed. rpPD31 was selected following colony hybridizations of a clone bank of VT-1 DNA using ^{32}P-labelled total DNA from ALS, periwinkle wilt (PW), elm leaf scorch (ELS) and ragweed (RG) bacteria. When rpPD31 was biotinylated, approx. 9 pg of target DNA, representing about 10^3 bacteria, immobilized on nitrocellulose filters was detected using alkaline phosphatase-labelled avidin. This probe hybridized with DNA of strains of (VT-1) PD, (AC-1) ALS and (ESG-1) ELS but not (PW-1) PW and (MiIV) RG DNA.

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DNA PROBES FOR DETECTING THE MAIZE-BUSHY-STUNT MYCOPLASMALIKE ORGANISM (MBS-MLO). M. J. Davis, J. H. Tsai, R. L. Cox, L. L. McDaniel, and N. A. Harrison. University of Florida, IFAS, Ft. Lauderdale Research and Education Center, Ft. Lauderdale, FL 33314.

The MBS-MLO was extracted from Dalbulus maidis (DeLong and Walcott) and partially purified using both differential and discontinuous Percoll density gradient centrifugation. DNA extracted from the MLO preparation was partially digested with Sau 3A, and DNA fragments were ligated into BamH I-cut pUC 8. Colonies of E. coli strain TB1 transformed with the recombinant plasmids were identified on X-Gal agar medium containing ampicillin and screened for MLO-DNA by colony hybridization using ^{32}P-labeled leafhopper-extract MLO DNA as a probe. ^{32}P-labeled DNA probes derived from selected clones hybridized with DNA from MBS-diseased corn and infectious leafhoppers but not from healthy corn, noninfectious leafhoppers, and Spiroplasma kunkelii.

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A RAPID ELISA FOR DETECTION OF FASTIDIOUS XYLEM-LIMITED BACTERIA IN TREES. J. D. Lei and J. M. Wells. Agdia Inc. 1901 Cedar St., Mishawaka, IN 46545 and USDA-ARS, Rutgers Univ., New Brunswick, NJ 08903.

Pierce's disease bacterium, grown on BCYE medium and fixed with glutaraldehyde, was injected into rabbits to produce polyclonal antiserum. A protocol for double-antibody sandwich ELISA, which required about 4.5 hr to complete, was formulated to detect fastidious xylem-limited bacteria (FXB) in fruit and ornamental trees. The assay was tested with major groups of bacteria and was found to be specific to FXB's. FXB's can be detected in petioles and small branches of trees affected by oak leaf scald disease, elm leaf scorch disease, and Pierce's disease. In trees affected by phony peach disease, highest concentrations of FXB's were detected in roots. The assay may be used as a general test for FXB's.

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INHIBITION OF MELANIN BIOSYNTHETIC REACTIONS BY ANTIBLAST COMPOUNDS IN PYRICULARIA ORYZAE. M. H. Wheeler and G. A. Greenblatt, USDA, ARS, SCRL, P. O. Drawer JF, and Texas A&M University, College Station, TX 77841.

HPLC was used to measure products made from melanin precursors by cell-free extracts. Nine compounds, known to prevent appressorial penetration of rice plants and melanin biosynthesis, inhibited enzymic reactions that reduce 1,3,6,8-tetrahydroxynaphthalene and 1,3,8-trihydroxynaphthalene to scytalone and vermelone, respectively; they did not inhibit enzymic reactions that dehydrate scytalone and vermelone. The compounds had the same order of effect in inhibiting reductase reactions as previously reported for inhibiting melanin biosynthesis and preventing appressorial penetration of leaf surfaces. Three of the four strongest reductase inhibitors (tricyclazole, fthalide and pyroquilon) are used commercially to prevent rice blast disease. The in vitro and earlier in vivo results suggest that the effect of melanin inhibitors on reductase activity can be used to predict their efficacy for preventing rice blast disease.

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PESTICIDE REGISTRATIONS FOR "MINOR" USES. J.E. Elson, W.L. Biehn, IR-4 Project, Cook College, Rugters University, New Brunswick, NJ, 08903.

Interregional Research Project No. 4 (IR-4) is a national coordinating program to provide requisite data to register pesticides for minor or special uses. To date, IR-4 has received 797 pesticide/commodity requests for disease or nematode control in food crops. IR-4 has 65 fungicide/ nematicide food-use projects underway and an additional 158 are eligible for research. Currently, 47 petitions for fungicides and nematicides are under review at manufacturers or at the Environmental Protection Agency (EPA). Since 1975, 47 IR-4 researched fungicide/nematicide uses were cleared by EPA. The IR-4 Ornamentals Program, initiated in 1977, has received over 3,500 different requests for disease and nematode control on ornamentals. IR-4 has contributed data in support of 849 ornamental registrations involving 19 fungicide products and two nematicide products. Presently there are 256 ornamental requests for disease and nematode control that are eligible for research.

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COMPARISON OF MANCOZEB AND PROPICONAZOL FUNGICIDES FOR THE MANAGEMENT OF FUNGAL BROWN SPOT OF WILD RICE. J. A. Percich and C. M. Huot, Dept. Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

Propiconazol and mancozeb when applied at 0.56 and 1.12 kg a.i./ha, respectively, resulted in lower yields and higher disease severity ratings in inoculated than in uninoculated but fungicide-treated plants. Inoculated plants receiving one application of propiconazol followed by two of mancozeb (IPM) at boot and heading stages, respectively, resulted in the greatest yield of all inoculated plant treatments. The yield of IPM plants did not differ significantly from plants that were not inoculated but which received five applications of mancozeb (CMM). The IPM treated plants averaged yields 24% greater than those receiving one application of propiconazol at either boot (IPI) or heading (IMP) stages. Both propiconazol and mancozeb individually or sequentially resulted in significant yield increases of 27 to 64% when compared with the inoculated but untreated control.

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RESPONSE OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA TO FUNGICIDES: AN IN-VITRO STUDY. J. A. Freedman, J. P. Snow, and G. T. Berggren, Jr. Dept. of Plant Path. and Crop Phys., LSU Agric. Center, Baton Rouge, LA 70803.

Two isolates of the soybean stem canker pathogen Diaporthe phaseolorum var. caulivora were subjected to treatments of fungicides from 2 to 1000 ppm a.i. in potato dextrose agar. Ascospore germination, conidia germination and colony area were inhibited in varying degrees by the compounds. Benomyl and thiabendazole were characterized as fungicidal. Isolates responded differentially (fungistatic/fungicidal) to thiophanate-methyl, but the remainder of the fungicides were fungistatic. Linear regression analysis was used to calculate EC50 values, which ranged from less than 2 ppm for several fungicides to 250 ppm for thiophanate-methyl in reducing conidial germination of one isolate. Variation in responses of isolates to these fungicides suggests an explanation for the variation in results from fungicidal field trials for stem canker.

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M.E. Sherman, J. J. Bates, and S. P. Heaney
Hexaconazole: A New Fungicide for Disease Control in Turf
ICI, Americas Inc., Biological Research Center, Goldsboro, NC 27530 USA; ICI PPD, Jealott's Hill, Bracknell, Berkshire, UK

Hexaconazole is a new systemic triazole fungicide with eradicant and protectant activity against a wide range of turf pathogens. In evaluative trials in 1985 and 1986, hexaconazole demonstrated activity against dollarspot, brown patch, anthracnose, rust and red thread. Trials are in progress to collect data on other turf diseases. Hexaconazole used alone or in tank mixes with compounds targetted for phycomycete control will offer a valuable tool for turf disease control.

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AGGRESSIVENESS AND COMPETITIVENESS OF BENZIMIDAZOLE-TOLERANT MICROSCLEROTIAL STRAINS OF VERTICILLIUM DAHLIAE. J. S. Mayer and L. R. Schreiber. USDA-ARS-Nursery Crops Research Laboratory, 359 Main Rd., Delaware, OH 43015

Two benzimidazole-sensitive (S) isolates, H & O, of Verticillium dahliae from sugar maple were screened for tolerance at 5 ug/ml Lignasan BLP. Tolerant (T) strains, H1 and O1 which grew above 100 ug/ml were selected. T & S strains were inoculated singly or in combination (H+H1, or O+O1) into greenhouse-grown sugar maples 45-60 cm in height. Reisolations were made after 18 wk. T & S strains were equally aggressive as measured by foliar symptoms, number of infected plants, change in plant height, number of plants with, and extent of vascular discoloration, and stem colonization. T strains were isolated from stem and petiole tissue inoculated alone or in combination with S strains, indicating they were competitive as well as being equally aggressive in vivo.

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RETENTION OF PANICLES AND PETIOLES OF PISTACHIO INFECTED BY BOTRYOSPHAERIA DOTHIDEA. T. J. Michailides and J. M. Ogawa, Dept. of Plant Pathology, Univ. of California, Davis, 95616.

Rachises, fruits, and petioles of pistachio (Pistacia vera) naturally infected by Botryosphaeria dothidea were retained on trees longer than healthy ones. One year after infection, 2X more infected rachises and 24X more infected petioles were hanging on the trees than healthy ones and 75-80% of them had mature pycnidia. Similarly, on inoculated shoots, 15X, 20X, and 25X more infected rachises, petioles, and fruits, respectively, were retained on trees longer than uninoculated ones. In a commercial orchard, lowering the trajectory angle of sprinklers from 23° to 12° significantly reduced infected fruits and numbers of retained rachises and petioles, and thus inoculum. In addition, one fungicide application made during bloom reduced numbers of retained rachises and leaf petioles, in an orchard irrigated with sprinklers of high trajectory angle (23°).

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INVESTIGATION FOR GENETIC EXCHANGE BETWEEN AGROBACTERIUM RADIOBACTER STRAIN K84 AND A. TUMEFACIENS ON FIELD GROWN PLANTS. M. L. Canfield, H. Bouzar and L. W. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Strains of Agrobacterium were isolated from roots and tumors of field grown apple and cherry seedlings pretreated with A. radiobacter strain K84. These strains were evaluated with respect to pathogenicity and agrocin 84 production. Strains expressing both phenotypes were expected if genetic exchange between K84 and A. tumefaciens had occurred. Such an exchange could lead to a loss of biological control of crown gall by K84. Of 528 Agrobacterium strains tested, 162 were pathogenic on tomato plants. None of these pathogenic strains produced an antibiotic when screened in vitro against A. tumefaciens strains that are sensitive to K84. Of the remaining 366 nonpathogenic strains, 66 produced antibiotics in vitro and 19 of these antibiotic producing strains reacted identically to K84 in immunodiffusion tests against an antiserum specific to K84. Thus, no conjoint expression of the two phenotypes was found.

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A BASIDIOMYCETE WITH POTENTIAL FOR BIOCONTROL OF PYRENOPHORA TRITICI-REPENTIS IN WHEAT STRAW RESIDUE. W. F. Pfender, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

A fast-growing basidiomycete, antagonistic to Pyrenophora tritici-repentis (causal agent of wheat tan spot), was isolated from conservation-tillage wheat residue. Its ability to inhibit Pyrenophora ascocarp formation was tested in the laboratory, using nonsterile wheat straw precolonized (during normal parasitic growth) by Pyrenophora. The basidiomycete reduced Pyrenophora ascocarp production to approximately 15% of that in the check (nonchallenged) treatment. This reduction occurred over a range of three temperatures (4, 14, 26 C), and under two different moisture treatments. An isolate of Trichoderma, included as an additional check at 26 C, did not significantly reduce ascocarp production. The basidiomycete produces chitinase, and appears to be related to Laetisaria and Limonomyces.

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MORPHOLOGICAL AND CYTOLOGICAL EFFECTS ON CERATOCYSTIS ULMI OF AN ANTIFUNGAL ANTIBIOTIC PRODUCED BY BACILLUS SUBTILIS. C. R. Krause[1], J. M. Ichida[1], L. R. Schreiber[1], and G. F. Gregory[2](Retired), [1]USDA-ARS, Nursery Crops Research Laboratory and [2]U. S. Forest Service, Delaware, OH 43015

Investigations were conducted to determine the morphological and cytological effects on C. ulmi, the causal agent of Dutch elm disease, of an antibiotic (BS1) produced by an antagonistic isolate of B. subtilis. Partially purified BS1 was incorporated into potato dextrose broth then inoculated with germinated C. ulmi spores, incubated for 48 hr and prepared for electron microscopy. Fungal preparations exposed to BS1 exhibited twisted hyphae with bulging tips, irregular cell wall thickenings of varying electron densities and indistinct membranes and organelles. Untreated C. ulmi exhibited normal hyphal development including smooth cell walls of uniform thickness, straight hyphal growth and a regular configuration of organelles and osmophilic bodies.

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HOST AND ENVIRONMENTAL FACTORS USED TO MODEL JOHNSONGRASS CONTROL WITH THE BIOHERBICIDES COLLETOTRICHUM GRAMINICOLA AND GLOEOCERCOSPORA SORGHI. James K. Mitchell, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville 72701.

Inoculation studies were conducted in controlled environments with field isolates of C. graminicola and G. sorghi isolated from johnsongrass. G. sorghi was more virulent over a wider range of host ages, temperatures, and dew periods than C. graminicola. G. sorghi infected all Sorghum spp. tested, whereas the cultivars IS2057 and Wiley of S. bicolor were resistant to C. graminicola. A two-year survey of selected southern U.S. States for climatological conditions conducive for the control of johnsongrass suggested that only G. sorghi could be utilized as a biological herbicide to kill johnsongrass. A mathematical simulation model representing controlled environmental data for these two pathogens was highly correlated with field results in 1986. This model can be used to forecast the amount of damage which would occur to johnsongrass following innundative inoculations with these two fungi.

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COMPARISON OF *ENDOTHIA PARASITICA* ISOLATES FROM SELECTED SITES IN MICHIGAN AND WEST VIRGINIA. T. M. Likins and W. L. MacDonald, Dept. of Plant Pathology, West Virginia University, Morgantown, WV 26506-6057.

Isolates of *Endothia parasitica* containing double-stranded (ds) RNA have been associated with cankered American chestnut trees recovering from chestnut blight at some sites in Michigan; in West Virginia, this relationship has not been observed. To determine if dsRNA is associated consistently with cankers, 20 infected American chestnut stems were collected from each of 3 sites in both states and randomly sampled at 2 positions. Isolates were grown in liquid culture for 10 days and harvested, and dsRNA content was determined by agarose gel electrophoresis. In Michigan, dsRNA was extracted from all cankers at 1 recovering site, frequently from a second recovering site, and never from a nonrecovering third site. In West Virginia, dsRNA was associated frequently with *E. parasitica* from all sites. Electrophoretic migration rates were comparable for all Michigan and West Virginia isolates with similar banding patterns.

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EFFECTS OF BACILLUS SUBTILIS ON IN VITRO GERMINATION AND HYPHAL ELONGATION OF MONILINIA FRUCTICOLA. B. E. Hazen and J. R. Aist. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Germination of conidia and elongation of hyphae from germ tubes of Monilinia fructicola (Mf) on potato dextrose gelrite-coated slides were measured 2, 4, 8, and 24 h after treatment with 20 µl of 10^6-10^{10} cfu of Bacillus subtilis (Bs)/ml of culture medium or with the corresponding culture filtrate. Nearly all untreated conidia germinated within 2 h. Spores were lysed and all germination was inhibited by 10^7-10^{10} cfu Bs/ml. Viability of conidia (fluorescein diacetate method) was reduced by treatments greater than 10^7 cfu Bs/ml or the corresponding culture filtrates. Germination was delayed and only partially inhibited by 10^6 cfu Bs/ml and filtrates from cultures containing 10^7 cfu/ml or less; elongation was slower than in controls. These results help explain previous cytological observations of inoculated peach fruit.

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A COMPUTER SIMULATION MODEL TO PREDICT THE DISPERSAL OF BIOCONTROL FUNGI IN SOIL. J.P. Stack[1], G.R. Knudsen[2], & D.O. Koch[1], [1]Texas Agr. Expt. Sta., Texas A&M Univ., Coll. Sta., TX, 77843 and [2]Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, Corvallis, OR, 97333.

A computer model was developed to simulate dispersal in soil of two biocontrol fungi (Gliocladium roseum, Theilavia terricola). Percentage of carrier granules from which growth occurred, number of hyphae per carrier granule, hyphal length and branching, and hyphal contact with target propagules (sclerotia of Phymatotrichum omnivorum or Aspergillus flavus) were determined as functions of soil temperature and moisture. Appropriate frequency distributions were determined for each parameter. During simulations, values were selected randomly using Monte Carlo routines. The simulator outputs summary statistics and 3-D graphics which are compared to digitized maps of hyphal growth patterns in situ. Following validation, the model will be used to investigate the combined effects of biocontrol agent/carrier density and distribution, target propagule density and distribution, and environment on the probability of contact with the target propagule. The dispersal model will be incorporated into a larger model simulating the epidemiology of mycoparasitism.

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IMPROVED BIOCONTROL STRAINS OF TRICHODERMA HARZIANUM DEVELOPED BY PROTOPLAST FUSION. T. E. Stasz and G. E. Harman, Cornell Univ., New York State Agric. Exp. Station, Geneva, NY 14456

Protoplast fusion was accomplished between auxotrophic mutants of T. harzianum strains T12 and T95, and fusion progeny were isolated by forced nutritional complementation. Progeny varied in colony appearance, biological characteristics, and isozyme phenotype at four marker loci. Several progeny were fully prototrophic and grew more rapidly than the parental auxotrophic mutants and wild-type strains. Some of these progeny were extremely effective biocontrol agents. When applied in a solid matrix priming system, they provided a higher level of biocontrol against pre- and postemergence damping-off of cucumber caused by Pythium ultimum than the parental strains, and gave much higher levels of protection than that provided by thiram. Moreover, some strains were more highly rhizosphere competent than T95, the rhizosphere competent parent, and these provided protection against root rot of seedlings. These are the first highly effective strains developed by protoplast fusion.

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BIOLOGICAL SEED TREATMENT FOR CORN ROOT DISEASE CONTROL USING PARASITES AND SAPROPHYTES. K. K. Sabet, Thor Kommedahl, and P. M. Burnes, University of Minnesota, St. Paul, MN 55108.

A 4-day-old culture of Pseudomonas lindbergii (ATCC No. 31099) applied to corn kernels (Zea mays) in a methyl cellulose carrier increased root weight of 4-wk-old seedlings by 44% over untreated kernels and was more effective than Bacillus subtilis (26%) or a combination of both species (12%) in tests made in the greenhouse using field soil amended with Fusarium graminearum inoculum. In the field without added inoculum to soil, B. subtilis and Chaetomium globosum applied without a carrier to kernels resulted in 35-36% emerged plants at a time when no treatment gave a 10% stand; seed treatments increased ear yield 21% over control (avg 5 replicates; 30 plants/replicate) from C. globosum and 10% from B. subtilis. Fusarium equiseti and F. oxysporum on kernels gave 14-15%, and captan 13%, increases in yield over control. Soil crusting from 6 consecutive days of rain after planting favored biocontrol by C. globosum especially, by hastening seed germination.

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MOLECULAR CLONING OF GENES ENCODING VIRULENCE IN PSEUDOMONAS SOLANACEARUM. P. Xu, S. A. Leong and L. Sequeira, Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The suicide plasmid, pSUP2021, was used to introduce Tn5 into the genome of Pseudomonas solanacearum wild-type strain, K60. About 6,000 Tn5-carrying isolates were screened for loss of virulence on tobacco and eggplant. Tn5-containing EcoR1 fragments from eight avirulent mutants were cloned into pBR322 and pUC12. One of the pUC12 derivatives, pKD810, containing a 13.6 Kb fragment from mutant KD810, was transformed into K60. $Kan^{R}Ap^{S}$ derivatives were scored for pathogenicity on tobacco. All isolates were avirulent and were shown to carry the 13.6 Kb EcoR1 fragment in place of the wild-type fragment. With pKD810 as a probe, cosmids carrying the corresponding wild-type virulence gene were isolated from a genomic library of K60 prepared in pLAFR3. The homologous cosmid, pL810, when introduced into KD810 by transformation, restored virulence and normal growth of this mutant in tobacco.

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INHERITANCE OF A SEVERE BIPOLARIS LEAFSPOT ON PEARL MILLET. H. D. Wells and W. A. Hanna, USDA-ARS, Forage and Turf Research, University of Georgia, Coastal Plain Experiment Station, P. O. Box 748, Tifton, GA 31793

In 1983 a previously unrecognized severe Bipolaris leaf spot symptom was observed at Tifton, GA on pearl millet in progenies from a cross involving Pennisetum americanum inbred line 'Tift 23DB' X P. americanum subsp. monodii (a wild type). This cross also yielded near immunity to both rust caused by Puccinia substriata var. indica and Piricularia leaf spot caused by Piricularia grisea; therefore, it was imperative that the inheritance of the severe leaf spot be determined. The pathogen was Bipolaris setariae which normally causes only minor leaf spotting on pearl millet. Evaluation of F_2, F_3, F_4 and backcross data indicate that three or more loci are involved in the inheritance of resistance to this severe Bipolaris leaf spot. Resistance has been effectively stabilized in our breeding program.

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LINKAGE STUDIES IN BRASSICA OLERACEA OF MORPHOLOGIC AND ISOZYMIC MARKERS AND DISEASE RESISTANCE. A. W. May, P. H. Williams and P. W. Bosland. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Linkage relationships among 17 morphological markers, several isozymes and genes conditioning resistant interaction-phenotypes to Fusarium oxysporum f. sp. conglutinans, races 1 and 2, to Peronospora parasitica and to Albugo candida pathotypes are being studied using the rapid-cycling stock of Brassica oleracea, CrGC-3. Most of the genes under study are represented in one of the seven linkage groups identified by Sampson in broccoli. The inclusion of several new morphologic markers generated from ethylmethanesulphonate-treated seeds and a number of isozymes will produce a linkage map of value for detailed genetic analysis of B. oleracea. Stocks carrying each of the mapped genes will be available in the rapid-cycling CrGC-3 background through the Crucifer Genetics Cooperative.

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Chlorate-resistant, nitrate-utilizing (*crn*) mutants in *Fusarium moniliforme* (*Gibberella fujikuroi*). C.J.R. Klittich and J.F. Leslie. Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506.

Chlorate-resistant mutants are generated spontaneously by strains of *Fusarium moniliforme* cultured on minimal medium containing 1.5% potassium chlorate. Most of these mutants are unable to utilize nitrate (*nit* mutants), but some chlorate resistant mutants can utilize nitrate (*crn* mutants). Genetic analysis showed that *crn* mutations occur in at least three unlinked genes, designated *crn1*, *crn2*, and *crn3*. One of these loci (*crn1*) is closely linked to, and may be allelic with, *nit-3*, a regulatory locus that governs the nitrate reduction pathway. Strains carrying mutations at any of the *crn* loci have lower nitrate reductase levels than do their wild-type progenitors.

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CAN RUST UREDOSPORES ACT AS SPERMATIA? J. W. McCain and J.V. Groth, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Fresh uredospores of bean rust (Uromyces appendiculatus) were applied to pycnia, resulting in aecia in a minority of cases. The uredospores were from a nontelial isolate that has many differences from the telia-producing pycnial isolate in isozyme band pattern and virulence spectrum on our set of bean differentials. Three putative progeny exhibited isozyme bands for six enzymes that could not have been obtained through a self of the receptor parent. Virulence patterns of the above progeny on five differential hosts were intermediate. The fourth isolate was isozymically similar to the receptor parent, and was probably a self. This technique can be used to obtain progeny of crosses involving the genetically interesting non- or reluctant-telia-forming isolates of bean rust that are common in nature.

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THE USE OF RAPID CYCLING BRASSICAS AND RADISH IN TEACHING PRINCIPLES OF PLANT PATHOLOGY AND PLANT BIOLOGY. P. H. Williams, Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Stocks selected from the petite rapid cycling populations of the interrelated species Brassica nigra (bb), B. campestris (aa), B. oleracea (cc), B. juncea (aabb), B. napus (aacc), B. carinata (bbcc) and Raphanus sativus (rr) have been developed in conjunction with a plant-growing system for use in classrooms. A number of model exercises have been designed to provide students with "hands-on" experience with the plants and the pathogens TuMV, CaMV, Xanthomonas, Pseudomonas, Plasmodiophora, Albugo, Peronospora, Leptosphaeria and Fusarium. Students are able to sow seed, grow, inoculate, evaluate, select, pollinate, harvest seed and resow within 40 days. The material is ideal for hypothesis testing and independent student exploration as well as for class experiments and population studies. Plants may be grown at densities of up to 2000 per M^2. Materials are available through the Crucifer Genetics Cooperative.

688. Withdrawn

689

PERSISTENCE IN SOIL AND CONTROL OF COMMON BUNT (TILLETIA CARIES) OF WHEAT. Ervin Williams, Jr., Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-0285.

Common bunt infestations in Oklahoma usually result from seedborne spores. However, southwest Oklahoma farmers reported inadequate control by seed treatment with carboxin-thiram or TCMTB. Thus, duplicate tests were conducted in a heavily infested area and a bunt free area. Eleven percent and 27.8% infection were in uninoculated and inoculated checks, respectively, in the field with a history of bunt. No infections occurred in the uninoculated check at the bunt free location. Carboxin-PCNB17-17F, PCNB23.7L, maneb-TBZ50-2D and carboxin-thiram17-17F significantly reduced bunt in both areas. Carboxin-captan20-20D and TCMTB30EC resulted in significant reductions only in soilborne free plot. No treatment provided total control where soilborne spores were evident. Results suggest labeled rates of available fungicides may not provide sufficient bunt protection with heavy soilborne inoculum.

690

RESPONSE OF A632 TYPE MAIZE INBRED LINES IN HYBRID COMBINATION TO LEAF FRECKLES AND WILT CAUSED BY CLAVIBACTER MICHIGANENSE SUSP. NEBRASKENSE. M. L. Carson and Z. W. Wicks, III, Plant Science Department, South Dakota State University, Brookings, SD 57007.

Forty-two A632-type inbred lines of maize derived from third and fourth backcross generations in a modified backcross program (to incorporate resistance to Erwinia stewartii) were evaluated for their response (in hybrid combination with A619) to Leaf Freckles and Wilt (LFW) in field trials. The 42 hybrids and the check hybrid A632 x A619 were tested for two years using a split plot arrangement of inoculation treatments (inoculated vs. uninoculated) and hybrids (whole plots). Hybrids exhibited a wide range of reactions to LFW and yield losses ranged from 0 - 40%. A significant correlation between LFW ratings and yield losses was found (r = 0.64). However, examination of outliers from the regression revealed that some hybrids had consistently high LFW ratings yet sustained no significant yield loss, indicating tolerance to LFW.

691

EVIDENCE FOR A NEW MITE-ASSOCIATED WHEAT DISEASE IN EASTERN NORTH DAKOTA. Michael C. Edwards and Marcia McMullen, USDA-ARS Cereal Crops Research Unit and Dept. of Plant Pathology, North Dakota State University, Fargo, North Dakota 58105.

A wheat disease of unknown etiology has recently been discovered in eastern North Dakota. The disease was first observed in 1985 in conjunction with an outbreak of wheat streak mosaic virus (WSMV). Twenty fields in 4 counties were examined and sampled for the presence of WSMV. While WSMV was found to be widespread in these fields, it's presence was not confirmed in many of the samples. ELISA tests indicated symptomatic plants transplanted from the field to the greenhouse were not infected with WSMV. Repeated attempts at mechanical transmission to winter wheat, spring wheat, and barley failed. Since the wheat curl mite (Eriophyes tulipae) was present in affected fields,mite-transmission of the disease agent was attempted. Chlorotic spotting developed on plants fed upon by mites which had previously fed on symptomatic plants,but not on plants fed upon by mites which had previously fed upon healthy plants. EM examination of diseased plants has not yet revealed evidence of any known pathogen.

692

LIGHT MICROSCOPY OF GIBBERELLA EAR ROT IN MAIZE (ZEA MAYS L.). Michael G. Smart, USDA/ARS, Northern Regional Research Center, 1815 North University Street, Peoria, IL 61604.

Gibberella zeae (Schw.) Petch. (anamorph: Fusarium graminearum Schwabe) causes one of the more destructive ear rots of maize. Ears of field-grown dent maize were inoculated with F. graminearum 10 days after silk emergence, harvested at intervals, and examined by light microscopy. Hyphae grew into the rachilla ("pedicel") of the wounded kernel and onto adjacent kernels. The pericarp and testa of these kernels were penetrated, leading to endosperm liquefaction. Further hyphal growth occurred via rachillas, which were penetrated by hyphae on floral bracts (glumes) or from the rachis (cob). Major host responses were false plasmolysis ahead of the hyphae, accelerated black layer formation, tannin accumulation in the rachilla and rachis, and tyloses in the xylem. These results outline the little-known early events in the progression of Gibberella ear rot.

693

SUSCEPTIBILITY AND YIELD LOSSES OF SMALL GRAINS TO TAKE-ALL. C. S. Rothrock, Dept. of Plant Pathology, University of Georgia, Georgia Station, Experiment, GA 30212.

The relative susceptibility and yield loss of small grains to take-all were examined in the field in 1986. Low (1.8 g/m^2), high (3.6 g/m^2), and no inoculum were the main treatments. The number of cultivars used in subplots were wheat (4), oats (2), rye (2), barley (2), and triticale (5). All cultivars were susceptible to the disease except the two oat cultivars. Within each small grain no differences in disease severity were found, except for barley. The order of susceptibility of small grains in decreasing order were wheat, triticale, barley, rye, and oats. Take-all reduced yields of all cultivars in the infested plots compared to the noninfested plots except for the resistant oat cultivars, the two rye cultivars, and one barley cultivar. Yields were reduced 39% and 74% for wheat, 20% and 52% for triticale, and 22% and 35% for the one barley cultivar for low and high inoculum levels, respectively.

694

EFFECT OF FREE MOISTURE ON SOYBEAN STEM CANKER DEVELOPMENT. J.P. Damicone, G.T. Berggren, and J.P. Snow, Dept. Plant Path. & Crop Physiol., LA Agric. Exp. Sta., LSU Agric. Ctr., Baton Rouge, LA 70803.

The effects of the type and duration of free moisture on stem canker development in J77-339 soybean plants inoculated at the V_5 stage with ascospores and conidia (10^6/ml) of the causal fungus, Diaporthe phaseolorum var. caulivora, were studied in greenhouse mist chambers. Stem canker incidence, canker number/plant, plant death, and total canker length (R^2=0.81 to 0.95) increased linearly with wetting durations from 24 to 144 hr after inoculation. The latent period length decreased linearly (R^2=0.94) with wetting duration following inoculation. Addition of 8 hr/day interrupted wetting periods significantly increased disease incidence, canker number and length, and reduced the latent period compared with dry treatment following 0, 48, and 96, but not 144 hr of continuous wetting after inoculation. Stem canker development was related more to the total moisture duration than to the type (continuous vs. interrupted).

695

THE FREQUENCY OF STEM CANKER RESISTANCE IN MATURITY GROUP V THROUGH VIII SOYBEANS. D. V. Phillips and P. L. Raymer, Georgia Experiment Station, Experiment, GA 30212.

A total of 532 soybean lines from 15 private and 10 public breeding programs were tested for reaction to southern stem canker. Three replications of the 163 maturity group V lines, 200 group VI lines, 124 group VII lines and 45 group VIII lines were planted at each of two naturally infested locations. All lines were visually rated at the late R6 growth stage and again just before maturity for percent of diseased plants. A total of 33% of the lines tested were highly resistant (fewer than 5% dead plants) and 20% of the lines were highly susceptible (more than 20% dead plants). Maturity group V had lowest percentage of highly resistant (29%) and highly susceptible (12%) lines whereas group VI had the highest percentage of highly resistant (36%) and highly susceptible (27%) lines. Additional testing should be effective in eliminating highly susceptible lines and identifying highly resistant lines.

696

IN VITRO SCREENING OF DRY BEAN CALLI TO IDENTIFY RESISTANCE TO SCLEROTINIA SCLEROTIORUM. C. L. Hartman, D. A. Albaugh, G. A. Secor, and J. R. Venette. Dept. of Plant Path., North Dakota State University, Fargo, ND 58105.

Bean (Phaseolus vulgaris) lines demonstrating relatively high levels of resistance to white mold (Sclerotinia sclerotiorum) were identified by limited term inoculation tests in growth chambers.* Calli from selected lines were tested on tissue culture media containing oxalic acid (putative toxin) or culture filtrates from each of three fungal isolates. Calli of resistant lines PI287536 and PI417603 (Phaseolus coccineus) grew normally on media containing either culture filtrate or oxalic acid. Four other lines showing resistance, PI189567, PI226865, PI204717, and PI180753, were tested against oxalic acid medium and only PI189567 and PI226865 displayed some tolerance. No calli from the susceptible varieties Kentwood or Seafarer grew on media containing either fungal filtrate or oxalic acid.

*Hunter, J. E. et al. 1982. Plant Dis. 66:320-322.

697

EFFECT OF SOIL TEMPERATURE AND SOIL MOISTURE ON SOYBEAN ROOT ROT. C. M-S. Beaupré and M. W. Ferguson, South Dakota State University, Plant Science Department, Brookings, SD 57007.

Effect of soil temperature (ST) and soil moisture (SM) on soybean root rot was examined in greenhouse temperature tanks. Corsoy 79 seedlings were grown at five ST's (10, 15, 20, 25 and 30°C), and four SM levels (Ψ_m = -1.36, -0.59, -0.11, -0.03 bars) within each ST, and inoculated in separate experiments with either Fusarium oxysporum, Pythium sp., or both pathogens combined. Effects of fungi on plant height, percent discoloration of primary and secondary roots, and stem and root dry weights were compared to non-inoculated treatments after 21 days. Reduction of plant height by F. oxysporum was greatest at low SM (Ψ_m = -1.36 bars). Pythium sp. at low ST (10 and 15°C) and SM (Ψ_m = -1.36) combinations significantly decreased (P = 0.05) plant height and stem dry weight. F. oxysporum + Pythium sp. caused greatest reduction of dry weights at 10 and 15°C, and greatest secondary root discoloration and reduction of dry weights at Ψ_m= -1.36.

698

PILOT PROGRAM FOR DIAGNOSIS OF RATOON STUNTING DISEASE OF SUGARCANE IN LOUISIANA. K. E. Damann, Jr. and C. A. Hollier. Louisiana State University Agricultural Experiment Station and Louisiana Cooperative Extension Service, LSU Agricultural Ctr., Baton Rouge, LA 70803

Alkaline-induced metaxylem autofluorescence was used to diagnose ratoon stunting disease (RSD) in samples from commercial fields which were prospective seed sources. Frequency of diseased stalks and infested fields by cultivar was: CP 65-357, 33%, 75%; CP 74-383, 33%, 75%; CP 76-331, 22%, 55%; CP 70-321, 18%, 54%; CP 72-356, 16%, 55%; CP 72-370, 3%, 34%. Disease incidence across cultivars was 22% of the 3607 stalks and 59% of the 184 fields. Mean RSD incidence for an infested field was calculated at 38%. Plotting years after heat-treatment of seed versus incidence of RSD in the field for all cultivars approximated a straight line, increasing by approximately 10% annually through the fifth year. The 22% RSD incidence in standing cane across cultivars, plus a 10% increase presumably due to mechanical spread by the harvester prior to replanting, suggested that one-third of the seed planted in 1986 had RSD.

699

PHOMA LUPINI ON LUPINE IN THE PERUVIAN SOUTHERN HIGHLANDS. B. Salas, Univ. Nac. del Altiplano, Puno, Peru and R. W. Stack, North Dakota State Univ. Fargo, ND 58105.

Lupine (Lupinus mutabilis Sweet.) is an important highland crop in Peru. In the Peruvian southern highlands (above 3800 m) the most common disease of lupines is caused by Phoma lupini Ell. & Ev. The fungus infects all above-ground plant parts. On leaves it causes gray concentric zonate lesions. Stem lesions are elongate and reddish-brown. Pod lesions are circular and cream-colored surrounded by a grayish-purple margin while infected seeds are gray and shrunken. Black pycnidia often develop in lesions on pods, stems, and infected seeds. The disease is most severe after hailstorms, which are common in this area. In greenhouse studies Koch's postulates were fulfilled with typical symptoms produced on leaves, stems and pods. In these experiments infection was increased by wounding. In other greenhouse experiments planting of naturally infected seeds produced an average of 30% infected seedlings.

700

INFLUENCE OF SOYBEAN CYST NEMATODE (SCN) ON ROOT COLONIZATION BY MACROPHOMINA PHASEOLINA. C. A. S. Pearson, T. C. Todd, and F. W. Schwenk, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Two SCN-resistant and five SCN-susceptible soybean cultivars were planted in two northeastern Kansas soils infested with SCN race 3 and M. phaseolina. At both locations, cultivars were planted in carbofuran-treated and nontreated plots and the levels of nematode and fungus were monitored throughout the growing season. In loamy sand, application of carbofuran 15G (2.2 kg a.i./ha) at planting reduced midseason nematode populations an average of 74%. Nematode populations in loam soil were not significantly reduced by carbofuran (P=0.05). In loamy sand and loam soils, levels of M. phaseolina were correlated with nematode densities (r=0.60 and 0.80, respectively) and with yield (r=-0.82 and -0.80, respectively). SCN-resistant lines had significantly less charcoal rot than SCN-susceptible lines. Results indicate that SCN infection can increase soybean root colonization by M. phaseolina and thus may increase losses due to charcoal rot.

701

PHYSIOLOGICAL CHANGES IN PEACH TREES AS RELATED TO PRUNING DATE, TRUNK TREATMENT AND COLD HARDINESS. C. C. Reilly, W. R. Okie, and R. R. Sharpe. USDA,ARS, S/E Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

Bark plugs obtained from the trunk of peach trees pruned in fall (early Dec) or winter (early March) had different responses when subjected to a range of temperatures (4,-4,-8, -12 and -16C). Electrical conductivity, leakage of reducing sugars and prunasin and the release of cyanide were used as measures of cold hardiness. Measurements were taken on four dates during the winter with the last just prior to bud break. Clear plastic was wrapped around the trunks of some fall pruned trees to accelerate temperature fluctuations of the trunk tissue. The critical temperature for tissue damage of the trunk was -8C for all treatments but damage of fall pruned, plastic wrapped treatment was > fall pruned > winter pruned. Fall pruning and rapid and wide fluctuations of temperature on the trunk of peach trees appeared to reduce cold hardiness, a condition related to tree loss due to peach tree short life.

702

AVAILABILITY OF STRAWBERRY GERMPLASM RESISTANT TO TEN U.S. RACES OF PHYTOPTHORA FRAGARIAE AND SEVERAL ISOLATES OF VERTICILLIUM. J. L. Maas, Fruit Laboratory, USDA-ARS, Beltsville, MD 20705

Levels of resistance among eastern U. S. strawberry cultivars to races of P. fragariae and Verticillium were determined to expand the usefulness of various germplasm sources for future resistance breeding work. The need for these studies is indicated by the increased movement of nursery material from region to region and the identification of an exotic race of P. fragariae in Maine. Several eastern cultivars are resistant to western P. fragariae races, but none are resistant to one Verticillium isolate from California.

703

LATE YELLOW RUST OF RASPBERRIES IN NOVA SCOTIA. N. L. Nickerson, Agriculture Canada, Research Station, Kentville, Nova Scotia, Canada B4N 1J5

Late yellow rust, caused by Pucciniastrum americanum, occurred in all commercial red raspberry plantings surveyed in Nova Scotia in 1986. Disease incidence ranged from a trace to 70% of the fruit and 90% of the leaves infected. Aeciospores were released from white spruce (Picea glauca), the alternate host, from mid June to early July. The first evidence of infection in raspberries was the appearance of uredinia on calyces and on basal leaves of fruiting laterals early in July. Subsequently uredinia developed on fruit, the lower leaves of fruiting canes and the leaves of primocanes. No cane lesions were found. Infection studies confirmed that the uredinial stage of the fungus recycles on raspberries. Some possibilities for the control of late yellow rust in eastern Canada will be discussed.

704

A COMPARISON OF FOUR LEAF SPOT DISEASES ON DICHONDRA. R. D. Raabe and Annamaria Pisi. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Dichondra micrantha sometimes is used as a ground cover or lawn in northern California. Alternaria porri leaf spot is common on it. Recently leaf spots have yielded also a Cercospora sp., a Gloeosporium sp. and a Colletotrichum sp. To produce infections, conidia of A. porri were produced by using Walker's modification of Richard's medium. Conidia of Cercospora were produced using V-8 juice agar of Bair and Ayers. Gloeosporium and Colletotrichum conidia were produced on potato dextrose agar. All fungi proved to be pathogenic to dichondra. Alternaria tended to infect older leaves more and produced brown circular spots with concentric zones. Colletotrichum and Gloeosporium tended to attack younger leaves, producing light brown spots with a dark border. Cercospora produced spots similar to Alternaria on both young and old leaves but without concentric zones. This is believed to be the first report of Cercospora and Colletotrichum on dichondra.

705

NATURAL INFECTION OF GLOXINIA (SINNINGIA SPECIOSA) BY TOBACCO MOSAIC VIRUS. P. R. Desjardins, P. J. Sasaki, M. E. Hilf, R. J. Drake, S. A. Swiecki and W. M. Young, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

In 1946 Holmes (Phytopath. 36:643-659) experimentally infected Gloxinia (Sinningia speciosa Benth. & Hook) with tobacco mosaic virus (TMV), but reported that it was a localized masked infection with no systemic spread of the virus. We have found a strain of TMV naturally infecting Gloxinia which systemically invades the host and produces symptoms that are varied in different florist cultivars. A symptom common to all cultivars, however, is color breaking of the flowers. Young plants of one cultivar were stunted by the virus and exhibited interveinal chlorosis of the leaves. The original infected plant came from a greenhouse that also contained orchids, prompting us to see if the strain we are studying is a known orchid strain of the virus. To date virion length measurements in the electron microscope and dot-blot hybridization tests with a cDNA probe to a known orchid strain have not been successful in identifying the TMV strain.

706

ASSOCIATION OF SPIDER MITES AND INCIDENCE OF STALK ROTS OF CORN. D.J. Jardine, Department of Plant Pathology; and L.L. Buschman and P.E. Sloderbeck, Department of Entomology, Kansas State University, Manhattan, KS 66506.

Natural populations of the Banks grass mite, Oligonychus pratensis (98% of the total population), and twospotted spider mite, Tetranychus urticae, were manipulated using insecticides to produce varying pest population levels in corn. Evaluations were made just prior to harvest by splitting stalks of 20 plants of each treatment and visually rating the stalks for both the incidence and severity of stalk rot (Fusarium and Macrophomina). Severity ratings were as follows: 0 = no visible stalk rot; 1 = stalk rot present in the first discernible node; 2 = stalk rot visible in two nodes; 3 = stalk rot in three or more nodes. There was a significant (P = .005) positive correlation between spider mite populations and the incidence and severity of stalk rot (r = 0.90 and 0.87, respectively). Yield was negatively correlated with stalk rot severity (r = -0.59, P = .05).

707

TILLAGE AND OTHER EFFECTS ON FUSARIUM GRAMINEARUM IN CORN ROOTS. Thor Kommedahl, W. C. Stienstra, P. M. Burnes, K. K. Sabet, D. A. Andow, K. R. Ostlie, and J. F. Moncrief, University of Minnesota, Departments of Plant Pathology, Entomology, and Soil Science, St. Paul, MN 55108.

Seven to 17% fewer root-infecting, Fusarium colony-forming units (cfu) grew from mature plant roots from ridge-tilled than from chisel-plowed or no-till corn (Zea mays) for 1985 and 1986 at one farm (silt-loam) but were 2-5% fewer in no-till corn at another farm (clay-loam) added in 1986. Infection by Fusarium was greatest where corn rootworm activity was greatest. Tillage effects on infection by F. graminearum were inconsistent. F. graminearum in 1985 dropped from 38% of 750 Fusarium cfu in seedling roots to 3% of 6530 cfu in mature roots; in 1986 this species varied from 12 to 14% cfu on all roots. Other populations were: F. oxysporum (50%); F. acuminatum, F. equiseti, and F. solani (<10%); F. avenaceum, F. crookwellense, F. moniliforme, F. proliferatum, F. sambucinum, F. semitectum, and F. sporotrichioides (<1%).

708

ZLEAF: A GENERAL LEAF GROWTH AND ONTOGENY MODEL FOR ZEA MAYS. C.J.A. Gay and R.L. Nicholson, Department of Botany & Plant Pathology, Purdue University, West Lafayette, IN, 47907

A leaf growth and ontogeny model was designed for use in prediction of the development of the anthracnose disease of corn, caused by Colletotrichum graminicola. The model simulates the growth and maturation of each leaf of the plant. ZLEAF determines how many leaves have emerged from the whorl, their respective sizes, rates of growth and eventual senescence. The ontogenetic range from the oldest to the youngest tissue of each leaf of a representative plant is presented. The model increases the level of detail of information provided by a crop-level growth model (CERES-Maize, Jones & Kiniry, 1986, Texas A&M University Press) to which it is coupled. Factors affecting leaf enlargement in the crop model (temperature, solar radiation, nitrogen, soil moisture) are reflected in ZLEAF, which allocates available leaf material and characterizes its developmental state.

709

RELATIONSHIP BETWEEN NEMATODE CONTROL, ROOTSTOCK AND PRUNING TIME ON PEACH TREE PHYSIOLOGY AT BUDBREAK. A. P. Nyczepir, P. L. Pusey, and C. C. Reilly, USDA-ARS, Byron, GA 31008.

'Redhaven' peach budded to Lovell (L) and Nemaguard (N) rootstock had treatments consisting of pre-plant fumigation (F) vs nonfumigation (NF) and December (DP) vs March (MP) pruning. Methyl bromide (561 kg/ha) was applied under plastic to control Criconemella xenoplax (Cx) and Meloidogyne sp (RK). In December 1986, Cx populations were greatest under N-NF-DP and lowest under L-F-MP trees; RK-J_2 populations were greatest under L-NF-DP. In late February-mid-March, trunk cambial electrical resistance (CER) was obtained weekly. CER readings were lower in N-NF-DP than L-F-MP trees in late February-early March. In mid-March water potential, ul-sap exuded/g twig and prunasin content in sap were also greater in N-NF-DP. Results indicate that N-NF-DP were more metabolically active (or less dormant) than L-F-MP trees.

710

A RAPID METHOD OF ASSESSING ROOT-KNOT NEMATODE INFECTION POTENTIAL. D. A. Gunning and B. C. Hemming. Biological Sciences Department, Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198

A method for rapid assessment of root-knot nematode infection potential on tomato has been developed. The assay system utilizes one 24-well tissue culture plate per treatment. Each well holds 2 tomato seeds buried in 2.5g fine sand saturated with 1.5ml water. Consistent infection levels were achieved by inoculating at a rate of 50 juveniles/well. This unique assay affords several advantages over conventional means of primary gall analysis which include the following: 1) results are generated rapidly, 2) space requirement is minimal, 3) variability is reduced, 4) improved statistics are obtained as a result of high numbers of observations, and 5) small quantities of test solutions are required. While no single assay can control all the factors involved in the nematode infection process, the method described has been proven an effective means of measuring primary infection levels on tomato seedlings in response to chemical treatments and resistant plant varieties.

711

HOST-SPECIFIC PATHOTYPES OF THE PINEWOOD NEMATODE BURSAPHELENCHUS XYLOPHILUS. R.I. Bolla, K. Fitzsimmons and C. Weaver. Dept. Biology, UM-St. Louis, St. Louis MO 63121.

Variation in susceptibility of pines to pinewood nematode, Bursaphelenchus xylophilus, (PWN) throughout its range in North America suggest development of pathotypes. We have compared host specificity, host response, and DNA restriction endonuclease fragment patterns of a population of PWN from Scots pine in Missouri (MPSy-1) to one from white pine in Vermont (VPSt-1). MPSy-1 induces wilting of Scots pine, VPSt-1 causes wilting of white pine, and Austrian and loblolly pine are not affected. Genomic differences were analyzed by cleaving genomic DNA with restriction endonucleases, and comparative hybridization of the fragments to genomic DNA from one of the isolates. Distinct differences in restriction fragment patterns could be seen between MPSy-1 and VPSt-1. These results suggest that MPSy-1 and VPSt-1 are pathotypes of B. xylophilus and could explain the regional differences in host specificity and pathogenicity.

712

M5:AN EXPERT SYSTEM FOR DIAGNOSING MUSKMELON DISORDERS. R. X. Latin, G. E. Miles, and J. C. Rettinger. Departments of Botany and Plant Pathology and Agricultural Engineering, Purdue University, West Lafayette, IN 47907

M5 is a prototype computerized diagnostic consultant for diseases and disorders of muskmelons. The system was developed using a LISP-based knowledge engineering programming language and operates on an IBM XT compatible computer. The knowledge base contains rules for identifying 11 infectious and 7 noninfectious disorders. During a consultation the user is prompted by the system to indicate (from a menu) the types of symptoms observed. Because of the software's advanced searching techniques, not all prompts are required for each diagnostic case, resulting in the most rapid consultation possible. The system was validated by comparing a human expert's diagnoses with conclusions drawn by M5 when operated by extension and agribusiness personnel who are not expert at identifying melon diseases.

713

DIAGNOSTIC DETECTION OF PHYTOPHTHORA MEGASPERMA F.SP. GLYCINEA USING MONOCLONAL ANTIBODIES. M. W. Ferguson, A. R. Ayers and K. L. Wykoff, South Dakota State University, Brookings, SD 57007; Cedar Crest College, Allentown, PA 18100; Harvard University, Cambridge, MA 02138.

A large number of monoclonal antibody (Mabs) clones have been produced to Phytophthora megasperma f.sp. glycinea (Pmg). In ELISA tests, several clones cross-reacted with other Phytophthora spp., but did not react with other genera of root infecting fungi. Two such clones were used in ELISA tests to detect Pmg in both greenhouse inoculated and naturally infected field samples. ELISA tests with a wide range of dilutions of pure antigen showed the response to be linear. Usage of Mabs in fluorescent assays and diffusion tests were also examined. Preliminary results show some Mabs may be useful in soil assays.

714

KARNAL BUNT DISTRIBUTION AND YIELD LOSS COMPONENTS IN A NATURALLY INFECTED MEXICAN WHEAT FIELD. M. H. Royer, L. E. Datnoff, USDA-ARS, Frederick, MD; J. M. Prescott, CIMMYT, Mexico; and T. T. Matsumoto, CDFA, Sacramento, CA.

Fifty wheat spikes were randomly collected in 53 of 90 marked quadrats of 3 m^2 from a commercial wheat field naturally infected with Karnal bunt near Ciudad Obregon, Sonora, Mexico in 1986. All samples were threshed with a single-head thresher, except for eight quadrats in which 25 spikes were hand threshed. The proportion and weight of bunted kernels were not significantly different whether machine or hand threshed. The percentage of all kernels that were bunted per quadrat ranged from 14% to 46%, with an average of 25%. The weights of healthy kernels in each quadrat were used to obtain reference yield weights since no control quadrats were available for comparison. The extrapolated reduction in weight averaged 0.33%. Many bunted kernels had only a portion of the embryo end of the kernel bunted, and confirmed other reports of low kernel weight due to Karnal bunt.

715

BROWN STEM ROT SURVEY AND ESTIMATED LOSS IN 28 SOUTHERN MINNESOTA COUNTIES. W. C. Stienstra, J. D. Pokorny and S. L. Gould. University of Minnesota, Department of Plant Pathology, St. Paul, MN 55108.

Phialophora gregata was isolated from soybean (Glycine max) stems from 71% of the 56 fields surveyed in 28 southern Minnesota counties. Five stems at R5 or R6 growth stage at 10 sites in each field were scored for pith color. The severity of infection based on stem pith color ranged from 0 to 98%. Average infection severity for all fields sampled was 32%; however, those fields in a corn/soybean rotation from 1981-86 had a disease severity score of 64%. P. gregata was always isolated from plants from fields when stem color percentages were above 34%, while isolation success was 85% when stem color percentage ranged from 18 to 32%. Disease severity was independent of tillage. The estimated yield loss for the corn/soybean rotation infection level was 3.8 q/ha.

716

CASSAVA BLIGHT ERADICATION PROGRAM AT INARAJAN EXPERIMENT STATION, GUAM, G.C. Wall, Agricultural Experiment Station, University of Guam, Mangilao, Guam, 96923.

After the recent discovery of cassava blight (Xanthomonas campestris pv. manihotis), an island-wide survey revealed that the disease was too widespread for complete eradication. However, the Experiment Station at Inarajan and Guam's cassava collection (severely infected), were made free of blight by the following cultural and chemical control measures: 1) select healthiest individual plants from each cultivar 2) take cuttings from older wood (basal 1m) 3) surface-sterilize pruning tools with 10% bleach 4) eliminate (burn) plant refuse 5) deep-plow field and rotate to non-host for two years 6) dip cuttings in 10% bleach for 1 min., rinse and plant in greenhouse,observing for two months 7) eliminate any plants developing blight symptoms 8) eliminate (herbicide) wild and volunteer cassava around station's perimeter 9) transplant healthy cuttings from greenhouse to new field.

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SELECTION OF SOMACLONAL VARIANTS TO THE SUGARCANE RUST PATHOGEN, PUCCINIA MELANOCEPHALA. J. C. Comstock and Andrew Maretzki, Dept. of Genetics & Pathology, Hawaiian Sugar Planters' Association, P. O. Box 1057, Aiea, Hawaii 96701.

Approximately 2500 somaclones were derived from each of two sugarcane donor clones H70-0144 and H74-0922, respectively resistant and susceptible to Puccinia melanocephala. Both individual somaclones and donor clones were planted in a rust-conducive area. Somaclones which varied from donor clones in rust symptom expression were vegetatively repropagated for further replicated testing. Somaclones of H70-0144 and of H74-0922 tentatively designated as variants, as well as 30 randomly selected non-variant somaclones derived from each donor clone were planted in a screening test with four replications. Seven somaclones derived from rust resistant H70-0144 were classified as susceptible. However, none of the somaclones derived from rust susceptible H74-0922 were resistant in the second vegetative repropagation. Increased urediniospore production on the seven susceptible somaclones derived from H70-0144 over that of the donor clone also indicates a change from resistance to susceptibility.

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

The association of long flexuous virus particles with mealybug-wilt affected pineapple has been reported (Gunasinghe and German, Phytopathology 76:1073). The purification procedure has been improved by homogenizing in "Tris" buffer at pH 8.6, chloroform clarification, ammonium sulfate precipitation and sucrose density gradient centrifugation. Virus purified by this improved procedure was used to produce polyclonal antiserum which reacts specifically with mealybug-wilt infected plants and purified virus in Ouchterlony double-diffusion assays. The antiserum was used for serologically specific electron microscopy to capture virus from plant sap and "decorate" virus particles attached to grids.

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AFLATOXIN PRODUCTION IN SOLID MEDIA: TECHNIQUE FOR RAPID ESTIMATION OF TOXIN LEVELS. P. J. Cotty, USDA/ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179

The quantity of aflatoxin produced on agar media was estimated with a technique which nondestructively quantifies the agar's fluorescence. A carriage was constructed to carry culture tubes in a thin-layer chromatography (TLC) plate densitometer. Tubes containing 5 ml of a synthetic medium inoculated with A. flavus were excited 3 to 9 mm below the surface with a 0.8 x 6 mm beam of 365 nm light. The resulting fluorescence (450 nm and above) was photometrically measured and recorded in a manner similar to that utilized in quantitative TLC. Aflatoxin was extracted from the media with organic solvents and quantified with TLC. Fluorescence of the agar media in the tubes directly correlated with the quantity of aflatoxin extracted. The technique permits simple, rapid evaluation of the relative aflatoxin producing ability of individual fungal isolates in an agar medium. In addition, monitoring aflatoxin production, degradation and screening agrichemicals for influence on aflatoxin production are facilitated.

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AFLATOXIN CONTAMINATION OF PREHARVEST CORN KERNELS: INFLUENCE OF MICROBIAL INTERACTIONS. E. B. Lillehoj and J. H. Wall, SRRC, ARS, USDA, 1100 R. E. Lee Blvd., New Orleans, LA 70124

The interactions between three fungal species in silks of developing corn ears was studied in the context of subsequent infection of kernels by an aflatoxin-producing species. Newly-emergent silks were inoculated with either independent spore suspensions of Aspergillus parasiticus, Trichoderma viride, and Rhizopus nigricans or varied combinations of the three fungi. Treatment effects were determined by quantitative assay of mature kernels for aflatoxin. Kernels from ears inoculated with A. parasiticus contained higher levels of toxin than controls. Independent treatment with either T. viride or R. nigricans did not reduce the background levels of aflatoxin. In addition, mixed spore inocula with A. parasiticus and either T. viride or R. nigricans did not influence toxin accumulation. However, introduction of an inoculum with spores of the three test fungi reduced aflatoxin concentration of test kernels.

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APPLICATION AND EFFICACY OF BACILLUS SUBTILIS FOR BROWN ROT CONTROL IN COMMERCIAL PEACH-PACKING OPERATION. P. L. Pusey and M. W. Hotchkiss, USDA-ARS, Byron, GA 31008; R. A. Baumgardner and E. I. Zehr, CU, Clemson, SC; H. T. Dulmage, USDA-ARS, Brownsville, TX; C. C. Reilly, USDA-ARS, Byron, GA; C. L. Wilson, USDA-ARS, Kearneysville, WV.

Bacillus subtilis (B-3) potential for postharvest control of peach brown rot was assessed in pilot tests in 1986 on simulated commercial packing lines at Byron, GA, and Clemson, SC, and in an actual packing house in Musella, GA. B-3 was cultured in flasks at Byron or in a 250-L fermentor at Brownsville, TX. B-3 suspensions were prepared from fresh cultures or stabilized forms (wet slurry or dry powder) stored at 4C. Only fresh B-3 exceeded benomyl (1-2 ppm residue) in performance and stored forms did not differ (P=0.05) from benomyl in some cases. Dry-stored B-3 was the least effective. Comparing quantities of B-3 and benomyl when the two appeared equal, a range of 10^7-10^{10} CFU were required, depending on B-3 source, for every 1.0 mg benomyl used.

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POSTHARVEST MUCOR ROT CONTROL ON APPLES WITH PSEUDOMONAS CEPACIA. W. J. Janisiewicz and J. Roitman, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430, and Western Regional Research Center, USDA-ARS, Albany, CA 94710.

Pseudomonas cepacia isolated from apple leaves showed strong antagonistic activity against Penicillium expansum, Botrytis cinerea and Mucor sp. on Nutrient Yeast Dextrose Agar (NYDA) medium. When tested as a water suspension on wounded 'Golden Delicious' apples challanged with Mucor sp. spore suspensions, the antagonist significantly reduced the number of Mucor rot lesions. An antifungal compound was isolated from Nutrient Yeast Dextrose Broth (NYDB) medium in which the bacterium grew for 24 hr and from P. cepacia cells. The compound was purified on Amberlite X AD7, Sephadex LH-20, and high pressure liquid chromatography (HPLC) C-18 reverse phase column. All three pathogens were strongly inhibited on NYDA medium by a single peak fraction from HPLC separation. The compound is methanol soluble and tested positive with diazotized sulfonilic acid.

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POPULATION DYNAMICS OF TWO BIOLOGICAL CONTROL AGENTS ON APPLE AND THEIR COMPATIBILITY WITH POSTHARVEST TREATMENTS. W. J. Janisiewicz, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Population dynamics of Acremonium breve and Pseudomonas sp. (L-22-64), antagonistic against Botrytis cinerea (incitant of grey-mold) and Penicillium expansum (incitant of blue-mold) respectively, were studied on wounded 'Golden Delicious' apples over a 30 day period. The antagonists were applied to the apples either in water or water with Ortho-X 77 wetting agent. For both antagonists there was a rapid increase (100-1000 fold) in population over the first ten days in both treatments. This followed by a small decline, after which population stabilized. In in vitro tests, antioxidant diphenylamine (DPA) at a concentration of 2000 ppm had little effect on the survival of A. breve and caused a small decline in the viability of Pseudomonas sp. The negative effect of DPA on survival of antagonists was eliminated largely by addition of the X-77 wetting agent.

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PREHARVEST AFLATOXIN CONTAMINATION OF CORN KERNELS: INTRODUCTION OF ASPERGILLUS ORYZAE OR TRICHODERMA HARTZIANA INHIBITS AFLATOXIN PRODUCTION BY A. PARASITICUS. O.H. Calvert, H.C. Minor, & E.B. Lillehoj, Dept. of Plant Path. & Agron., Univ. of Missouri, Columbia, MO 65211; Microbiologist, SRRC, ARS, USDA, New Orleans, LA 70179.

Aflatoxin B_1 was determined for pin-board-injured mature kernels of field-inoculated ears of 2 Zea mays hybrids. Injured kernels were aerosol inoculated with 2,500 (100%), 2,250 (90%) or 250 (10%) of Aspergillus parasiticus, A. oryzae or Trichoderma harzianum. Conidial inoculum was introduced at 2 stages of kernel dev., 20 d and 28 d after silking. Kernels on each of 5 ears/rep (15 ears/trt.) were inoculated independently or variedly combined. The ears were harvested 30 d after the 2nd inoculation. Regardless of the conidial concentration, kernels inoculated on day 20 with A. oryzae or T. hartzianum, followed by re-inoculation on day 28 (same ears) with A. parasiticus, little aflatoxin was detected (average 25 ng/g, B_1). Conversely, kernels inoculated on day 20 with A. parasiticus, followed by the re-inoculation on day 28 with A. oryzae, or T. hartzianum (average 9,807 ng/g B_1) was produced. Preinoculated kernels with nonaflatoxin producing fungi are protected from aflatoxin contamination by A. parasiticus.

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OCCURRENCE OF ECHINULIN AND VOMITOXIN IN SWINE FEED. R. F. Vesonder, NRRC, 1815 N. University, Peoria, IL 61604, and R. Lambert, University of Illinois, Urbana, IL 61801.

A specimen of feed obtained from herd episodes of swine disorders, i.e., refusal to eat, loss of sows and litters, was investigated for causative principles. Mycological examination by dilution plating of 20-g samples onto agar plates revealed predominantly species within the Aspergillus glaucus group. Three Aspergillus isolates were grown on rice at 25°C for 2 weeks. Two isolates produced 50 µg/g of echinulin, but none was detected from the third isolate. Echinulin offered to mice in their drinking water at 100 ppm produced a 30% refusal response. In addition to echinulin at 8 ppm, the feed was found to contain 0.3 ppm of vomitoxin, a known swine refusal factor. This high incidence of A. glaucus in the feed probably developed in storage due to improper moisture content. These results may cause concern that refusal response of low levels of vomitoxin may be enhanced by other mold metabolites that develop during improper storage of the feed.

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CATEGORIZATION OF ANASTOMOSIS INTERACTIONS THAT OCCUR BETWEEN ISOLATES OF RHIZOCTONIA SOLANI. D.E. CARLING AND R.H. LEINER. Univ. of Alaska, 533 E. Fireweed, Palmer, AK 99645

Current studies of anastomosis in Rhizoctonia solani make use of categorization systems proposed by Matsumoto (1932) and Flentje and Stretton (1964). Study of these systems reveals cytological characteristics of categories are incompletely defined, implied or simply not mentioned. Various interpretations of both systems are possible and confusion has resulted. A system (0, 1, 2, 3) patterned after Matsumoto's no reaction, contact fusion, imperfect fusion and perfect fusion, is proposed. Category 0: no interaction; Category 1: contact between hyphae, apparent hyphal attachment, no evidence of membrane fusion, occasional death of one or both anastomosing cells; Category 2: wall and membrane fusion (fusion point readily identifiable), a pore at the point of fusion with a diameter less than that of hyphae, and death of anastomosing cells; Category 3: wall and membrane fusion (point of fusion not obvious), a pore at the point of fusion nearly equal to hyphal diameter, and anastomosing cells that frequently do not die.

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EFFECTS OF LEAF BLAST AND PANICLE BLAST ON GRAIN YIELD OF RICE. B. S. Kim. and E. W. Park. Department of Agricultural Biology, Seoul National University, Suweon, Korea.

Effects of leaf blast and panicle blast on rice grain yield and yield components, i.e., 1000 grain weight, number of heads per hill, number of grains per head, and grain fertility, were evaluated using rice cultivar Nakdong in 1985, and Nakdong, Jinju, and Jinheung in 1986. Path coefficient analyses indicated that the amount of total grain yield of each rice cultivar was affected most greatly by the number of heads per hill and least by the number of grains per head. The most important path resulting in grain yield loss of three cultivars was via the number of heads per hill which was greatly reduced by leaf blast. Grain yield loss due to panicle blast was most significant on Jinheung whereas it was almost negligible on Nakdong as compared with loss due to leaf blast. Panicle blast showed greater negative correlation with grain fertility than leaf blast. Leaf blast, as the inoculum source for panicle blast, showed significant indirect effect on grain yield of Jinju and Jinheung.

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A COMPUTER SIMULATION MODEL FOR RICE LEAF BLAST. W. J. Choi, E. W. Park, Department of Agricultural Biology, Seoul National University, Suweon, Korea, and E. J. Lee, Institute of Agricultural Sciences, Office of Rural Development, Suweon, Korea.

A computer simulator LEAFBLST for rice leaf blast progress was developed based on the data from growth chamber experiments and from the literatures. LEAFBLST was written in FORTRAN IV and composed of six subroutines representing conidial germination, infection, latent period, lesion number, lesion expansion, and spore deposition, and four subroutines for initialization, leaf area calculation, and numerical and graphic output. Since the program was assembled on a modular basis, it can be easily modified and improved with more extensive data from further studies in the future. LEAFBLST was tested with data from two nursery plots located at Suweon, Korea. The results indicated that LEAFBLST was a useful tool for determining effects of environmental factors on leaf blast progression although it needed to be improved to be used for forecasting leaf blast development in practice.