

**The American Phytopathological Society**

**ABSTRACTS OF PRESENTATIONS  
at the 1985 Annual Meeting**

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## ABSTRACTS

1  
Induction of epicarp lesion of pistachio fruit by mechanical injury. J. K. Uyemoto, R. M. Dostock, and J. H. Ogawa, Dept. of Plant Pathology, University of California, Davis, 95616.

Fruit of pistachio (*Pistacia vera* L.) develop a disorder called epicarp lesion (EL). Symptoms of EL are brown to black lesions on fruit surfaces often associated with a dried resin exudate within the lesion. Symptomatic fruit delisce or fail to produce marketable kernels resulting in yield losses over 50% in some orchards. Recently, hemipterous insects were shown to incite EL. Likewise, needle punctures made through the pericarp caused EL. EL did not develop when mechanical injuries were limited to the epicarp and mesocarp tissues. During endocarp lignification, in late May, incidence of EL decreased sharply and, after completion of lignification (late June), fruit no longer developed EL. This suggests that EL is a host response to necrogenic factors present in the endocarp.

2  
AN ANGULAR LEAFSPOT OF PECAN FOLIAGE. A. J. Latham, H. J. Amling and S. A. McCraney, Ala. Agric. Exp. Stn., Auburn University, AL 36849.

An angular leafspot of young 'Cape Fear' and some seedling pecans has been observed in Alabama orchards since 1974. The disease has been found during the high summer temperatures of July into September on the youngest leaves. Lesions start with necrosis of the smallest veins and may progress to include laminar tissue resulting in reddish-brown leafspots. No fungus has been isolated from diseased leaves. Vacuum infiltration of leaflets with a *Pseudomonas* sp. isolated from symptomatic leaves failed to reproduce the disease. Extraction of 'Cape Fear' twigs with 0.1 M KOH did not yield a xylem-limited organism. Leaves growing from wood from healthy trees grafted onto trees with diseased foliage did not develop the angular leafspots, nor did foliage on 'Cape Fear' seedlings exposed to ozone. Light and electron microscope examinations of leaf cross sections have shown plugged bundle-sheath cells with cell plugging extending into spongy mesophyll cells.

3  
DEVELOPMENT AND TESTING OF NEW OPEN-TOP CHAMBERS FOR EXPOSING LARGE, PERENNIAL PLANTS TO AIR POLLUTANTS IN THE FIELD. R. H. MANDL, J. A. LAURENCE, AND R. J. KOHUT, BOYCE THOMPSON INSTITUTE, ITHACA, NY 14853-1801.

Open-top chambers were designed for use with grape vines grown on a continuous trellis system. A rectangular chamber, 24' x 9' x 14' high, and a 15' diameter, 14' high circular chamber were developed using wind tunnel models and were field tested during two growing seasons. Both chambers have restrictions near the top to help exclude 90% of ambient pollutants under most wind conditions. Distribution of introduced pollutants is as good or better than that of standard open-top chambers. The environment within the chambers is similar to the ambient environment. Reduction in light intensity of 10 to 50% due to shading by the top structures may occur depending on time of day and position within the chamber. Growth of vines in the chambers was similar to those outside, but fewer flowers were found in chambers, probably due to lower light intensity.

4  
DILUTE SOLUTIONS OF NITRIC-SULPHURIC ACID ENHANCE GROWTH OF WHEAT AND CORN. Wayne E. Jardner and Paul D. Evenson, Dept. of Plant Science, South Dakota State University, Brookings, SD 57007

Dilute aqueous solutions of nitric-sulphuric acid (1:1, V:V) were applied at 5 pH levels (5.6, 3.5, 2.5, 2.0 and 1.8) along with tap water to spring wheat and D88 corn in 3-leaf stage to maturity in greenhouse experiments. Seed weight, seed number, spike number and leaf necrosis significantly increased in seven wheat cultivars in soil application only and in soil-foliar applications as pH decreased. There were significant cultivar by application and cultivar by pH by application interactions. Plant height, green leaf color, dry weight, ear set and leaf necrosis significantly increased with combined soil-foliar acid treatment to corn as pH decreased. In both wheat and corn, increasing acidity was beneficial to overall growth and the fertilizer effect of nitrate-sulphate overcame moderate to severe foliar injury at pH 2.0 and 1.8.

5  
Effects of Simulated Acid Precipitation on Disease Dynamics in Four Pathosystems in the Field. C. L. Campbell, J. Pawloski Sinn, R. I. Bruck, and S. B. Martin, Jr., Department of Plant Pathology, North Carolina State University, Raleigh, NC. 27695.

Investigations on the effects of varying levels of acidity in simulated rain on disease dynamics in plant pathosystems were initiated in 1984. Pathosystems studied were alfalfa leaf spot (ALS), peanut early leaf spot (PLS), potato late blight (PLB), and soybean brown spot (SBS). Twenty field plots accommodated two crops in adjacent sub-plots. Simulated rain pH levels were 2.8, 3.6, 4.2, 4.8, and 5.6, with four plots/pH level. ALS and PLB systems were treated three times/wk at 1 cm/event; SBS and PLS were treated two times/wk with 3 cm/event. Ambient rain was excluded by moveable fiberglass panels. Disease severity was assessed three times/wk. Increasing level of acidity in rain increased rate of disease progression and final disease for ALS, reduced rate of disease progression for PLS, and had no significant effect on these parameters in PLB or SBS. Thus, disease response to level of acidity in simulated rain appears to be system dependent.

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THE EFFECT OF SIMULATED ACID RAIN ON *EXSEROHILUM TURCICUM* AND *CORYNEBACTERIUM MICHIGANENSE* PV. *NEBRASKENSE*. W. L. Pedersen, T. R. Phillips and L. J. Brandenburg, Department of Plant Pathology, University of Illinois, Urbana, 61801.

Field plots of the maize hybrid FR632 x FR619 were inoculated with a conidial suspension of *Exserohilum turcicum* race 2 (2200 viable conidia/ml applied at 1200 l/ha) at pH 3.0 and 5.6. The number of lesions per plant was 35% greater at pH 3.0 than at pH 5.6. A second set of plots of the same hybrid were inoculated with *Corynebacterium michiganense* pv. *nebraskense* by mascerating greenhouse infected leaf tissue in sterile simulated acid rain (pH 3.0 & 5.6), dipping a plastic block imbedded with pins into the bacterial suspension and wounding the plants with the pins. The number of plants with symptoms of Goss' bacterial blight was 30% and 100% for pH 3.0 and pH 5.6, respectively.

agation using recently hardened wood. Time from cutting scion wood to attempted propagation may be several days, during which time the scion wood suffers severe water stress. More than 20 species of fungi have been isolated from surface sterilized scion wood from Colombia, Costa Rica, Ecuador, Puerto Rico, and Trinidad. These fungi were on the surface or in the tissues of scion wood pieces. Following removal of cortical tissue and reesterilization, five fungal species, *Botryodiplodia theobromae*, *Fusarium decemcellulare*, *Gloeosporium* sp., *Pestalotiopsis* sp., and *Phomopsis* sp., were residents in the vascular cylinder of apparently healthy cacao. When scion wood was inoculated with these fungi, *B. theobromae*, *F. decemcellulare*, and *Phomopsis* sp. invaded the vascular cylinder. These fungal residents of cacao wood, when stimulated by water stress to which the scion wood is subjected, cause failures in attempted propagation.

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COMPUTER GRAPHICS SIMULATION OF GROWTH AND SPORULATION OF *ERYSIPIHE POLYGONI* ON BEANS. James A. Oufon and T.T. Fujimoto, Rohm and Haas Co., Spring House PA 19477.

*Erysiphe polygoni* was grown on bush bean seedlings for seven days under controlled conditions. Each day data on germ tube number, hyphal growth, the timing, position and angle of branching, and the timing and position of conidial formation was obtained. This data was used in a computer graphics simulation of colony growth and sporulation. Branching and sporulation frequencies were highly correlated with observed frequencies. Randomness in position and timing of branches and conidia was simulated using random number generators. Uses of the simulation will be discussed. With 16 hr day (22 C) and 8 hr night (19 C), hyphae grew 325  $\mu$ m per day, branched after a 24 hr lag period on average every 12 hrs at a mean 45° angle and formed conidiophores after a 72 hr lag period once every 231  $\mu$ m which bore conidia in a diurnal fashion.

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COMPARATIVE STUDIES OF A *TILLETIA* SP. FROM *POA REFLEXA* AND *TILLETIA FUSCA*. M. K. Guillemette and J. A. Hoffmann, USDA-ARS, Crops Research Laboratory, Logan, UT 84322.

A specimen of *Tilletia* from *Poa reflexa* had been previously assigned to *T. fusca* on the basis of similar teliospore morphology. *T. fusca* occurs commonly on certain bromes and fescues but has not been reported on *Poa*. A comparison of teliospores of *T. fusca* from different hosts with those of the bunt from *Poa* showed a high degree of morphologic similarity. However, teliospores of the bunt from *Poa* germinate slower and in lower percentages than those of *T. fusca*, do not require light for optimal germination, and produce fewer primary sporidia (2-6 vs. 8-20 in *T. fusca*) which do not fuse. This suggests that the bunt from *Poa* may represent a distinct, undescribed species.

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A FLUORESCENCE MICROSCOPY METHOD TO DISTINGUISH *TILLETIA CARIES* AND *T. CONTROVERSA* TELIOSPORES. V. Stockwell and E. J. Yrione, USDA-ARS, Dept. Bot and Plant Path, OSU, Corvallis, OR 97331.

Mature teliospores of wheat bunt pathogens, *Tilletia caries* (TCT) and *T. controversa* (TCK), are difficult to identify by standard light or electron microscopy methods, however they are distinguishable by epifluorescence microscopy. Aqueous spore suspensions of TCT or TCK were air-dried on glass slides, mounted in nonfluorescent immersion oil, and viewed with an epifluorescence microscope (485 nm excitation, 520 nm barrier filter). The reticulated wall layer, W2, of TCK teliospores fluoresced yellow-orange, the cytoplasm was nonfluorescent, and the spores were spherical. The W2 layer of TCT teliospores did not fluoresce, the cytoplasm often had yellow fluorescing bodies and the spores were aspherical. The differences in the W2 layers were consistent in numerous samples and races of the pathogens. This procedure provides a rapid, easy, sensitive method for distinguishing teliospores of TCK from those of TCT. It may be useful in detecting dwarf bunt spores (TCK) in international wheat shipments.

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THE NATURE OF SCION WOOD DETERIORATION IN *THEOBROMA CACAO*. L. H. Purdy, Univ. of Florida, Gainesville, FL 32611, and P. K. Soderholm, USDA/ARS, Subtrop. Hort. Res. Sta. Miami, FL 33158. Exchange of germplasm of *Theobroma cacao* is by vegetative prop-

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RELATIVE PATHOGEN GROWTH AND LESION DEVELOPMENT IN SOYBEAN PLANTS INOCULATED WITH *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA*. B. L. Keeling, USDA-ARS, P. O. Box 123, Stoneville, MS 38776.

Plants of the susceptible soybean breeding line J77-339 were grown in field plots and inoculated with the stem canker pathogen (*Diaporthe phaseolorum* var. *caulivora*). Plants were inoculated by inserting an infested toothpick into 30 day old plants 10 cm above the ground or into 60 day old plants 6 to 8 cm below the apical meristem. Plants were harvested at intervals (20 to 60 days) after inoculation and examined for the extent of lesion development and for the presence of the pathogen in the plant tissue. In all plants examined, lesion development extended beyond growth of the pathogen. In plants inoculated near the ground, pathogen growth extended 19 to 27 % as far as the lesion. Pathogen growth in plants inoculated near the top extended 21 to 64 % as far as the lesion. These results suggest that a phytotoxin may be involved in this host-pathogen relationship.

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LEAF SPOT OF TOBACCO CAUSED BY *RHIZOCTONIA SOLANI*. H. D. Shew and C. E. Main, Dept. of Plant Pathology, N. C. State University, Raleigh 27695-7616.

A new leaf spot disease was observed on flue-cured tobacco in North Carolina in 1984. Symptoms began as small circular watersoaked spots that rapidly expanded into light green to tan lesions 2 to 6 cm in diameter with an irregular margin. Tissue within the lesions was almost transparent, often displayed a pattern of concentric rings, and frequently dropped out leaving a shot hole effect on the leaves. Fungal mycelium was present frequently at the margins of lesions on the underside of leaves and occasionally a hymenial layer and basidiospores of *Thanatephorus cucumeris* were observed. *Rhizoctonia solani* was isolated consistently from these lesions. Koch's postulates were completed using attached and detached tobacco leaves inoculated with a mycelial suspension, basidiospores and artificially infested soil. The perfect stage of the fungus was produced readily by all isolates of the pathogen collected. This is the first report of the disease in the United States.

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A METHOD FOR UNIFORM INOCULATION OF *ORYZA SATIVA* L. SEEDLINGS WITH *RHIZOCTONIA SOLANI* KUHN. S.D. Thompson and M.W. Andres, ROHM and HAAS CO., 727 Norristown Road, Spring House, PA 19477.

A method for uniform inoculation of rice seedlings with *Rhizoctonia solani* was developed for use in fungicide screening. Mycelium was produced in 500 ml flasks containing 150 ml potato dextrose broth on a shaker for 6 days at 22 C and a photoperiod of 16 hr. A slurry consisting of a blended mixture of three components was prepared: 100 ml deionized water, 20 g pure rice flour, 23 g fungal mycelium (wet weight). Four ml of this slurry were applied with a pipette onto the soil surface of a 5 cm square pot containing an average of 15 rice seedlings. After inoculation pots were placed in a humidity cabinet for two 48 hr periods at 28 and 25 C, respectively with a photoperiod of 16 hr. The height of mycelial growth was observed and compared to a fungicide control. The method permitted uniform and reproducible levels of disease.

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PATHOGENICITY OF ALBERTA ISOLATES OF THE *ARMILLARIA MELLEA* COMPLEX. K.I. Mallett and Y. Hiratsuka\*.

Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T5P 2G6. \*Northern Forest Research Centre, Canadian Forestry Service, Edmonton, Alberta, Canada, T6H 3S5.

Three biological species of the *Armillaria mellea* complex have been found in Alberta. North American biological species I, V, and a species tentatively named the Foothills type were identified. Two-year-old lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) grown in a greenhouse were inoculated with isolates of *Armillaria mellea* sensu stricto, biological species I, V, and the Foothills type. Seedlings were inoculated by placing infested autoclaved branch segments of trembling aspen next to seedling roots. All species were pathogenic but varied in virulence. *A. mellea* sensu stricto and biological species V infected 100% and 91% of the seedlings, respectively. Biological species I and the Foothills type were less virulent, each infected 33% of the seedlings.

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STEM CANKER ON BLACK WALNUT CAUSED BY *FUSARIUM SPOROTRICHIOIDES*. J. E. Cummings and J. E. Kuntz, Department of Natural Resources, Madison WI 53711; and Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

*Fusarium sporotrichioides* Sherb., a pathogen newly recorded on black walnut, causes a narrow elongate trunk canker in southwestern Wisconsin and northeastern Minnesota. Incidence of cankers is highest on saplings in plantations in valleys and along stream beds. Cankers have marginal callus and surround a branch stub or wound. Stem-wound inoculation of greenhouse seedlings caused epinasty of leaves and wilting of leaflets. The optimum temperature for sporulation in greenhouse conditions was 24°C. On wound-inoculated saplings in the field, bark and wood were colonized followed by canker development. In a heavily infected stand, new cankers developed at pruning wounds on trees pruned monthly from May through September. Observations and evidence suggest removal of understory brush and infected trees and pruning during the dormant season are the best silvicultural controls.

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INFECTION OF YOUNG LODGEPOLE PINE SEEDLINGS WITH *ENDOCRONARTIUM HARKNESSII*. Eric ALLEN, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6E 2G6; and Yasuyuki HIRATSUKA, Northern Forest Research Centre, Canadian Forestry Service, Edmonton, Alberta, Canada T6H 3S5

Young lodgepole pine seedlings, 11 to 76 days old, were artificially inoculated with *Endocronartium harknessii* (J.P. Moore) Y. Hirat. Easily recognizable tissue discoloration was observed as early as 7 days after inoculation. Immature epicotyl tissue was the only site of infection that led to successful gall formation. In this tissue, *E. harknessii* entered by direct penetration of the epidermis. Occasional successful infections were also observed on hypocotyls, where the fungus entered through stomata and by direct penetration, and on needles, where it also entered through stomata. Infections on needles and hypocotyls did not result in gall development.

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CYTOLOGY OF AN AUTOECIOUS SOFT PINE BLISTER RUST (*PERIDERMIIUM YAMABENSE*) IN JAPAN. Yasuyuki HIRATSUKA, Northern Forest Research Centre, Canadian Forestry Service, Edmonton, Alberta, Canada T6H 3S5

An autoecious (pine-to-pine) blister rust (*Peridermium yamabense* Sano and Takahashi) on a Japanese soft pine (*Pinus pumila* Reg.) was monokaryotic throughout its life cycle. Hyphae in the pine tissue, young and mature aeciospores, and young germ tubes each possessed a single nucleus per cell. The nuclei in the germ tubes usually divided once and were separated by a septum. Tip cells were swollen to form fusiform "vesicles". Possible taxonomic and nomenclatural implications of the results will be discussed.

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CHARACTERIZATION OF HOST RESPONSE TYPES FOLLOWING INOCULATION OF SLASH PINE WITH THE PITCH CANKER FUNGUS. T. R. Meyer, and G. M. Blakeslee. Dept. of Forestry, Univ. Florida, Gainesville, FL. Branches of 3-yr-old field-grown slash pines (ca. 3 m ht.) were

inoculated with conidia of *Fusarium moniliforme* var. *subglutinans* to determine the roles of host genotype and pathogen isolate in disease expression. Three geographic isolates of the fungus were inoculated into separate branches on trees of 12 half-sib families with three replications. Three categories of host response were identified based upon external and gross internal symptoms; 1) immune-like response - absence of apparent symptoms, 2) callus response - compartmentalization of colonized host tissue within a border of hypertrophic callus, 3) diffuse canker - rapidly expanding area of necrosis lacking evidence of resistant host response. Variation in host response was not significant among pathogen isolates nor in isolate-family interactions. Families differed significantly ( $p < 0.0001$ ) in response type, indicating that symptom expression is a function of host genotype. These response types have been noted in previous studies on seedlings and on mature slash pines.

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THE OCCURRENCE OF *CERATOCYSTIS FAGACEARUM* - CONTAMINATED NITIDULIDS IN CENTRAL TEXAS. D. N. Appel, K. Andersen, T. Kurdyla, Dept. of Plant Pathology & Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843, and R. L. Lewis, USDA Forest Service, Hardwood Insect and Disease Research Laboratory, Stoneville, MS 38776

From 9 March to 28 September, 1984, 1653 free-flying nitidulids were trapped in central Texas oak stands and subsequently assayed for contamination with the oak wilt pathogen, *Ceratocystis fagacearum*, using a spermatization technique. A total of 24 beetles were found to be contaminated. Eleven of the contaminated beetles were caught in March and April when fungal mats were forming on diseased Spanish oaks (*Quercus texana*). Another 11 contaminated beetles were trapped in May, one month following the cessation of mat formation on monitored trees. Of the remaining contaminated beetles, one was trapped in June and one in August. The contaminated nitidulids consisted of three species, *Cryptarcha concinna*, *Colopterus maculatus*, and *Lobiopa undulata*.

## 21

THE RELATIONSHIP OF TEMPERATURE AND SHRINKAGE IN LONDON PLANE TREES WITH FROST CRACKS. E. B. Himelick & Dan Neely, Illinois Natural History Survey, 607 E. Peabody, Champaign IL 61820

Frost cracks in the stems of trees are longitudinal, radial separations of the wood and bark that gape at subfreezing temperatures. When stem temperatures return to the freezing point, the frost cracks close. The stem circumference and crack width on 20 established *Platanus acerifolia* trees in Urbana, Illinois, were measured during four consecutive winters. Below 0°C the tree circumference is directly correlated and crack width is inversely correlated with stem temperature. At temperatures of -20°C the area of the crack may constitute 2.5 percent of the stem. At a given time the wood temperatures at depths of 50 to 250 mm vary minimally both daily and seasonally. These observations support the hypothesis that frost cracks are the result of cellular drying as water migrates out of the walls and freezes in the lumens. With freezing and/or drying, wood shrinks tangentially twice as much as it does radially, resulting in the tension stress that causes wood failure.

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ASSOCIATION OF *VERTICILLIUM TRICORPUS* WITH *VERTICILLIUM DAHLIAE* AND POTATO EARLY DYING. Davis, J.R. and L.H. Sorensen, Univ. of Idaho Res. & Ext. Center, Aberdeen ID 83210.

Surveys throughout the potato growing areas of southern Idaho and eastern Oregon indicate negative correlations between *Verticillium tricorpus* Isaac and *Verticillium dahliae* Kleb - lower *V. dahliae* populations with higher populations of *V. tricorpus*. For a period involving several years (from 1978 to 1984), assays of replicated field plots have demonstrated negative correlations between populations of *V. tricorpus* in soil and potato early dying. Along with these associations, positive correlations were observed between *V. tricorpus* populations and potato yield. A greenhouse study provided evidence for cross-protection against *V. dahliae* by *V. tricorpus*. When *V. tricorpus* was inoculated in potato 7 days before *V. dahliae*, the severity of wilt was significantly reduced (0.05 P level) and the time required for symptom development was increased. Yield was increased with disease suppression.

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RELATIONSHIPS BETWEEN SOIL INOCULUM DENSITY OF *VERTICILLIUM DAHLIAE* AND SYSTEMIC COLONIZATION OF POTATO STEMS IN COMMERCIAL

FIELDS. P. C. Nicot and D. I. Rouse, Dept. of Plant Pathology, University of Wisconsin, Madison, WI 53706.

The incidence of stem colonization by *Verticillium dahliae* was assessed in commercial potato fields at various times during the growing season, and related to the inoculum density of the fungus in soil taken early in the season from the vicinity of the plants to be examined. A linear logit model adequately described this relationship for potato stems examined later than 90 days after planting (DAP) in 1983. The fungus could be isolated after 90 DAP from nearly 100% of the stems examined in areas where the soil inoculum density was greater than 5-7 propagules per gram of soil. Greater probabilities of isolating *V. dahliae* from stems, for given soil inoculum levels of the fungus, were occasionally associated with the presence of *Pratylenchus penetrans* in root systems of examined plants. Soft rot *Erwinia*, which were frequently isolated from stems, and *Colletotrichum coccodes*, had no effect on the frequency of isolation of *V. dahliae* from potato stems.

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PARASITIC FITNESS AND INTRASTRAIN DIVERSITY OF BENOMYL-SENSITIVE AND BENOMYL-RESISTANT SUBPOPULATIONS OF *VENTURIA INAEQUALIS*. M. Lalancette, K. D. Hickey, and H. Cole, The Pennsylvania State University Fruit Research Laboratory, Biglerville, PA 17307.

Benomyl-sensitive and benomyl-resistant isolates of *Venturia inaequalis*, obtained from two commercial apple orchards, were inoculated onto potted 'McIntosh' trees in the greenhouse. The sensitive and resistant subpopulations from the Schultz orchard had similar mean latent periods, sporulation capacities, colonization abilities, and infection efficiencies. However, the resistant subpopulation from the Bernard orchard was less fit than the sensitive subpopulation for these same four fitness parameters. We speculate that removal of benomyl from the spray program in the Bernard orchard may allow reversion of the population to sensitivity, but this would not occur for the Schultz orchard. Unlike the means, the diversities (Simpson's Diversity Index) of the distributions for each fitness parameter were not consistent. The sensitive subpopulations had higher diversity indices for some parameters but lower indices for others.

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ASSESSMENT AND PROGRESS OF SUGARCANE SMUT IN TIME. M. T. Momoi, L. H. Purdy, and R. A. Schmidt. Dept. of Plant Pathology, Univ. of Florida, Gainesville, Florida 32611.

Four cultivars of sugarcane were inoculated by the "needle-wound-paste" method with teliospores of *Ustilago scitaminea* Syd. and were planted in Canal Point, Florida. Incidence of smut was assessed based on percentages of infected plants, infected stalks and infected stalks per plant. Disease progress curves based on incidence of infected stalks or infected stalks per plant were similar. In the field experiments, assessment based on infected stalks is preferred. Disease progress curves were fit better by the Bertalanffy (n=1) and Compertz equations than by Mitscherlich (n=1) and logistic equations. The sugarcane-smut pathosystem had a low epidemic rate from Bertalanffy (n=1), 0.002, 0.025, 0.042, and 0.048 units per week for cultivars CP 75-1553, CP 57-603, CP-75-1091, and CP 65-357, respectively, but long latent and infectious periods. Physiological and morphological resistance were detected and different levels of physiological resistance were observed among cultivars.

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ULTRAVIOLET RADIATION AND SURVIVAL OF *SEPTORIA TRITICI* (*MYCOSPHAERELLA GRAMINICOLA*) CONIDIA. F. J. Gough and R. K. Mibey, USDA-ARS, Plant Science and Water Conservation Laboratory P.O. Box 1029, and Department of Plant Pathology, Oklahoma State University, Stillwater, 74076.

Extruded cirri of *Septoria tritici* exposed for 96 hr to continuous ultraviolet (UV = 254 nm) radiation (0.10 mW/cm<sup>2</sup>) contained viable conidia. Washed conidia and conidia mixed with conidial matrix (from the wash water) were exposed on nitrocellulose filters to 254 nm radiation (0.10 mW/cm<sup>2</sup>) for 0, 0.5, 1, 2, 3, 5, and 6 min; transferred in water to V-8 agar; and incubated for 10 days. A significant linear correlation (r = -0.82) was obtained between exposure and survival values. No conidia survived 6 min of exposure. Added matrix partially protected conidia from UV at exposures longer than 1 min. The results were the same whether the conidia were incubated in either continuous light (4800 lux) or darkness.

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DISEASE GRADIENTS OF BEAN RUST, L. A. Maffia<sup>1</sup>, R. D. Berger<sup>1</sup>, A. I. Khuri<sup>2</sup>, and T. A. Davoli<sup>2</sup>, <sup>1</sup>Plant Pathology Dept. and <sup>2</sup>Statistics Dept., Univ. of Florida, Gainesville 32611.

Severity and incidence of rust (*Uromyces phaseoli*) on bean (*Phaseolus vulgaris*), that developed from point, line, and area sources were fitted to six gradient models. The model of Kiyosawa and Shiyomi (Ann. Phyto. Soc. Jpn. 38:41-51) provided a good fit to the data. The equation  $\log(y) = \log(a) - b * \text{distance}$  was used to generate and compare parameters by Zellner's procedure (J. Am. Stat. Asso. 57:348-368). The weaker the strength of the source, the steeper was the gradient. However, the slopes were similar in the last half of the epidemic. Gradients based on severity were steeper than gradients for incidence of diseased leaves, which were steeper than those for incidence of diseased plants. The gradients in vectors predominately downwind from the source were flatter than those in upwind vectors. The slopes flattened as the maximum asymptote was reached.

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COMPARISON OF DISEASE PROGRESS RATES IN THE *Uromyces phaseoli* - *Phaseolus vulgaris* PATHOSYSTEM. L. A. Maffia<sup>1</sup>, R. D. Berger<sup>1</sup>, A. I. Khuri<sup>2</sup>, and J. E. Gallo<sup>2</sup>, <sup>1</sup>Plant Pathology Dept. and <sup>2</sup>Statistics Dept., Univ. of Florida, Gainesville, FL 32611.

There is a need for a statistically sound methodology to compare parameters of a set of regression lines. The SYSREG technique of SAS (1982) is appropriate, as it provides a means to compare correlated regression lines, using Zellner's procedure (J. Am. Stat. Asso. 57:348-368). This technique was used to compare epidemic rates of bean rust, calculated using the model  $-\log(-\log(y)) = a + k * t$ . The epidemic rates based on incidence of infected plants were faster than those for incidence of infected leaves or severity. For three inoculum sources (area, line, and point), the fastest rates were observed in epidemics originating from the point source in all assessments. The rates, in general, did not differ between epidemics of spring and fall, and were similar in the vectors that were predominantly downwind or upwind.

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PRODUCTION OF LOW MOLECULAR WEIGHT CARBOXYLIC ACIDS BY *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* AND THEIR POSSIBLE INVOLVEMENT IN PATHOGENESIS. David J. Robeson and Douglas R. Cook, ARCO PCRI, 6560 Trinity Ct., Dublin, CA 94568.

*X. campestris* is a pathogenic bacterial species comprising a large number of pathovars which are only distinguished with certainty by their host reaction. One such pathovar, pv. *campestris* (black rot of crucifers), is an economically important pathogen in many parts of the world. Several low molecular weight carboxylic acids produced by other pathovars have been reported as phytotoxins. During an investigation of pv. *campestris* for phytotoxins we observed the production of a range of carboxylic acids, but only when cultures were supplied with the appropriate precursor. For example, 3-methylthiopropionic acid only accumulated following growth in the presence of methionine. All compounds expressed only weak phytotoxicity and furthermore toxicity was virtually eliminated when assays were performed in buffered media. We conclude that these metabolites are unlikely to play a role in pathogenesis.

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BACTERIOGIN PRODUCTION BY *XANTHOMONAS CAMPESTRIS* PV. *GLYCINES*. W. F. Fett and G. T. Maher. Eastern Regional Research Center, USDA, ARS, 600 E. Mermaid Lane, Philadelphia, PA 19118

*Xanthomonas campestris* pv. *glycines* causes bacterial pustule disease of soybean. All sixteen strains of pv. *glycines* tested produced bacteriocins on nutrient agar. The bacteriocins were inhibitory towards strains of *X. c.* pv. *glycines* and other *X. campestris* pathovars. Optimal activity was obtained at 20°C and 2 days of incubation. Several agar media supported bacteriocin production. Bacteriocins were sensitive to heat (75° for 30 min) and to protease but were insensitive to chloroform. To our knowledge this is the first report of bacteriocin production by a xanthomonad. We propose the name of glycinecins for the *X. c.* pv. *glycines* bacteriocins.

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SURVIVAL OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN INFECTED

DEBRIS IN SALINAS, CALIFORNIA. M. L. Derie and N. W. Schaad, Dept. Pl., Soil & Ent. Sci., Univ. of Idaho, Moscow, ID 83843.

Eight stems of broccoli (*Brassica oleracea* var. *italica* L.) plants inoculated with *Xanthomonas campestris* pv. *campestris* (Xcc) were harvested after 120 days and each divided into two 7 to 10 cm long sections. After initial sampling, four sections were buried 8 to 13 cm and four placed on the surface of soil in Salinas, Calif. on 12/21/83 at site I and on 1/17/84 at site II. At specified intervals, sections were removed and sent to Moscow, Id. for dry weight determination and for assaying on three semiselective agar media, SM, SX, and BSCAA. Buried sections were positive for Xcc up to 145 days in site I, and 59 days in site II, whereas sections at the surface were positive through 360 and 333 days in sites I and II, respectively. Half-life of Xcc in buried and surface stems was 16.6 and 64.1 days at site I, and 11.8 and 61.5 days at site II, respectively. These data indicate that Xcc is likely to survive in debris up to one year in soil and up to four years at the surface in Salinas, Calif.

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THE HYPERSENSITIVE RESPONSE IN TOMATO TO *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*. J. B. JONES AND J. W. SCOTT. Gulf Coast Res. & Ed. Ctr., Bradenton, FL.

Bacterial spot of tomato, incited by *Xanthomonas campestris* pv. *vesicatoria* (XCV), is an extremely destructive disease in Florida. Nearly 300 tomato genotypes were screened in the field for bacterial spot resistance. One genotype, Hawaii 7998, was highly resistant. In electrolyte leakage studies, leaflets of Hawaii 7998 and 'Walter', a known susceptible were injected with  $10^8$ ,  $10^7$ ,  $10^6$ , or  $10^5$  c.f.u./ml of XCV. After 24 h electrolyte leakage increased from 45 and 44  $\mu$ MOS to 97 and 107  $\mu$ MOS in Hawaii 7998 leaf disks that had been infiltrated with  $10^8$  and  $10^7$  cfu/ml, respectively; however in 'Walter' leaf disks, no electrolyte leakage increase was observed. Populations of XCV in leaves injected with  $10^5$  c.f.u./ml. were consistently 50 to 100 times lower in Hawaii 7998 leaflets than in 'Walter' leaflets from day 4 through day 15 after infiltration. These data provide strong support for a hypersensitive reaction in Hawaii 7998 and the claim that it is the first recognized source of absolute resistance in tomato to bacterial spot.

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CHARACTERIZATION OF *ERWINIA AMYLOVORA* THROUGH FATTY ACID PROFILING. T. van der Zwet, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430 and M. Sasser, Plant Science Department, Univ. of Delaware, Newark, DE 19711.

A total of 143 isolates of *Erwinia amylovora* from various rosaceous hosts were collected from 5 states and 10 foreign countries. To determine composition of the cellular fatty acids of the bacteria, isolates were grown on trypticase soy broth with agar added. Extracts were prepared by saponification of whole bacterial cells, liberation of fatty acids, and gas liquid chromatography. Fatty acid peaks were identified by a computer-calculator. The most abundant fatty acids were straight-chain 12:0, 14:0 and 16:0; unsaturated 16:1 and 18:1; and 14:0 3OH and 17:0 cyclopropane. Minor fatty acids included straight-chain 15:0, 17:0 and 18:0. The saturated even-carbon chains comprised about 50%, the unsaturated acids 30%, cyclic acids 5%, hydroxy substituted chains 4%, and saturated odd-carbon chains 2% of total fatty acids. Fatty acid class analysis and a low standard deviation between acids indicated relative uniformity within the *E. amylovora* group.

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COMPETITIVE INTERACTIONS BETWEEN ANTIBIOTIC PRODUCER AND SENSITIVE *ERWINIA* STRAINS DURING POTATO TUBER SOFT ROT DEVELOPMENT. Paige E. Axelrood and M. N. Schroth, University of California, Berkeley, CA 94720.

Equal numbers of antibiotic producing strains of *Erwinia carotovora* subsp. *betavasculorum* (ECB-P) ( $nal^R rif^B$ ) and antibiotic sensitive strains of *Erwinia carotovora* subsp. *carotovora* (ECC-S) ( $nal^S rif^R$ ) were inoculated singly and in combination into aerobic wounds in whole potato tubers. ECB-P in single or mixed treatments and ECC-S in single strain treatments reached population densities of  $1 \times 10^{10}$  CFU/g of rotted tissues by 48 hr at 24°C. Inhibition of ECC-S was evident within 24 hr in mixed ECB-P/ECC-S inoculations. By 48 hr, ECC-S population densities were one hundredfold lower than that at inoculation time and were one millionfold lower than when ECC-S was inoculated alone. Growth of ECC-S, in ECB-P/ECC-S joint inoculations was similarly inhibited at 28°C and when initial ECC-S inoculum was 10X that

of ECB-P. Antibiotic resistant ECC-R strains were not inhibited in ECB-P/ECC-R joint inoculations. A diffusible inhibitory substance was found in ECB-P soft rot tissue.

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APPEARANCE OF STREPTOMYCIN-RESISTANT *ERWINIA AMYLOVORA* IN MISSOURI APPLE ORCHARDS. W.H. Shaffer and R.N. Goodman, Dept. of Plant Pathology, University of Missouri-Columbia, 65211.

Streptomycin has been used to control fireblight in Missouri apple orchards since the mid-1950's. Despite several reports from growers that streptomycin had become ineffective, resistance of *Erwinia amylovora* to the antibiotic was never detected in these orchards. The lack of control was usually a result of improper spray timing or inadequate applications by the grower. However, for a second consecutive year, *E. amylovora* resistant to >500  $\mu$ g of streptomycin/ml has been recovered from stem tissue of Jonathan and other apple varieties that showed typical fireblight symptoms. A field trial was conducted during 1984 in one orchard where resistance to streptomycin had been detected in 1983. Treatment with five streptomycin spray applications at 100  $\mu$ g/ml was compared with a nontreated control. The mean numbers of blighted twigs/tree 355 and 294, respectively, were not significantly different ( $P=0.05$ ). Resistance to streptomycin appears to be localized in three neighboring orchards in west-central Missouri.

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Infrared thermometry for determination of root rot severity in beans. Tu, J. C. and C. S. Tan. Research Station, Harrow, Ontario, Canada NOR 1G0

Leaf and air temperatures taken with an infrared thermometer between 1400 and 1500 h on a sunny day showed that green leaves of plants with root rot were warmer than those of healthy plants in soil maintained at field capacity. Increases in leaf temperature corresponded closely to increases in severity of root rot. Such correlation was more apparent in plants with one or two trifoliate leaves than in older plants. When plants were grown under water stress, the differences between leaf and air temperatures were much greater in diseased plants than in healthy plants. When plants were stressed almost to the wilting point and water was added to return soil to field capacity, decreases in leaf temperatures of healthy plants were larger than those of diseased plants. In diseased plants, decreases in leaf temperatures subsequent to watering were inversely related to the severity of root rot. Thus leaf temperatures can be used for the early detection of root rots and for estimating their severity without removing plant from the soil.

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FREQUENCY AND DIVERSITY OF MYCOPHAGOUS VAMPYRELLIDAE IN TOBACCO FIELD SOIL. T. R. Anderson, Agriculture Canada, Research Station, Harrow, Ontario NOR 1G0

Soil from within rows of burley tobacco was bioassayed at intervals during the growing season to assess the population and diversity of Vampyrellidae in field soil. Populations ranged from a high of 34/g of soil in June to a low of 3/g of soil in September. The average population of *Vampyrella* sp. was low (4/g of soil) but constant during the sample period. Populations of

*Theratomyxa* spp. were high in June (22/o of soil) but decreased as the season advanced. Two distinct isolates of *Theratomyxa* were identified as isolates "a" and "b". Trophozoites of isolate "a" move in an amoeboid manner and form large double-walled resting cysts. Trophozoites of isolate "b" generally float and seldom form double-walled resting cysts. Both isolates lyse conidia of *Bipolaris sorokiniana* within a digestive cyst and make small perforations in walls of conidia.

### 39

**INFLUENCE OF SOIL MATRIC POTENTIAL ON <sup>14</sup>C-EXUDATION FROM FUNGAL PROPAGULES.** A. B. Filonow, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

The <sup>14</sup>C-exudation from labeled conidia of *Bipolaris victoricae* or sclerotia of *Macrophomina phaseolina* was measured in a sandy loam soil at 0, -50, -100 and -200 mbar matric potential. Propagules in a filter membrane 'sandwich' were incubated in soil in a Buchner funnel with fritted glass plate, serving as a tensiometer, and modified to allow the passage of moist air over the soil. The <sup>14</sup>CO<sub>2</sub> from soil respiration of exudate was measured every 24 h. After 5 days the propagules were removed, germination assessed, and <sup>14</sup>C in the propagules determined. <sup>14</sup>C-exudation was greatest in soil at 0 mbar and decreased with decreasing soil moisture. At 0 mbar <sup>14</sup>C exuded from conidia and sclerotia were 20.9% and 5.5%, respectively. For both fungi the effect of matric potential on soil respiration was in the order: -50 > -100 > -200 mbar. Soil with conidia evolved 2-3.5 times more <sup>14</sup>CO<sub>2</sub> than that from soil with sclerotia. Germination of both fungi (0-11%) was suppressed during the 5 day incubation.

### 40

**DEVELOPMENT OF THE FUNGAL MICROFLORA ASSOCIATED WITH LIVE ROOTS OF COTTON (*GOSSYPOLIUM HIRSUTUM*).** G.A. Fisher and O.C. Huisman, Dept. Plant Pathology, University of California, Berkeley, CA 94720.

The growth of fungal colonies in the rhizosphere was estimated, using two chitin assays and a modified ELISA procedure, for cotton plants grown in *Verticillium dahliae* (Kleb.) infested soil. The chitin content of cotton roots was found to increase with increasing distance from the root tip. This increase, interpreted using chitin contents of fungi grown in vitro, suggests at least a four-fold increase in the fungal biomass between the root tip and tissue ten centimeters back from it. Similar trends were shown using serological assays which employed antisera specific to the soluble protein fractions of *V. dahliae* and *Penicillium* spp. Roots obtained from the field had a larger fungal component than roots grown in the greenhouse in field soil.

### 41

**EFFECT OF ROTATION CROPS ON PLANT VIGOR AND YIELD OF POTATO.** Gene D. Easton and Michael E. Nagle, Department of Plant Pathology, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350

Plots of field corn, sweet corn, wheat, sudan grass, wheat + sudan grass and potato cv. Russet Burbank were replicated six times and cropped under sprinkler irrigation on *Verticillium dahliae* infested soil two consecutive years. Each spring, all crop residue and potato tubers were rototilled into the soil. The third year, all plots were fertilized with 336 Kg N/ha and planted to potatoes. Soil assays showed all plots were infested with *V. dahliae*. Potato plants were taller and yielded 140-280 q/ha more following rotation crops than following two previous years of potatoes. Potato plants in all plots were infected with *V. dahliae*, but appeared healthy until late September. Root pathogens other than *V. dahliae* apparently caused yield losses in plots cropped two previous years to potatoes.

### 42

**EFFECT OF SOIL DISTURBANCE AND NH<sub>3</sub> ON THE ACTIVITY OF SEVERAL SOILBORNE FUNGI.** D.M. Huber, S.Y. Liu, and D.W. Nelson, Purdue University, W. Lafayette, IN 47907; North Carolina State University, Raleigh, NC; and University of Nebraska, Lincoln, NB.

Activity of *Fusarium*, *Rhizoctonia*, *Pythium*, *Trichoderma*, and *Gliocladium* in soil distributed by an 8-mm wide NH<sub>3</sub> knife was evaluated with the plate profile technique. Anhydrous ammonia

with or without nitrapyrin was injected with the knife and a nylon string implanted to indicate the point of NH<sub>3</sub> release. Physical disruption of the soil stimulated activity of *Fusarium* as fungistasis was nullified in the disrupted soil. Fungitoxicity was limited to one week in the zones of highest NH<sub>3</sub> concentration. *Fusarium*, *Rhizoctonia*, and *Trichoderma* were more active with NH<sub>3</sub> than without. *Pythium* and *Gliocladium* activity was not affected by soil disruption or N amendment. Fungistasis may be an important mechanism of biological control of diseases caused by *Fusarium* in no-till programs.

### 43

**PRODUCTION OF AFLATOXINS IN FIELD CORN WITH *ASPERGILLUS FLAVUS* AND *A. PARASITICUS* AS INOCULUM.** David M. Wilson, William W. McMillan and Neil W. Widstrom, University of Georgia and USDA-IBPMRL, Coastal Plain Experiment Station, Tifton, GA 31793

*Aspergillus flavus* Link and *Aspergillus parasiticus* Speare have been used to study aflatoxin production in corn but few studies have directly compared these fungi under field conditions. Experiments were designed to compare aflatoxin B<sub>1</sub>, G<sub>1</sub> and total aflatoxin (B<sub>1</sub> + B<sub>2</sub> + G<sub>1</sub> + G<sub>2</sub>) production in preharvest inoculated ears. Developing ears were inoculated with *A. flavus* (NRRL 3357) and *A. parasiticus* (NRRL 2999). Four replications of two inoculation methods were used with each isolate. Spores were applied to silks or introduced by wounding with a contaminated knife. Aflatoxin concentrations were determined in shelled kernels from each plot after harvest. There were no significant differences in the B<sub>1</sub> contamination between isolates but there were differences in B<sub>1</sub> between inoculation methods. Isolates reacted differently for G<sub>1</sub> and total aflatoxins depending on the inoculation technique used.

### 44

**THE INFLUENCE OF *ASPERGILLUS FLAVUS* ON PARTICLE SIZES OF MAIZE KERNELS AND AFLATOXIN LEVELS.** C. S. Stauffer, J. R. Wallin, and M. S. Zuber, Dept. of Plant Path. and USDA-ARS, and Dept. of Agronomy, Univ. of Missouri-Columbia.

Eight replications of a four parent maize diallel of inbreds Mo17, N107, N78, and Mo5 and 100 ears of B73 X Mo17 were inoculated with *Aspergillus flavus* in the field. All kernels were coarse ground and separated into three particle sizes with U.S. standard sieves #20 and #60. Particle size 1 would not pass through a #20 screen, size 2 passed through a #20 screen but not through a #60 screen, and size 3 passed through a #60 screen. Significant differences were found in aflatoxin levels among particle sizes. Size 2 contained the highest levels of toxin, and size 1 had the lowest levels. These results indicate that particle size variation in ground maize may play a role in sampling error when measuring aflatoxin levels in the samples.

### 45

**MAIZE YIELD AND THE INCIDENCE OF AFLATOXIN** J.R. Wallin, II, Minor, and G. E. Rottinghaus, Dept. of Plant Pathology, USDA-ARS; Dept. of Agronomy and Dept. of Veterinary Biomedical Sciences, Univ. of Missouri-Columbia.

High levels of aflatoxin (AFB<sub>1</sub>) have long been suspected of being associated with low yields caused by drought stress. To date this has not been documented. In 1982, the average maize yield of 4 hybrids at 6 locations ranged from 102 to 158 bu/acre with an average of 144 and the AFB<sub>1</sub> levels ranged from 0ppb at 5 locations to a trace at 1 location. In 1983, 6 hybrids were tested. The average bu/acre yield of the 6 hybrids for the 7 locations ranged from 7 to 121 and the AFB<sub>1</sub> levels were the highest where the yield was 78 bu/acre or less. This situation prevailed at 6 locations where the levels ranged from 28 to 70ppb. In 1984, 12 hybrids were analyzed for toxin at 7 locations. The average yield for the 12 hybrids ranged from 48 to 151 bu/acre. Yields were low, 48 and 56 bu/acre, at 2 locations where AFB<sub>1</sub> levels were the highest, 37 and 38ppb.

### 46

**AMINO ACID STIMULATION OF AFLATOXIN PRODUCTION IN GROWTH AND STATIONARY PHASE CULTURES OF *ASPERGILLUS FLAVUS*.** S. F. Marsh and G. A. Payne, Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC 27695-7616.

Aflatoxin production by *Aspergillus flavus* occurs mainly during stationary phase. Although amino acids are known to stimulate aflatoxin production, it is not known if their effect is on

growth or stationary phase. Mycelial mats from three NRRL strains (3251,3357,6513) were transferred at the end of growth phase (4 days) into one of two resuspension media (RM): RM1 without amino acid and RM2 with one of six amino acids (ala, asp,asn,glu,gln,pro). Mycelium transferred into RM2 was initially grown in SLS medium (Reddy et al., 1971) containing the same amino acid. Aflatoxin B<sub>1</sub>, B<sub>2</sub>, and mycelial dry weight were measured after 4 days in resuspension culture. The presence of pro or glu during growth or stationary phase supported the highest B<sub>1</sub> yields for all strains. Although mycelial dry weights were higher from RM2, aflatoxin production in RM1 and RM2 was not different. It appears that amino acids added during stationary phase do not increase aflatoxin production.

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REDUCTION OF AFLATOXIN LEVELS IN MAIZE DUE TO IRRIGATION AND TILLAGE. G. A. Payne, D. K. Cassel, and C. R. Adkins, Depts. of Plant Pathology and Soil Science, North Carolina State University, Raleigh, NC 27695-7616.

The effect of irrigation and tillage on infection and aflatoxin production by *Aspergillus flavus* was studied in NC over a 4 year period. Aflatoxin levels in nonirrigated, naturally infected plots in 1980, 1981, 1982, and 1983 were, respectively, 94, 1,249, 95, and 84 ug/kg. Corresponding aflatoxin levels for irrigated plots were 25, 78, 63, and 45 ug/kg. Inoculation of corn silks resulted in more aflatoxin than natural infection. Irrigation in silk-inoculated plots reduced aflatoxin levels from 517 to 110 ug/kg in 1982 and from 1,111 to 288 ug/kg in 1983. In silk-inoculated plots, subsoiling reduced aflatoxin levels from 517 to 113 ug/kg in 1982, and from 1,111 to 315 ug/kg in 1983. Numbers of infected kernels were also greater in nonirrigated plots. These studies indicate that drought conditions result in greater aflatoxin levels and that either irrigation or tillage to reduce water stress reduces aflatoxin levels.

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RATE OF AFLATOXIN ACCUMULATION IN INOCULATED MAIZE EARS UNDER FIELD CONDITIONS. G. A. Payne and W. M. Hagler, Dept. of Plant Pathology and Dept. of Poultry Science, North Carolina State University, Raleigh, NC 27695-7616.

Maize ears were either silk-inoculated (SI) or wound-inoculated (WI) with *Aspergillus flavus* and analyzed weekly for levels of aflatoxin. Aflatoxin appeared in the WI-treatments within one week after inoculation and peaked at 6, 7, and 9 weeks after inoculation, respectively, at Rocky Mount and Clayton in 1982, and at Clayton in 1983. Aflatoxin levels peaked one week later in the SI-treatments. In 1983, the increase in aflatoxin was associated with an increase in the number of infected kernels and a decrease in kernel moisture from 59 to 16%. As kernel moisture decreased below 16%, aflatoxin levels declined from 2,764 on Sept 16 to 1,690 ug/kg on Oct 7 in the WI-treatments and from 1,534 ug/kg to 1,060 ug/kg in the SI-treatments. Aflatoxin levels and the number of infected kernels increased later in the season as kernel moisture increased due to late season rains. These data indicate that aflatoxin levels fluctuate during the season and are related to kernel moisture.

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DEOXYNIVALENOL IN *Fusarium*-INFECTED WHEAT AND IN BREAD MADE FROM INFECTED WHEAT. H.K. Abbas, C.J. Mirocha, R.J. Pawlosky, and D.J. Pusch, Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

Samples of wheat naturally infected by *Fusarium graminearum* were obtained from 1982 crops of Nebraska, Missouri, Kansas, and Minnesota and analyzed for deoxynivalenol (DON). DON was found throughout all the milling fractions (bran, shorts, reduction flour, and break flour) of the milled wheat. The DON recoveries for each mill run were 90-98%. Regardless of DON concentration, these samples gave similar fractional distributions of DON. DON concentration was greatest (21 ppm) in the bran, and smallest (1 ppm) in the break flour. Cleaning and milling did not remove DON, and DON was not destroyed in the bread baked from the naturally contaminated wheat; however the effect on DON concentration in the samples analyzed was variable--the reduction ranged from 24 to 71%. The percentage reduction in cleaned wheat ranged from 6-19%. DON concentrations in the following commercially made breads, caraway rye, seedless rye, and pumpernickel, were 45 ppb, 39 ppb, and 0 ppb, respectively.

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EFFECT OF GIRDLING SOUR ORANGE SEEDLINGS ON MYCORRHIZAL DEVELOPMENT. R.M. Davis and J.E. Fucik. Texas A&I Univ. Citrus Center, P.O. Box 1150, Weslaco, TX 78596.

Six-month-old nonmycorrhizal or mycorrhizal (*Glomus fasciculatus*) sour orange seedlings grown in sand supplemented with 0, 50, or 100 ug P/g soil were completely girdled 5 mm below the basal leaves. Six wks later root extracts and exudates were analyzed and half of the nonmycorrhizal plants were inoculated with *G. fasciculatus*. Reducing sugars were generally greater in exudates from girdled nonmycorrhizal plants than from nongirdled or mycorrhizal plants. In root extracts levels of reducing sugars in nonmycorrhizal plants decreased with increasing soil P and were greater from girdled plants than from nongirdled plants. Levels of amino acids were not consistently affected by any treatment. Roots of the girdled plants at 0 ug P/g soil become mycorrhizal to the same degree as in the nongirdled plants but the fungus did not appreciably infect girdled plants at 50 or 100 ug P/g soil.

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INCIDENCE OF VA MYCORRHIZAL FUNGI ASSOCIATED WITH CITRUS IN FLORIDA J. J. FERGUSON and N. C. Schenck, University of Florida, Gainesville 32611

Fifty mature citrus groves in 5 central and southern Florida counties were assayed for VA mycorrhizal fungi. Spores extracted from soil by wet sieving were identified and both washed roots and root-soil mixtures from each sample were used to establish pot cultures with bahiagrass (*Paspalum notatum*) to recover species not sporulating abundantly in soil. A total of 34 species were recovered, with *Glomus* spp. being the most common, and *G. deserticola* and *G. intraradices* being the most common in soil samples and bahiagrass pot cultures, respectively. Nine species of Acaulospora were recovered from soil samples, with *A. mellea* and *A. morrowae* being the most common. Eight species of *Gigaspora*, occurring less frequently than either *Glomus* or Acaulospora spp., and one species each of *Entrophospora* and *Sclerocystis* were also recovered. Most samples had 4 species present and total spore numbers ranged from 6 to 1050 per liter of soil.

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THE EFFECT OF THE ADDITION OF SODIUM CHLORIDE AND CALCIUM PHOSPHATE TO SOIL ON ENDOMYCORRHIZAE OF GUAYULE (*PARthenium ARGENTATUM*). C.M. Pfeiffer and H.E. Bloss, Dept. of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721.

The effects of the addition of 100ug/g of Na as sodium chloride and 750ug/g of P as calcium phosphate on the intensity of infection and the fungal structures present in Guayule roots inoculated with *Glomus intraradices* were observed. The addition of either NaCl or Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> decreased the incidence of arbuscules and vesicles while the incidence of hyphae remained unchanged. The addition of NaCl decreased the intensity of infection of Guayule roots compared with the control and the Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> treatments. The addition of both NaCl and Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> had a synergistic effect on the mycorrhizae. The numbers of hyphae, arbuscules and vesicles observed in the roots and the intensity of infection decreased with the addition of both NaCl and Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>.

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PLANT RESPONSES TO RHIZOPOGON VINICOLOR ELICITORS. Margaret E. Coleman and Anne J. Anderson, Department of Biology, Utah State University, Logan, UT 84322.

A plant's fitness may be enhanced by positive selection for the ability to recognize pathogen components, termed elicitors, which trigger pleiotropic defense responses. Elicitors have been purified from culture filtrates of *Rhizopogon vinicolor*, a fungus that forms mutualistic ectomycorrhizae with Douglas-fir but not with lodgepole pine. Partially-purified *R. vinicolor* elicitors induced browning of both compatible Douglas-fir and incompatible lodgepole pine callus. These elicitors were also active on non-host red kidney bean cotyledons. Elicitor treatments of less than 5 ug of carbohydrate per cotyledon induced accumulation of low molecular weight and condensed phenolics. The most potent *R. vinicolor* elicitors were carbohydrate-rich fractions which did not adsorb to DEAE-Sephadex. Fractions which adsorbed to DEAE-Sephadex exhibited weaker elicitor activity.



TALLGRASS PRAIRIE FORBS AND MYCORRHIZAE. B. A. Deniele  
Herick, J. M. Zajicek and C. E. Owensby, Departments of Plant  
Pathology, Horticulture and Agronomy, Kansas State University,  
Manhattan, KS 66506.

Because tallgrass prairie forbs are generally deep-rooted to avoid competition with grasses and VA mycorrhizal fungi are generally found in the upper 50 cm soil, the mycorrhizal status of prairie forbs was studied. Excluding Asclepias and Liatris spp., forb roots removed from cut banks or an excavated pit were mycorrhizal to depths up to 210 cm. Though Glomus fasciculatum, G. constrictum, Sclerocystis coremioides, Gigaspora heterogama, G. albida and an Endogone sp. were found in upper soil layers of the excavated pit only G. fasciculatum spores were observed from 100 cm to 220 cm depths. When the impact of VA mycorrhizae on prairie forbs and grasses was studied in sterilized and nonsterile prairie soil in the greenhouse, a significant growth response was observed in sterilized soil with all plant species tested. However, this growth response was suppressed in nonsterile soil.

## 55

COMPARATIVE VAM ESTABLISHMENT ON A PREDICTABLE VERSUS AN UNPREDICTABLE SITE: EARLY SUCCESSIONAL MYCOTROPHY ON MOUNT ST. HELENS AND KEMMERER WY. M.F. Allen, E.B. Allen, and R. Vali, Ecology Center, Utah State University UMC 45, Logan, UT 84322.

Succession theory suggests that on a predictable site the biota may regulate establishment but on unpredictable sites the abiotic environment influences succession. We monitored plants which were inoculated with VAM at both types of sites. On Mount St. Helens (a predictable site), the early successional plants were predominantly mycotrophic species. Initially plant survival was greatest on gopher mounds with VAM. During the drought of 1984, tephra plants had greater drought stress than VAM plants on the mounds. On a Wyoming strip mine (an unpredictable site), non-mycotrophic weeds were the initial colonizers. Following inoculation, Salsola kali, the dominant invader, had reduced cover. The competing mycotrophic grasses had reduced drought stress. We suggest that at Mount St. Helens, VAM are important for initial plant establishment whereas at the Wyoming site, VAM are important for the transition from initial colonizers to later successional species.

## 56

TOXICITY OF COPPER FUNGICIDE IN SOIL TO CITRUS AND VESICULAR-ARBUSCULAR MYCORRHIZAE. J. H. Graham, L. W. Timmer and D. Fardeimann, Univ. of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850.

Carrizo citrange seedlings were planted in a limed sand soil (pH 6.8) with double-actd extractable (DAE) copper levels from 3-248  $\mu\text{g g}^{-1}$  soil [Cu added as  $\text{CuSO}_4 \cdot 3\text{Cu}(\text{OH})_2 \cdot \text{H}_2\text{O}$ ]. Growth of seedlings and colonization by Glomus intraradicis was reduced logarithmically with Cu level. Minimum toxic levels of DAE Cu ranged from 19-34  $\mu\text{g g}^{-1}$  soil. Leaf P content decreased linearly with DAE Cu for mycorrhizal seedlings without added P but not for non-mycorrhizal plants with supplemental P. The Cu-induced reduction in P uptake by mycorrhizal plants was more closely related to the inhibition of hyphal development in the rhizosphere than to development of vesicles and arbuscules in the root. Thus, Cu-induced P deficiency was attributed to inhibition of P uptake by mycorrhizal hyphae in soil. In a citrus grove soil with DAE Cu  $>80 \mu\text{g g}^{-1}$  and pH <5, stunted replants had reduced mycorrhizal colonization compared to unaffected trees.

## 57

TIMING ONION LEAF BLIGHT SPRAYS WITH A DEDICATED DISEASE PREDICTOR IN MICHIGAN. M. L. Lacy, Department of Botany and Plant Pathology, Michigan State University, E.Lansing, MI 48824.

Field testing of a disease prediction system for onion leaf blight (Botrytis squamosa) developed by Lacy and Pontius (Phytopathology 73:670-676) was carried out in Michigan onion fields. This prediction system uses average temperatures and humidities from the previous three days to predict spore releases. Spore trapping indicated that the system accurately predicted epidemiologically significant spore releases. Sprays were applied to different sections of onion fields either weekly or when the sporulation index value (SIV) was  $>50$  (provided a spray had not been applied in the previous 7 days). Using the predictor, spray applications were reduced by two to six over weekly sprays during a 5 yr period with no significant

differences in disease severity. This prediction model has been installed by Reuter-Stokes Inc. (Cleveland, OH) in a dedicated computerized field instrument which will be available for the first time in 1985.

## 58

RELATIONSHIP BETWEEN THREECORNERED ALFALFA HOPPER DAMAGE AND POD AND STEM BLIGHT AND STEM ANTHRACNOSE DISEASES OF SOYBEAN IN LOUISIANA. J.S. Russin and D.J. Boethel, Dept. of Entomology, Louisiana State Univ., Baton Rouge, LA 70803-1710

Soybean (Glycine max) plants of three maturity groups (V,VI, VII) were examined at harvest for characteristic main stem girdles caused by the threecornered alfalfa hopper (TCAH) (Spissistius festinus) and for disease caused by Phomopsis sojae (pod and stem blight)(PSB) and Colletotrichum dematium var. truncatum (stem anthracnose)(SA). Incidence of PSB and SA did not differ significantly between TCAH-girdled and non-girdled stems for each maturity group. To better explore influence of TCAH on colonization by PSB and SA, greenhouse-grown soybeans that were girdled by TCAH were inoculated with these fungi and sprayed *in situ* after 7 da with Paraquat for visualization of fungal infections. Lengths of stem sections colonized by each of these fungi were measured 7 da later and did not differ significantly on girdled or non-girdled stems.

## 59

EFFECT OF SOYBEAN PLANTING DATE AND SOIL NUTRIENTS ON INCIDENCE OF RED CROWN ROT AND POPULATIONS OF INSECTS ASSOCIATED WITH ROOTS. J.S. Russin, N.N. Truxclair, Jr., D.J. Boethel, Dept. of Entomology, and E.C. McGawley, Dept. of Plant Path. and Crop Physiol., Louisiana State Univ., Baton Rouge, 70803-1710.

Red crown rot (Calonectria crotalariae) was observed in a study conducted to determine effects of planting date (5/30, 6/21, 7/12/84) on insect populations in 'Brugg' soybean. Incidence of perithecia on stems was recorded at harvest and was associated with soils that were found to have significantly higher levels of K, Ca, Mg, Zn, and organic matter. Season-long populations of bean leaf beetle (BLB) also were higher on these soils. Significant differences by planting date were obtained in these plots for percentage of stems with perithecia (82.5, 30.0, and 2.5%) and for distance from soil line to perithecia highest on stem (114, 39, and 12 mm) for planting dates 5/30, 6/21, and 7/12, respectively. Populations of BLB and banded cucumber beetle (BCB) also showed significant differences by planting date, but populations were highest on the 7/12 planting, thus exhibiting a trend opposite to that exhibited by C.crotalariae.

## 60

ASSESSMENT OF FARMER UTILIZATION OF TOBACCO PEST MANAGEMENT PROGRAMS IN NORTH CAROLINA. H. W. Kirby, Department of Plant Pathology, University of Illinois, Urbana, IL, 61801, and C. E. Main, Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27650.

Personal interviews were conducted with 82 randomly selected tobacco farmers and 84 tobacco farmers involved in extension supported integrated pest management (IPM) programs in eight eastern North Carolina counties during 1980-1981 to determine if IPM program technology was being adopted and used by program farmers. Results of the survey indicated that there were significant differences between the two groups including differences in crop rotation practices, soil sampling and nematode management, information sources, nematicide selection, biographical and farm size data. Group membership was correctly predicted for 81% of the respondents by discriminant analysis based upon 15 significant variables identified by factor analysis.

62

A MORPHOLOGICAL RESISTANCE TO BACTERIAL SOFT ROT IN TOMATO FRUIT. Jerry A. Bartz. Plant Pathology Dept., Univ. of Florida, Gainesville, 32611.

The ranking of tomato cultivars for resistance of fruit to bacterial soft rot after inoculation by infiltration was correlated with the physical resistance of the stem scar to water intrusion. Over 85% and 94% of the fruit that absorbed  $>0.05$  and  $>0.15$ g/fruit of an aqueous suspension of *Erwinia carotovora* pv. *carotovora* ( $1 \times 10^6$  cfu/ml), respectively, were diseased within 14 days after inoculation, whereas less than 24% were diseased when the weight increase was less than 0.05 g. Fruits of Florida MH-1 were relatively resistant when wound inoculated by contaminated needles but were the most susceptible of 24 cultivars when inoculated by infiltration. MH-1 fruit absorbed up to eight times more suspension than the most resistant entry. Significant field-location and picking-time effects were observed but did not obscure the larger differences among cultivars.

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THE IMPACT OF MACHINE-HARVESTING AND FUNGICIDES ON ROT AND PHYSIOLOGICAL BREAKDOWN OCCURRING IN COLD-STORED CRANBERRIES. P. R. Bristow and G. E. Windom, Washington State University (WREC), Puyallup, 98371.

Fungal rots accounted for nearly one-half (11.6%) of the total number of machine-harvested McFarlin cranberries that rotted (24.3%) when stored for 12 weeks at 3°C; physiological (sterile) breakdown (PB) accounted for the rest. Harvesting berries by hand reduced the incidence of PB but not fungal rots. The incidence of PB in hand-picked fruit increased with time in storage; 0.2, 1.8 and 5.8% after 4, 8 and 12 weeks, respectively. Surface disinfecting berries prior to storage did not reduce fungal rot. Fungicides, including chlorothalonil (2.3-5.8 kg/ha) and mancozeb (3.6 kg/ha), applied after anthesis but no later than 53 days before harvest (2-4 applications) reduced fungal rots occurring in storage by 50-80% but had no effect on PB. *Fusicoccum putrefaciens* was the most frequently isolated fungus (52.5%). Relative frequencies of the fungi isolated were only slightly affected by the fungicides.

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PHYTOTOXICITY IN CALIFORNIA CITRUS FRUIT FUMIGATED WITH METHYL BROMIDE. L. G. Houck and J. F. Jenner, HCRL, USDA, ARS, 2021 South Peach Avenue, Fresno, California 93727

Methyl bromide (MB) is being considered as an alternative fumigant to ethylene dibromide for quarantine control of tephritid fruit flies in citrus fruit. Commercially washed, waxed and packed California citrus fruits were treated with 40 g/m<sup>3</sup> MB for 2 hr at 20 C, a recently approved treatment for citrus imported into the U.S. Lemons were more susceptible to injury than Minneola tangelos or navel oranges. Grapefruit were injured less than the other citrus. Curing fruit for 1 wk at 15 C prior to fumigation reduced injury by 15-20% in lemons, oranges and tangelos, and by 4% in grapefruit. Post-fumigation aeration at 15 C for 1 wk before storage at cooler temperatures also reduced injury, but to a lesser degree than curing. MB fumigation increased decay by 10-15% in lemons, oranges and tangelos, and 2% in grapefruit. Treatment with 40 g/m<sup>3</sup> MB under the conditions tested is not practical for California lemons, oranges or tangelos, but may be marginally useful for grapefruit.

65

GLUCOSE CONCENTRATION IN GROWTH MEDIA AFFECTS SPORE QUALITY OF *MONILINIA FRUCTICOLA*. Douglas J. Phillips and Dennis A. Margosan, Horticultural Crops Research Laboratory, USDA, ARS, 2021 South Peach Avenue, Fresno, California 93727

Spores of two isolates of *Monilinia fructicola* were produced at 15 and 25 C on potato dextrose agar containing 2, 15 or 30% glucose. Spore volume, nuclear number (nuclei per spore) and spore aggressiveness (rot causing ability) changed with temperature and sugar concentration. Temperature affected the spore volume, nuclear number and aggressiveness 4 to 20 times more when they were produced on 2 or 15% glucose than when they were

produced on 30% glucose. Spores grown at 15 C and with 15% glucose had the greatest average volume (970  $\mu^3$  per spore) and aggressiveness (24 mm lesion after 3 days from an inoculation of 350 spores). Nuclear number, however, was greatest in spores grown in 30% glucose at 15 C and 15% glucose at 25 C. The sugar concentration of the growth substrate and the temperature during spore production are important interacting variables when evaluating the potential for *M. fructicola* spores to incite disease.

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MUCOR PIRIFORMIS PROPAGULE LEVELS IN PEAR ORCHARDS IN THE PACIFIC NORTHWEST. Themis J. Michailides and R. A. Spotts, Oregon State University, Mid-Columbia Exp. Station, Hood River, OR 97031

*Mucor piriformis* causes postharvest decay of pear fruit in cold storage in the Pacific Northwest. Propagule levels of 6.3-3,380 per gram dry soil (gds) were found in soils collected from five pear orchards one month before harvest. Leaf, fruit, and air samples collected during harvest revealed no propagules of *M. piriformis*. At harvest, 2.5 - 5% of decayed fruit on the ground were infected with *M. piriformis*. Two months later the percentage increased to 23-50. Soil propagule levels increased to 735-6,832/gds. Soil adhering to the outer surface of picking bins had 1,042-8,333 propagules/gds. These results show that 1) soil is the primary source of inoculum, 2) propagule levels increase after harvest. Bins of fruit with adhering soil are routinely dipped in a tank of water before storage and this appears to be how pear fruit is contaminated with *M. piriformis*.

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RELATIONSHIP BETWEEN INOCULUM CONCENTRATION OF THREE DECAY FUNGI AND PEAR FRUIT DECAY. R. A. Spotts, OSU Mid-Columbia Experiment Station, Hood River, OR 97031

The relationship between inoculum concentration of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* and decay of 'd'Anjou' pear fruits was studied. Wounded and nonwounded fruits were immersed in water containing up to 2000 conidia/ml for 5 min, at 10 C, followed by a water rinse. Relationships between inoculum concentration and decay were nonlinear and were best described with polynomial regression equations. At each inoculum concentration, *P. expansum* caused more decay of wounded fruits than *B. cinerea* or *M. piriformis*. However, *M. piriformis* caused the highest percentage of stem end decay. A fine spray rinse significantly reduced decay caused by all three fungi when compared to nonrinsed fruits. When pear fruits were stored at -1 C and inoculated at various times up to 22 weeks after harvest by dipping stem ends into inoculum, decay developed in all samples.

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EFFECT OF BENOMYL ON FUNGI CAUSING SIDE ROT OF PEAR. David Sugar and Kate Powers, Oregon State University, Southern Oregon Experiment Station, Medford, OR 97502.

Both *Phialophora malorum* and *Cladosporium herbarum* are associated with side rot of cold-stored pears in Oregon. *P. malorum* is not sensitive to benomyl (0.6g/l, which is used in packinghouses to control several postharvest pathogens. Lesions on pears from the orchard or packinghouse prior to benomyl application yielded 90% *C. herbarum* upon isolation, while fruit treated with benomyl predominantly yielded *P. malorum*. Wounded fruit inoculated with a mixture of *P. malorum* and *C. herbarum* conidia developed lesions from which both fungi were isolated, but only *P. malorum* was recovered from benomyl-treated fruit. Wounded fruit inoculated with *P. malorum* and *Penicillium expansum* yielded only *P. expansum* upon isolation, while benomyl-treated fruit yielded only *P. malorum*. Sterile filtrate of *P. expansum* cultures did not inhibit infection by *P. malorum*. Field experiments on control of *P. malorum* may require postharvest benomyl application to eliminate the competitive influence of other decay fungi.

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COMPATIBILITY OF *BACILLUS SUBTILIS* BIOCONTROL OF PEACH BROWN ROT WITH COMMERCIAL POSTHARVEST PROCEDURES. P. L. Pusey\*, C. L. Wilson\*\*, M. W. Hotchkiss\* and J. D. Franklin\*\*, USDA-ARS, \*S.E. Fruit & Tree Nut Res. Lab., Byron, GA 31008, and \*\*Appalachian Fruit Res. Sta., Kearneysville, WV 25430.

Objective was to determine whether use of *Bacillus subtilis* (B-3), as a substitute for chemical control of peach brown rot,

would be compatible with postharvest application of other materials. Unaltered B-3 cultures and aqueous cell suspensions (both consisting of  $10^7$ - $10^8$  CFU  $ml^{-1}$ ) were each applied to fruit in combination with dichloran (for *Rhizopus* control) and commercial fruit waxes. Following treatment, fruit were challenged with *Monilinia fructicola* spores and subsequently incubated at 20 or 25 °C in a moist chamber. Treatments consisting of B-3 in combination with dichloran and wax (oil or water-base) resulted in greater reduction ( $P=0.05$ ) of brown rot than dichloran/wax mixture alone. This was also shown for treated fruit held in simulated cold storage for up to 21 days prior to fungal spore challenge and high temperature incubation.

## 70

PREVENTATIVE CONTROL OF RED THREAD AND PINK PATCH IN PERENNIAL RYEGRASS. B. B. Clarke, P. M. Halisky, C. R. Funk and R. E. Engel. New Jersey Agric. Exp. Stn., Rutgers University, Cook College, New Brunswick, NJ 08903.

In field turf trials at Adelphia, NJ, fungicides were evaluated for the control of pink patch (*Limonomyces roseipellis*) and red thread (*Laetisaria fusciformis*) on 'Pennant' perennial ryegrass. Foliar sprays were applied in the fall and turf was visually rated for percent disease the following spring. Of 8 fungicides tested, Banner and Bayleton completely controlled both diseases, while Chipco 26019, Vorlan, Terraclor and Rubigan were also effective. Daconil was ineffective against pink patch and only partially effective against red thread. In trials on 'Diplomat' perennial ryegrass, 150 nitrogen and granular fungicide combinations were evaluated for the control of red thread. Although nitrogen treatments alone provided poor control, they significantly enhanced disease suppression in 83% of the Bayleton and 30% of the Dyrene, Daconil and Cadmium-Thiram combinations. Control was independent of nitrogen source but strongly dependent on the date and rate of application. NJAES #K-11130-3-85.

## 72

ISOLATION, PATHOGENICITY, AND INHIBITION OF GROWTH WITH FUNGICIDES OF A LEPTOSPHAERIA KORRAE - LIKE FUNGUS FROM KENTUCKY BLUEGRASS IN MICHIGAN. M. E. Otto and J. M. Vargas Jr., Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824

A fungus resembling *Leptosphaeria korrae* was consistently isolated from dead patches and rings of Kentucky bluegrass from four locations in Michigan. Laboratory inoculations and isolations on Fylking Kentucky bluegrass at 15, 24, and 28 °C confirmed the pathogenicity of two isolates. One isolate was induced to form pseudothecia on culms of red fescue plants propagated in moist sand in the greenhouse. Four isolates were tested in laboratory bioassays of PDA amended with 1, 10 and 100 µg/ml concentrations of various turfgrass fungicides. At 10 µg/ml, fenarimol, propiconazol, and benomyl completely inhibited fungal growth, thiophanate-methyl and thiophanate-ethyl showed nearly complete inhibition, and iprodione and vinclozolin provided approximately 50% inhibition. At 100 µg/ml, triadimefon was not effective in inhibiting growth of the fungus.

## 73

DEFINITION OF BENTGRASS LEAF HEALTH AT THE TIME OF INFECTION BY CURVULARIA LUNATA. J. J. Muchovej, Dept. de Fitopatologia, Univ.

Fed. de Vicosa, 36570 Vicosa, MG Brazil and Houston B. Couch, Dept. Plant Pathology, Virginia Tech, Blacksburg, VA 24061.

The state of health and physical integrity of 'Penneagle' bentgrass (*Agrostis palustris*) leaves at the moments of infection and colonization by *Curvularia lunata* was studied. Individual plant groups were grown until 30 and 120 days after seed germination respectively. At these points, all possible combinations of both the normal temperature regimen of 21°C and a sub-group exposed to 38°C for 18 hr were made by clipping and not clipping the leaves of additional sub-groups at 0, 1, 2, 16, 32 and 132 hr before infestation with aqueous conidial suspensions of *C. lunata*. These studies showed that *C. lunata* is not able to infect intact bentgrass leaf surfaces. Ingress into the leaves can only be accomplished through cut leaf tips or already existing necrotic tissue within the laminar area. Once infection has occurred, colonization of tissue can only be achieved in cases of advanced leaf age or heat-induced senescence.

## 74

ARTIFICIAL INFECTION OF TALL FESCUE WITH *ACREMONIUM COENOPHIALUM* BY MEANS OF CALLUS CULTURE. M. C. JOHNSON<sup>1</sup>, L. P. BUSH<sup>2</sup> AND M. R. SIEGEL<sup>1</sup>, Depts. of Plant Pathology<sup>1</sup> and Agronomy<sup>2</sup>, University of Kentucky, Lexington, KY 40546.

Calli initiated from peduncle tissue of endophyte-free tall fescue plants and maintained on a modified MS medium were inoculated with the endophytic fungus, *Acremonium coenophialum*. This was accomplished by making a single clean incision into the middle of 1-2 cm diameter calli with a scalpel and inserting a small amount ( $<1mm^2$ ) of mycelium followed by a 10 wk incubation. Approximately 12% of the regenerated plants from these calli were determined to be infected by microscopic examination of leaf sheaths and by ELISA. Additional evidence for the successful establishment of *A. coenophialum* in these plants was the accumulation of pyrrolizidine alkaloids and conferment of resistance to the oat bird cherry aphid. *A. coenophialum* was reisolated from leaf sheaths of the artificially inoculated plants.

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INFLUENCE OF CLIPPINGS MANAGEMENT PROGRAMS ON DISEASE AND WEED INCIDENCE IN COMMON BERMUDAGRASS. P. F. Colbaugh and W. E. Knopp Texas Agricultural Experiment Station, Texas A&M University Research and Extension Center, Dallas, Texas 75252.

Yearly collections of turfgrass clippings in large metropolitan areas account for a significant part of available landfill used for solid waste disposal. A turfgrass clippings management program called the Waste Saver Lawn Care Program was initiated to eliminate the need for clippings disposal. Benefits of recycling clippings are counterbalanced by a greater potential for weed, insect and disease problems. Common bermudagrass field plots receiving 48.9 kg N/ha fertility at 4 or 6 week intervals were subjected to three types of mowing operations to determine their influence on weeds and common diseases during June to October 1984. Plots were mowed once or twice weekly at 3.5 cm using a mulching mower or standard rotary mower with clippings either bagged or left standing. Broadleaf weed counts following 16 weeks were lower on plots where clippings were removed; however, damaging disease activity was not observed with any of the treatment programs under conditions of limited rainfall.

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EXOTIC DISEASES IN ZOYSIAGRASS COLLECTED IN THE ORIENT. N. R. O'Neill, J. J. Murray, R. E. Davis, and S. S. Hurtt. Agricultural Research Service, USDA, Beltsville, MD 20705.

Approximately 800 zoysiagrass specimens were collected in the Orient and increased in the greenhouse in 1982. Symptoms of three exotic diseases were found on 200 of 2600 plants. A mosaic was associated with pinwheel inclusions indicative of potyviruses; the agent of a dwarfing disease was not identified. The most severe symptoms were irregular chlorotic streaks and spots on leaves which frequently rolled along one margin. Expanding leaf tips were caught in leaf sheaths, forming loops. The agent of this chlorotic leaf roll was rapidly transmitted in the greenhouse among contiguous pots of mature zoysia, to seedlings, and to healthy plugs from the field. Warm temperatures (29.5 °C) enhanced symptom severity, especially in plants previously maintained at 18.5 °C. Hot water treatment (20 min at 49 °C) of zoysiagrass nodes eradicated chlorotic leaf roll in all accessions. The causal agent was subsequently found to be a previously undescribed species of eriophyid mite.

EFFECT OF FUNGICIDES ON THE INCIDENCE AND SEVERITY OF DOWNY MILDEW ON CANTALOUPE. M. E. Miller\*, M. J. Jeger and E. L. Cox\*, \*Texas Agricultural Experiment Station, Weslaco, TX 78596 and Department of Plant Pathology and Microbiology, College Station, TX 77843.

Chlorothalonil and mancozeb were alternately applied to cantaloupe cvs 'Perlita', 'Hale's Best', 'Maqum 45', 'Dulce', 'TAM Uvalde' and 'Dulce x TP 21' to control downy mildew caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rost. Incidence and severity ratings were recorded for five weeks by counting the number of leaves with lesions and estimating the percentage of leaf area killed, respectively. Fungicide treatments had different effects on incidence and severity of downy mildew. Incidence ratings were not significantly different ( $P=0.05$ ) between treated and nontreated plots even though disease incidence increased significantly with time within all cultivars. Severity ratings, however, increased with time and differed significantly between treated and nontreated plots within all cultivars.

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MYCELIAL GROWTH AND INFECTION OF CARROT ROOTS BY RHIZOCTONIA CAROTAE. Z. K. Punja, Campbell Institute for Research and Technology, Route 1, Box 1314, Davis, CA 95616.

Processing carrots grown at Napoleon, CA and stored for 3-4 mo at 3 C developed natural infections due to *Rhizoctonia carotae* Radar, the causal organism of crater rot (CMI Description No. 408). Symptoms of disease, which included pitting of the roots, were reproduced within 2 mo after inoculation of roots with mycelial plugs of each of eight isolates of the pathogen; sclerotia formed on the surface of roots. Linear growth in culture was greatest (0.75 cm/day) on malt and V-8 juice agars at 20 C. In a defined liquid salts medium, maximum dry weight after 10 days at 20 C occurred with asparagine as the nitrogen source and with glucose:pectin (1:1) as carbon sources. The fungus produced oxalic acid during growth and endo-polygalacturonase (PG) activity was detected in culture and in infected tissue. Necrosis and death of the tissue occurred in advance of fungal penetration of cells and resulted from secretion of oxalic acid and endo-PG; crystals of calcium oxalate were abundant. No specialized infection structures were produced by the pathogen.

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RESERVOIR SOURCE OF TOMATO SPOTTED WILT VIRUS AND ITS INSECT VECTOR IN HAWAII. J. J. Cho, R. Mau, D. Gonsalves\*, and W. C. Mitchell. University of Hawaii, Kula 96790 and Honolulu, HI 96822 and \*Department of Plant Pathology, NYS Ag. Expt. Stn., Cornell University, Geneva NY 14456.

Tomato spotted wilt has seriously affected production of lettuce, tomato, and bell pepper in the major agricultural areas of the state. Comprehensive field surveys to identify important tomato spotted wilt virus (TSWV) source plants via ELISA and insect vector sources have been initiated. Seven new hosts have been implicated through ELISA and further confirmatory tests are being conducted. These hosts include: *Melilotus officinalis*, *Portulaca oleracea*, *Arctium lappa*, *Alternanthera sessilis*, *Leonotis nepetaefolia*, *Ipomoea congesta* and *Brassica pekinensis* var. pak choy. Four weed species found in abundant numbers within and outside of major Kula farming areas on Maui are major reservoirs for TSWV and its insect vector. These weeds include: *Malva parviflora*, *Verbascum enceloides*, *Bidens pilosa* and *I. congesta*.

81

A TOMATO DECLINE OF UNKNOWN ETIOLOGY IN IMPERIAL VALLEY, CA. F. E. Laemmlein, A. F. Van Maren, R. M. Endo\*, and R. A. Valverde\*, Cooperative Extension, El Centro, CA 92243 and Department of Plant Pathology\*, University of California, Riverside, CA 92521.

A tomato disorder designated as "tomato decline" has affected fresh and processing tomato production in Imperial Valley, CA since 1977. Entire fields are often affected. Initial symptoms appear as cessation of growth after 1-2 flower clusters have set. Leaves are reduced in size, cup downward, then become thick and brittle. As the disorder progresses, leaves yellow, develop necrotic margins, and older leaves die prematurely. Fruit set is greatly reduced, fruits size poorly and sunburn easily due to lack of foliage. Root systems are reduced but appear normal in other respects. To date no pathogens including viral pathogens or other factors have been consistently associated with the disorder. Field and greenhouse trials are underway to identify the factor(s) causing the decline.

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OCCURRENCE OF SNAP BEAN BACTERIAL BLIGHT PATHOGENS ON WILD LEGUMES. M. J. Goode, D. J. Hagedorn, and J. E. Cross, Dept. of Plant Pathology, Univ. of Ark., Fayetteville, AR 72701; Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706; Agrow Seed Co./Sub-Upjohn Co., Kalamazoo, MI 49001.

Bacterial isolates were obtained from leaf and stem lesions on hairy vetch, *Vicia villosa*, and common vetch, *V. sativa*, growing wild at 12 sites in close proximity to, or ranging up to 5 miles from commercial snap bean, *Phaseolus vulgaris*, fields in Arkansas and Tennessee. Pathogenicity studies were made by inoculating young bean plants and detached pods. Weakly to highly pathogenic isolates of *Pseudomonas syringae* pv. *syringae* were obtained from every site. Weakly to highly pathogenic isolates of *Xanthomonas campestris* pv. *phaseoli* were obtained from leaf lesions on kudzu, *Pueraria thunbergiana*, growing at 9 sites in Arkansas, Mississippi, Tennessee, Louisiana, and Alabama. Highly pathogenic isolates of *Pseudomonas syringae* pv. *phaseolicola* were obtained from 6 of the 9 sites representing every state. Weakly to highly pathogenic isolates of *X. campestris* pv. *phaseoli* were obtained from every site.

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ASSOCIATION OF COMMON RAGWEED WITH SCLEROTINIA ROT OF CABBAGE IN NEW YORK STATE. H. R. Dillard and J. E. Hunter, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

In 1984, *Sclerotinia sclerotiorum* caused significant losses in several fields of cabbage (*Brassica oleracea* L. var. *capitata*) in New York. A wet growing season provided ideal environmental conditions for disease, and prevented normal cultivation for weed control. *Sclerotinia* rot of cabbage frequently was associated with the presence of common ragweed (*Ambrosia artemisiifolia* L.), a known host of *S. sclerotiorum*. Ragweed flower parts and whole plants were infected and bridged the infection to cabbage. In laboratory tests, ascospores of *S. sclerotiorum* infected male and female ragweed flowers, and cabbage leaves became infected when in contact with these flowers. Ragweed pollen alone served as a substrate for *S. sclerotiorum*, but only resulted in leaf infections when present in large clumps. Ascospores did not infect ragweed or cabbage leaves without an exogenous food base.

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CHARACTERIZATION OF SEVERAL ISOLATES OF CORYNEBACTERIUM INSIDIOSUM AND THEIR APPLICATION IN SCREENING BACTERIAL WILT RESISTANCE IN ALFALFA. John Kao, Jeff Johnson and Ike Kawaguchi, PLANT GENETICS, INC., 1930 FIFTH Street, Davis, CA 95616.

Gram-positive coryneform bacteria were isolated from field-grown plants showing bacterial wilt symptoms in California. Twelve isolates were compared with seven isolates received from other laboratories for their biochemical characteristics, pigmentation, plasmid content and virulence on alfalfa, Cultivar Ranger. The

nineteen isolates differed slightly in the seven biochemical characteristics tested; however, they varied considerably in their virulence. Two major types of pigmentation were observed: the apricot-orange type; and the yellow-blue type. Virulence on alfalfa was found in both types of bacteria. Although isolates varied in their plasmid content, several isolates from widely separate locations had similar plasmid content. An experiment designed to study the responses of eight alfalfa cultivars to the infection caused by two virulent isolates, each representing one of two pigmentation types, showed that the resistance ratings agreed in most cases with the previously published ratings.

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EFFECT OF *CORYNEBACTERIUM FLACCUMFACIENS* ON YIELD OF ZORNIA GLABRA AND PHASEOLUS VULGARIS IN COLOMBIA. Jillian M. Lenné, A. Chavarro and C. Lopez, Tropical Pastures Program, CIAT, A.A. 6713, Cali, Colombia.

The incidence and severity of bacterial wilt caused by *C. flaccumfaciens* was studied in several *P. vulgaris* varieties and the tropical pasture legume *Z. glabra* CIAT 7847 at the CIAT Research Station, Santander de Quilichao, Colombia. Dry matter losses of 81.4% in *Z. glabra* and grain yield losses of 46.0% and 71.3% in *P. vulgaris* varieties PI 13677 and Porrillo synthetic, respectively, were measured. Levels of bacterial infection of up to 52.5% and 88.8% were registered in seed of *P. vulgaris* and *Z. glabra*, respectively. The correlation coefficient between % bacterial infection and % seed germination was -0.87. The pathogenicity of isolates of *C. flaccumfaciens* from *Z. glabra* to *P. vulgaris* was confirmed.

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CORRELATION BETWEEN POPULATIONS OF *CLAVIBACTER XYLI* SUBSP. *XYLI* AND SUSCEPTIBILITY TO RATOON STUNTING DISEASE IN SUGARCANE. M. J. Davis, and N. A. Harrison, University of Florida, IFAS, REC, 3205 College Avenue, Ft. Lauderdale, Florida. 33314.

Populations of *C. x.* subsp. *xyli* in sap extracts were enumerated by direct-counts of fluorescent-antibody stained cells. Extracts were obtained by centrifugation of stalk internodes. The third internode above the ground was sampled. Two internodes (plant crop) or one internode (first ratoon crop) were taken from the oldest stalks of each plant on three occasions at 6 wk intervals during the latter half of each growing season. Four to 15 infected plants of each of 10 cultivars were studied. Generally, pathogen populations developed at a faster rate and to a greater extent in susceptible cultivars. When the overall populations (cells/ml) for the cultivars were compared to yield-reduction (kg/stool) data, there was a high correlation ( $r = 0.90$ ; null hypothesis: slope = 0,  $P = 0.0002$ ).

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DETECTION OF RATOON STUNTING DISEASE BACTERIUM, *CLAVIBACTER XYLI*, IN SUGARCANE BY DOT BLOT HYBRIDIZATION. J. M. Kamso-Pratt and K. E. Damann, Jr., Dept. of Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, La. 70803.

We have demonstrated that dot blot hybridization has the potential to be an effective system to detect *Clavibacter xyli* in sugarcane sap. Pure cultures of the bacterium were used to isolate DNA by alkaline lysis, phenol extraction, and cesium chloride centrifugation. Chromosomal DNA was digested with Pst I, cloned into the plasmid pUC 19 and used to transform *E. coli* JM 83 cells. The cloned inserts and *C. xyli* chromosomal DNA were radiolabelled by nick translation and tested for their ability to hybridize with purified DNA and cultured cells of *C. xyli*. All the probes hybridized with *C. xyli* DNA and cells. Hybridization also occurred with sap from infected sugarcane but no hybridization occurred with sap from healthy sugarcane or with *Agrobacterium tumefaciens* which was used as a negative control.

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USE OF BIOLUMINESCENCE TO MONITOR AGROBACTERIUM, ERWINIA, PSEUDOMONAS AND XANTHOMONAS IN PLANTS. Shaw, J.J., Close, T.J., Engbrecht, J.\*, Kado, C. I., Department of Plant Pathology, University of California, Davis, CA 95616, \*Scripps Institute of Oceanography, La Jolla, CA.

Monitoring phytopathogenic bacteria *in planta* generally requires disruptive analysis of the host tissue. Insertion of bioluminescence genes into species of *Agrobacterium*, *Pseudomonas*, *Xanthomonas* and *Erwinia* permits the detection of bacteria during the course of an infection with photographic film or a photomultiplier, leaving the host and the bacteria essentially undisturbed. The bioluminescence genes (*lux*) of *Vibrio fischeri* were inserted into broad host range cloning vectors (Glose, T. J., Zaitlin, D., Kado, C. I., 1984, *Plasmid* 12:111-118). Constitutive expression of the *lux* genes was obtained by substituting the promoter of an antibiotic resistance gene for the *lux* regulatory region. Following introduction of the *lux* genes, species of the above genera emitted visible light *in vitro* and their colonization of plant tissues was observed as changes in patterns and levels of light.

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PROPERTIES OF A PECTOLYTIC STRAIN OF *CYTOPHAGA* SP. ASSOCIATED WITH POST-HARVEST ROT OF BELL PEPPERS. C.H. Liao and J.M. Wells USDA-ARS, Rutgers University, New Brunswick, NJ 08903.

An aflagellated, orange-pigmented bacterium tentatively identified as a member of the genus *Cytophaga*, was isolated from a bell pepper showing maceration symptoms. This bacterium (strain PF062) possessed cultural, and biochemical properties that were different from any other soft-rotters previously described. The cultured organism, capable of causing soft-rot of pepper, potato, and squash was Gram-negative, thin elongated rod (0.2-0.3 x 3-5  $\mu$ ); and was motile by gliding on solid surface. A variety of extracellular depolymerases (amylase, cellulase, and protease) were produced by the strain PF062. The pectate lyase and polygalacturonase were induced in media containing pectin, polygalacturonic acid, or polypectate. Very little or no activity of pectin esterase, and pectin lyase was found. This bacterium could be readily differentiated from *Flavobacterium pectinovorum* (ATCC 19366) by its spreading growth on agar media; its inability to reduce sucrose; and its stronger macerating ability.

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A SURVEY OF GEORGIA AND FLORIDA FOR THE PRESENCE OF COPPER TOLERANT PHYTOPATHOGENIC BACTERIA. R. D. Gitaitis, Coastal Plain Station, Tifton, GA, T. B. McInnes, Dept. of Plant Pathology, Univ. of Georgia, Athens, GA, and J. B. Jones, Gulf Coast Research Station, Univ. of Florida, Bradenton, FL.

Reactions to copper by 150 strains of bacteria representing 20 species were varied when assayed onto nutrient agar amended with different levels of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . *Pseudomonas cepacia*, *P. viridiflava*, and *Xanthomonas campestris* pv. *vesicatoria* (XCV) grew in the presence of 128 ppm of copper. Strains of *P. syringae* pv. *tomato* and *P. cichorii* tolerated up to 32 ppm of copper, whereas strains of *X. campestris* pv. *pruni* and pv. *vignicola* were sensitive to 8 and 4 ppm of copper, respectively. However, when assayed in solutions of equivalent levels of copper that were prepared from either  $\text{CuOH}_2$  or  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , only XCV was tolerant to copper. Consequently, an assay in liquid containing water-soluble copper may be better correlated with detecting bacteria tolerant to copper bactericides in the field rather than an assay on agar.

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MULTIPLE DISEASE RESISTANCE IN POTATO CULTIVARS WITH *Solanum phureja* AND *S. demissum* BACKGROUND. E. R. French, International Potato Center (CIP), Apartado 5969, Lima 100-Peru.

*Solanum phureja* PI 310439 and PI 310491 selected for resistance to *Pseudomonas solanacearum* in Colombia (CCC 1339 and CCC 1386, respectively) were utilized in crosses with late blight (*Phytophthora infestans*) resistant Mexican varieties (*S. demissum* background) to develop tetraploid cultivars (BR clones) by L. Sequeira and P. R. Rowe at the University of Wisconsin. Field testing in Peru resulted in varieties *Caxamarca* (BR63.74), *Molinera* (BR63.65) and *Amapola* (unknown BR). Further distribution of these, sister lines and additional crosses made at CIP led to the selection of BR69.84 (Domoni) in Fiji, BR63.15 in Peru, BR 63.76 in Kenya, and CIP 377847.5 and CIP 377852.2 in Sri Lanka. These eight cultivars were resistant to *P. solanacearum* and *P. infestans* in one or more of seven countries, four were resistant to PLRV, three were heat tolerant, two *Alternaria* blight resistant and one PVY resistant.

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MONOCLONAL ANTIBODIES USED TO IDENTIFY SOURCES OF CABBAGE BLACK

ROT. A. M. Alvarez, A. A. Benedict, C. Y. Mizumoto, and K. J. Trotter. University of Hawaii, Honolulu, HI 96822.

Xanthomonas campestris was detected by ELISA on seeds, leaves, and plant debris of crucifers and on weeds and lettuce using rabbit antiserum raised to one strain of Xc pv. campestris. Cultures also were isolated on starch-methionine medium and tested with monoclonal antibodies (MCA) to determine their serotypes. Strains recovered from symptomless cabbage seedlings and those showing mild chlorosis were in previously identified Xcc serogroups. Most Xanthomonas strains recovered from non-cruciferous weeds and lettuce failed to react with crucifer-specific MCA. However, 4 strains isolated from mildly necrotic lettuce leaves were identified as Xcc with MCA and caused black rot on cabbage. In contrast, the commonly occurring lettuce pathogen, Xc pv. vitiensis, caused no symptoms on cabbage and could be distinguished from the 4 Xcc strains by reactions with 5 MCA. Thus, lettuce harbored both pathogens of X. campestris and may serve as an additional source of Xcc strains.

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OCCURRENCE OF BACTERIAL WILT ON POA ANNUA AND OTHER TURFGRASSES. D. L. Roberts, J. M. Vargas, Jr. and R. Detweiler, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

A disease of unknown etiology occurred on annual bluegrass (Poa annua L.) at locations in Michigan and Pennsylvania in 1984. Affected plants quickly wilted from leaf tips to the crown. A bacterium was consistently isolated from diseased tissue and shown by inoculation to be the causal agent. Bacteria were also isolated from diseased Nimissilia creeping bentgrass (Agrostis palustris Huds. cv. Nimissilia) and from diseased Seaside creeping bentgrass from Ohio. The three bacteria from annual bluegrass, 'Nimissilia' and 'Seaside' exhibited biochemical-physiological, cultural, morphological, pathological and growth characteristics similar to those of Xanthomonas campestris pv. graminis, the cause of bacterial wilt of Toronto creeping bentgrass. Host range studies suggested that these bacterial pathogens were highly specific for their respective hosts.

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PATHOGENICITY OF BACILLUS CIRCULANS TO DATE PALM (PHOENIX DACTYLIFERA) SEEDLINGS. J. V. Leary, Department of Plant Pathology, University of California, Riverside, CA 92521.

Bacillus circulans was reported earlier to be a consistent and destructive contaminant of date palm tissue cultures. Seeds of seven varieties of date palm were soaked for 60 min in a suspension of  $10^9$  cells of a B. circulans culture which had been obtained from a tissue culture specimen. After 10-12 weeks, the percentage germination, seedling height and seedling fresh weight was significantly decreased for some varieties when compared to those parameters for control seeds soaked in water. In addition, seedlings of two varieties developed tip necrosis which eventually spread the length of the coleoptile, resulting in death of the seedling. The progress of the necrosis and death is similar to an unknown disorder seen in date palm offshoots in the field.

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PURPLE TOP AS A CAUSE OF CHIP DARKENING IN PROCESSED POTATOES. E.E. Bantlari, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; P. Orr, USDA Potato Research Laboratory, East Grand Forks, MN 56721; and D. Preston, Potato Extension, University of Minnesota, East Grand Forks, MN 56721.

Field experiments for two years in which aster leafhoppers (Macrostelus fascifrons), infected with aster yellows mycoplasma-like organism (AY-MLO) were introduced into caged plots, resulted in purple top in the exposed potato plants. Tubers from the purple top plots and healthy check plots were harvested, stored and processed, and only the chips from tubers from the purple-top plots were discolored. In both years the results of these experiments suggested there are differences in varietal susceptibility to the intensity of discoloration. It appears also that this purple top-related problem becomes evident only after tubers have been stored three or more months. Tubers from plots exposed to non-infected aster leafhoppers or from non-exposed check plots produced chips that were bright and considered normal for the trade.

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BACTERIAL GALLS OF GYPSOPHILA PANICULATA CAUSED BY ERWINIA HERBICOLA IN CALIFORNIA. D. A. Cooksey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Symptoms resembling crown gall disease were observed on Gypsophila paniculata plants in southern California. Rooted cuttings from several nurseries had galls at the pruned end that were 1-2 cm in diameter. The galls were soft, light brown in color, and were usually rotted. Mature field-grown plants had large galls up to 10 cm in diameter just below the soil line that were soft and friable. The presence of large galls was often associated with stunting and reduction in flower production. Many plants with galls eventually wilted and died. Decomposition of galls and a soft rot of the stem were associated with the wilted plants. Although Agrobacterium was isolated from galls and soil around infected plants, no galls were produced by inoculation of these isolates to wounded Gypsophila or tomato plants in greenhouse tests. However, gall symptoms were reproduced in repeated greenhouse tests when Gypsophila was inoculated with strains of Erwinia herbicola recovered from galls and aerial plant parts of field-grown Gypsophila.

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RELATIVE SUSCEPTIBILITY OF RED RASPBERRY CLONES TO CROWN GALL. C.L. Zurowski, R.J. Copeman and H.A. Daubeny. Plant Science Dept., #248-2357 Main Mall, University of B.C., Vancouver, B.C. V6T 2A2 and Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C. V6T 1X2.

The relative susceptibility of 13 clones of red raspberry to crown gall was evaluated in an outdoor, replicated pot trial. Rooted cuttings were planted in pasteurized greenhouse mix artificially infested with four levels of a biovar 2 strain of Agrobacterium tumefaciens isolated from a galled raspberry root obtained locally. Although no clone was immune at the highest inoculum level used, the cultivar Willamette having a mean gall number per plant of 0.4 was considered highly resistant. Cultivars Nootka and Canby with mean gall numbers of 9.8 and 12.6 respectively, showed intermediate resistance. Cultivar Haida and B.C. selection 72-1-7 had significantly more galls than Skeena, an important commercial cultivar. The remaining cultivars and selections were as susceptible as Skeena. These data explain why crown gall was not a problem in the Pacific Northwest when Willamette was the only widely grown cultivar.

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PATTERNS OF FUNGAL COLONIZATION OF ROOT SYSTEMS OF TOBACCO SEEDLINGS. J.T. English and D.J. Mitchell, Plant Pathology Dept., Univ. Florida, Gainesville, FL 32611.

Populations of fungi associated with tobacco root systems were examined periodically over a 4-wk period of seedling growth in raw and autoclaved soils to provide insight into microbial community development as associated with root infection by Phytophthora parasitica var. nicotianae. Populations of detectable fungi in both the rhizosphere and rhizoplane were consistently greater for root systems developing in raw soil than in autoclaved soil. Fungal diversity associated with roots was consistently greater in raw soil than in autoclaved soil; the dominant fungi varied with time and soil type. The distribution of fungal hyphae at the rhizoplane of seedlings, as determined by direct observation, was found to be highly aggregated. Such aggregation was greater in autoclaved soil than in raw soil as shown by the higher values of Lloyd's index of patchiness (LIP) asso-

ciated with the former treatment. Mean crowding (mean LIP) of hyphae within colonized regions of the rhizoplane was greater in raw soil than in autoclaved soil.

#### 100

**INFLUENCE OF NUTRITION AND CARBON DIOXIDE ON SCLEROTIAL FORMATION AND CORD DEVELOPMENT IN *SCLEROTIUM ROLFSSII* AND *S. DELPHINII*.** Z. K. Punja, Campbell Institute for Research and Technology, Route 1, Box 1314, Davis, CA 95616.

The influence of a step-down in the level of carbon (C) or nitrogen (N) and of nutrient deprivation, imposed after initial growth of *Sclerotium rolfssii* and *S. delphinii* had occurred, on subsequent formation of sclerotia and cords was investigated using split-plate (compartmentalized) petri dishes. One-half of the dish received a salts medium (with 20 g/L glucose and 1 g/L  $\text{NH}_4\text{NO}_3$ ; C:N ratio of 23:1) which was inoculated (side I). In the other half (side S), the level of glucose was stepped down to 15, 10, 5, or 0 g/L, or  $\text{NH}_4\text{NO}_3$  was reduced to 0.75, 0.5, 0.25, 0.125, or 0 g/L. Sclerotial production on side S was rated after 3 wk of incubation at 24-28 C with 12 hr/day light. Greatest sclerotial production occurred when mycelia grew from side I onto side S containing 20 g/L glucose and 0.25 g/L  $\text{NH}_4\text{NO}_3$  (C:N = 92:1). Cords developed on side S only under nutrient-deprived conditions (no C or N) but few sclerotia formed. Sclerotial formation was inhibited in cultures exposed to  $\text{CO}_2 > 4\%$ .

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**NATURAL SENEESCENCE OF WHEAT ROOT AND CROWN TISSUES IN RELATION TO PARASITISM BY *MICRODOCHIUM BOLLEYI*.** R. T. Kane and R. W. Smiley, Dep. of Plant Pathology, Cornell Univ., Ithaca, NY

*M. bolleyi* is frequently isolated from diseased root and crown tissues of wheat, but it is generally regarded as a minor pathogen. Cytological studies were conducted to determine the nature of the association between *M. bolleyi* and cells of the root system that display natural senescence phenomena. Senescence of cells in root and crown tissues was monitored by loss of nuclei as indicated by several staining techniques. Natural senescence was observed in seminal and coronal root cortices, in scutellar tissues, and in leaf bases of 15-37 day old plants. Colonization of root cortices by *M. bolleyi* was initially intercellular, but became intracellular once cells had senesced. Colonization of coleorrhiza, epithelium, and cortical cells of the scutellar node was also correlated with natural senescence. Penetration of the endodermis and stele had not occurred in 62 day old plants. Apparently, *M. bolleyi* is a weak parasite that is adapted to colonization of naturally senescent cell layers.

#### 102

**ASSOCIATION OF *MICROBISPORA* SPP. WITH ROOTS OF APPLE SEEDLINGS.** S. W. Westcott III and S. V. Beer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853 USA.

In studies of the etiology of apple replant disease (ARD), small lesions were consistently observed only on roots of apple seedlings that had been grown in soils conducive to ARD. The lesions contained profusions of hyphae about 1  $\mu\text{m}$  in diameter, similar to actinomycete hyphae. Isolation from lesions routinely produced a mixture of actinomycetes similar to *Microbispora amethystogena* Nonomura & Ohara and/or *M. diastatica* Nonomura & Ohara. When apple seedlings were planted in steamed ARD-conductive soil, amended with ARD-conductive soil (1-5% V/V), together with single strains of *Microbispora*, lesions developed and actinomycetes similar to the introduced strains were isolated. Lesions did not develop on roots from non-conductive or steamed conductive soils amended only with mycelia of *Microbispora*. Although it is clear that *Microbispora* spp. were associated with lesions on apple roots, the actinomycetes alone did not cause the lesions. Apparently, interaction with other factors is required for infection of roots by *Microbispora* spp.

#### 103

**PATHOGENESIS OF BLUEGRASS BY PHIALOPHORA GRAMINICOLA, IN RELATION TO ROOT CORTICAL DEATH.** R. W. Smiley and D. E. Giblin, Dept. of Plant Pathology, Cornell Univ., Ithaca, NY 14853.

Root cortical death (RCD) in grasses and cereals is a natural process closely associated with autolysis of nuclei. *P. graminicola* (Pg), a necrotrophic parasite of grass roots, induces no vascular necrosis at 14-22 C, but does so on

susceptible Kentucky bluegrass cultivars in unshaded turfs at  $>24$  C. The association of Pg with RCD in bluegrass was studied under shaded and nonshaded conditions at 14, 24, and 29 C, using numbers of nuclei as an indicator of RCD. Anucleation rates in the cortical layer nearest the endodermis were directly proportional to temperature, and Pg colonized the stele only in roots with few cortical nuclei. Shading reduced the rate of anucleation at 29 C, had no effect at 24 C, and enhanced it at 14 C. RCD was a useful and quantitative indicator of environmental effects which influence the susceptibility of grasses to pathogenesis by Pg.

#### 104

**INFLUENCE OF AN UNIDENTIFIED ECTOTROPHIC FUNGUS ON SIX CEREAL SPECIES AT THREE TEMPERATURES.** R. W. Smiley and M. C. Fowler, Dept. of Plant Pathology, Cornell Univ., Ithaca, NY 14853.

In Germany, an unidentified, dematiaceous fungus (UDF) is a minor pathogen of corn roots and suppresses take-all of wheat via competition with *Gaeumannomyces graminis* var. *tritici* (Ggt) at 22 C. A similar fungus in New York is associated with a high temperature disease of turfgrass, and is isolated from turf by using wheat in trap-crop bioassays. Pathogenicity of the UDF to cereals was studied at 14, 24 and 29 C. Inoculations with UDF caused little or no root rot at any temperature on spring wheat, barley, oats, triticale and rye. The UDF enhanced growth of rye at 14 C, wheat and barley at 24 C, and oats at 29 C, and it suppressed growth of rye at 24 and 29 C. UDF rotted cortices of corn at 24 C, but did not affect growth at any temperature. Dual-inoculation of spring wheat with UDF and Ggt reduced growth less than did Ggt alone at 24 C, was equivalent to Ggt alone at 14 C, and had an additive negative effect on yield at 29 C.

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**PATHOGENICITY OF PHIALOPHORA GRAMINICOLA TO SIX CEREAL SPECIES AT THREE TEMPERATURES.** R. W. Smiley and M. C. Fowler, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Biocontrol of take-all (caused by *Gaeumannomyces graminis* var. *tritici* - Ggt) on wheat in several countries is attributed in part to competition by *P. graminicola* (Pg), based on studies conducted at 15-22 C. Summer patch of turfgrasses in North America is incited by Pg at  $>24$  C, and the incitant is isolated from turf by using wheat in trap-crop bioassays. Pathogenicity of Pg to cereals was therefore studied at 14, 24 and 29 C. Pg severely reduced growth of barley, triticale, and spring and winter wheat at 29 C, had intermediate effects at 24 C, and no effect at 14 C. Corn sustained moderate to severe cortical rot at 29 C, but growth was not affected by Pg at any temperature. Pg did not cause root rot or reduce growth of oats or rye at any temperature, and enhanced the growth of rye at 14 C. Dual-inoculations of spring wheat with Pg and Ggt did not suppress take-all at 14 and 24 C, and had additive negative effects on yield at 29 C.

#### 106

**EFFECTS OF DURATION AND TIME OF INITIATION OF FLOODING ON SOYBEAN EMERGENCE.** R. S. Ferriss, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Carboxin-thiram treated or untreated soybean seeds were planted in untreated or pasteurized soil. Soil was flooded for 0 to 5 days starting immediately after planting, or was flooded for 3 days starting 1 to 4 days after planting. Soil moisture was then returned to near optimum for emergence (EM). When flooding started immediately after planting, EM from untreated seeds declined steadily with time to  $<20\%$  at 5 days flooding in both soils. Emergence from treated seeds did not decline to below 80% until  $>4$  days flooding in both soils. The relative effects of soil and seed treatment were influenced greatly by the starting time of flooding. In untreated soil, seed treatment greatly increased EM only when flooding started  $<2$  days after planting. In pasteurized soil, seed treatment increased EM only when flooding started  $<24$  hr after planting. The mechanism(s) of flooding damage appears to depend on the seed germination stage at the start of flooding.

#### 107

**GROWTH PROMOTION OF APPLE ROOTSTOCKS BY RHIZOBACTERIA.** A.J. Caesar and T.J. Burr, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Strains of root-zone bacteria applied to apple rootstock liners increased growth of the liners when planted in apple replant soil, in greenhouse and field experiments. Liners of clonal rootstocks M7 and M26 dipped in a bacterial formulation of gum xanthan and coated with talc exhibited significant increase in total plant weight, ranging from 38-121% in greenhouse studies and 27-66% in field studies. Significant growth increases were correlated with reduced colonization of liner roots by various fungi (e.g., *Cylindrocarpus* sp., *Rhizoctonia solani* and *Pythium* spp.). There was also an average increase of 43% in the number of lateral root origin points on rootstocks. Growth promotion may be due to a reduction in fungal root colonization, and possibly a protection of the rootstock axis, allowing lateral root development.

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PHENOTYPIC COMPARISON OF APPLE PLANT GROWTH-PROMOTING BACTERIA FROM NEW YORK SOILS. A. J. Caesar and T. J. Burr, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Strains of rhizobacteria which increased growth of apple seedlings in the greenhouse or apple rootstock liners in greenhouse or field studies were compared for characteristics associated with growth-promoting ability in studies by others. Assays such as *in vitro* broad spectrum antibiosis, osmotolerance, motility, EDDHA tolerance and apple root adherence, indicate that New York strains vary substantially with regard to these parameters. Data indicate that for New York strains no definitive trait or set of traits could be associated with growth-promoting ability. Selection of rifampicin resistant (100 ppm) mutants resulted in a loss of *in vitro* broad spectrum antibiosis in a high proportion of single colony strains derived from wild type parent strains exhibiting *in vitro* broad spectrum antibiosis. The phenotypic traits of these mutants in comparison to their parent strains is discussed.

#### 109

TAKE-ALL OF WHEAT AS AFFECTED BY TILLAGE AND DOUBLECROPPING. C. S. Rothrock, Dept. of Plant Pathology, Georgia Experiment Station, Experiment, GA 30272.

Plots were established in the fall of 1982 to evaluate the effects of tillage and wheat/soybean doublecropping on take-all of wheat. The experimental area had been doublecropped using conservation tillage since the fall of 1977 and take-all has been observed in this area since 1982. Treatments included tillage (conventional vs. no) and cropping system (wheat/fallow vs. wheat/soybean). In 1983 the percentage of infected plants was 28%. Tillage did not significantly influence disease severity or incidence. Disease incidence in 1984 increased to 76% and both disease severity and incidence were higher in plots with conventional tillage. No differences were found between the amount of organic material sieved from soil between the two tillage treatments. Take-all was not affected by wheat/soybean doublecropping.

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EFFECTS OF SOLARIZATION OF SOIL AMENDED WITH CABBAGE RESIDUES ON *FUSARIUM OXYSPORUM* f. sp. *CONGLUTINANS* RACE 5. Jose Ramirez-Villapudua and D. E. Munnecke, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

*F. oxysporum* f. sp. *conglutinans* (Foc) was practically eliminated and cabbage yellows was undetectable in a field in which plant amendments were combined with soil solarization. Chopped cabbage heads were dried, collected, and mixed in soil. Plots were covered with clear polyethylene tarps for 4 wk in August. Both solarization and cabbage amendments reduced disease severity and populations of Foc, but not as much as the combination of solarization and cabbage amendments. Disease severity and Foc populations in pots buried in the field were markedly reduced by amendments of cabbage, kale, and mustard; moderately reduced by alfalfa hay; and increased by wheat straw, chicken manure, and steer manure. Fungitoxic gases were detected from cabbage-amended soils. Control may be due to an increase in rate of production of gases (C), and to an increase in time of treatment due to tarping (T), thus producing fungitoxic CxT products.

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INDUCED SUPPRESSIVENESS IN SOLARIZED SOILS. A. Greenberger, Anat Yaguev and J. Katan. Volcani Center, ARO, Bet Dagan 50250 and Dept. of Plant

Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Soil solarization often produces a long term effect in disease control. This might result from induced suppressiveness which delays reinfestation. In many but not all tested soils, incidence of *Fusarium*, *Sclerotium rolfsii* and *Verticillium wilt* diseases was lower in solarized and subsequently inoculated soils than in non-treated inoculated ones and populations of lytic and antibiotic microorganisms were higher. Suppression of chlamydsopore formation by *Fusarium* and reduced fungitoxicity to *S. rolfsii* were frequently observed. This suppressiveness in solarized soils was found against five different pathogens thus denoting a general nonspecific mechanism possibly different from the more specific suppressiveness naturally occurring in certain soils.

#### 112

EFFECT OF SOIL SOLARIZATION WITH CLEAR PLASTIC AND SHALLOW FLOOD ON THE SURVIVAL OF *RHIZOCTONIA SOLANI* SCLEROTIA. Fleet N. Lee, University of Arkansas, P.O. Box 351, Stuttgart, Arkansas 72160.

Survival of *Rhizoctonia solani* sclerotia collected from rice field debris was studied under weed free summer fallow regimes of either undisturbed soil, soil continuously flooded to a 1-5 cm depth or soil mulched with 2 mil clear plastic. Sclerotia placed in nylon bags were allowed to float on the water surface in flooded plots or were buried at 0 (soil surface), 2.5, 5.0 and 7.5 cm depths. Data were collected from 3 trials conducted in 1983 on silt loam soils near Stuttgart, Arkansas. Sclerotia viability after 58 days in the undisturbed soil was 96, 79, 79 and 61% at 0, 2.5, 5.0 and 7.5 cm soil depths, respectively. In flooded plots, 93% of the floating sclerotia remained viable while 6 (2 trials only), 9, 15 and 21% remained viable at 0, 2.5, 5.0 and 7.5 cm depths, respectively. Viability under the plastic mulch was 1, 0, 6 and 10% at 0, 2.5, 5.0 and 7.5 cm, respectively. Results were confirmed at one test location in 1984. Sclerotia viability remained greater than 68% in two 1983-84 winter fallow tests.

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REDUCED INFECTIVITY OF EXTRACTS FROM NECROTIC LESIONS OF HYPERSENSITIVE TOBACCO WITH INDUCED RESISTANCE TO TOBACCO MOSAIC VIRUS. D. A. Roberts, Department of Plant Pathology, University of Florida, Gainesville, Florida 32611.

The infectivity of juice from necrotic areas caused by tobacco mosaic virus (TMV) in leaves of hypersensitive tobacco (*Nicotiana tabacum* L. 'Samsun NN') with induced resistance (systemic) was less than that from equivalent necrotic areas in control leaves. In each of five experiments, five lesions (5.0 mm<sup>2</sup>) from control leaves or 25 lesions (1.0 mm<sup>2</sup>) from resistant leaves contained approximately 25 mm<sup>2</sup> of necrotic, and 475 mm<sup>2</sup> of non-necrotic leaf tissue. Discs from each source were ground in 1.0 ml of 0.05 M neutral phosphate buffer with a mortar and pestle. Inocula from leaves with induced resistance caused 41% fewer lesions in mechanically inoculated leaves of NN tobacco than did inocula from control leaves. Differences in each experiment were significant (P=0.01) by the  $\chi^2$  test. Induced resistance thus seems to result in repressed rates of replication or in the inactivation of TMV.

#### 114

LIGHT AND ELECTRON MICROSCOPY OF THE JORDANIAN ISOLATE OF BROAD BEAN WILT VIRUS. A.M. Al-Musa, H.A. Al-Hajj and L.O. Monayer, University of Jordan, Amman, Jordan.

The Jordanian isolate of broad bean wilt virus serotype 1 induced two types of inclusions which were visible in the light microscope: Polyhedral and elongated crystals and amorphous inclusions. The crystalline inclusions appeared later than and disappeared before the amorphous ones. In the electron microscope the crystals are formed by densely packed spherical particles whereas the amorphous contains scattered virus particles: arrays of virus particles (usually in pairs) and vesicles. No tubules were detected.

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COMPARISON OF THE CAPSID AND NONCAPSID PROTEINS OF MAIZE STRIPE VIRUS, RICE HOJA BLANCA VIRUS AND HELIANTHUS HOJA BLANCA VIRUS.



B. W. Falk<sup>1</sup>, F. J. Morales<sup>2</sup>, J. D. Tsai<sup>3</sup>, and A. J. Niessen<sup>2</sup>.  
<sup>1</sup>University of Florida, JREC, Belle Glade, 33430, <sup>2</sup>FLREC, Fort Lauderdale, 33314, and <sup>3</sup>CIAT, Cali, Colombia.

Virion capsid proteins [ca 52,000 Mr (52K)] and virus-specific noncapsid proteins [ca 16,000 Mr (16K)] were readily identified from plants infected with maize stripe virus (MStpV), rice hoja blanca virus (RHBV) and *Echinochloa* hoja blanca virus (EHBV). All of the proteins were good immunogens in rabbits, and antisera produced to these 6 proteins reacted specifically by ELISA with extracts of their respective infected but not healthy plants. Western blot analysis showed the capsid proteins to be serologically unrelated to the noncapsid proteins. Serological and one-dimensional peptide mapping tests showed the RHBV and EHBV capsid proteins to be closely related. The noncapsid proteins of RHBV and EHBV also were closely related. The MStpV capsid and noncapsid proteins were distinct from those of RHBV and EHBV.

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ISOZYME PATTERNS IN RELATION TO THE INTERACTIONS OF BARLEY AND BARLEY STRIPE MOSAIC VIRUS. F. C. Wu and R. G. Timian, Department of Plant Pathology and USDA-ARS, North Dakota State University, Fargo, ND 58105.

Electrophoretic patterns of various isozyme systems of barley seedlings were studied in relation to the interactions of barley and barley stripe mosaic virus (BSMV). Isozyme patterns of healthy resistant, healthy susceptible, and BSMV-infected cultivars were compared. Changes as a result of infection by highly virulent (CV52(ND18)) and moderately virulent (CV42(ND159)) BSMV strains were also examined. The six barley cultivars used in these experiments were Black Hullless (CI 666), Larker (CI 10648), Manchuria (CI 1251), Silver King (CI 905), Moreval (CI 5724), and CI 4885. Alterations in activities of some of the isozymes were found to be correlated with BSMV infection as well as with the virulence levels of virus strains. Differences in the zymograms were also observed among uninoculated resistant and susceptible cultivars.

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INCIDENCE OF BEET WESTERN YELLOWS VIRUS AND POTATO LEAFROLL VIRUS IN POTATO LEAFROLL-AFFECTED POTATO PLANTS. M. M. Sibara and S. A. Slack, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

A total of 519 potato plants representing 18 different cultivars with leafroll symptoms were surveyed by ELISA for potato leafroll virus (PLRV) and beet western yellows virus (BWV) from 1982-85. In addition to Wisconsin samples, 60 samples were collected from the Maine, Minnesota, Nebraska and North Dakota winter test plots in Florida during 1985. Three antisera (one each specific for BWV and PLRV and one non-specific) were used in tests. The incidence level for these viruses in symptomatic plants using the specific sera was 4% for BWV alone, 16% for PLRV alone, 65% for BWV and PLRV; and using the non-specific serum was 85%. BWV was detected in the majority of samples tested from each state. Positive assays were not recorded for apparently healthy (symptomless) field plants collected as test controls. Neither virus was detected in 15% of the symptomatic samples, which suggested that a significant component of the potato leafroll complex was not detected by these sera.

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MULTIPLICATION AND TRANSLOCATION OF POTATO LEAFROLL VIRUS AND BEET WESTERN YELLOWS VIRUS IN SELECTED POTATO CULTIVARS. M. M. Sibara and S. A. Slack, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

The concentration of potato leafroll virus and beet western yellows virus in field-grown potato cultivars was monitored throughout the growing season by ELISA. Cultivars tested were Atlantic, Katahdin, LaChipper, Red LaSoda and Russet Burbank. For all cultivars, virus concentration increased in inoculated leaves for about 1 mo, then decreased to significantly lower levels with leaf maturation. Virus concentration was consistently higher in older leaves below the inoculation site in contrast to younger leaves above the inoculation site. Virus concentration in comparable tissues was lowest in the field-resistant cultivar Katahdin and highest in the susceptible cultivar Russet Burbank. Virus incidence in plants grown from harvested tubers was highest for the earliest inoculation date for all cultivars and decreased with each succeeding inoculation date. Virus incidence in these plants was consistently lowest for the cultivars Katahdin and LaChipper and highest for the cultivar Russet Burbank.

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PARTIAL CHARACTERIZATION OF MAIZE CHLOROTIC MOTTLE VIRUS. S. A. Lommel, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Maize chlorotic mottle virus (MCMV) interacts synergistically with maize dwarf mosaic virus resulting in a severe disease of corn, corn lethal necrosis. MCMV is a 30 nm sphere with a sedimentation coefficient of 112 S and a CsCl density of 1.362 and 1.251; the less dense particle lacks viral RNA. MCMV is composed of a single capsid protein of MW 24,600 daltons and a single RNA species of MW 1.47 X 10<sup>6</sup> daltons. Northern blot hybridization and dsRNA analysis suggest that a subgenomic RNA is produced *in vivo*, and this RNA may be packaged at low levels within the virion. Rabbit reticulocyte lysate translation of virion RNA yielded three major polypeptides: P45, P28, and P25. The P25 polypeptide was immunoprecipitated by MCMV antiserum. Comparisons of physical properties suggest that MCMV could be placed in the sobemovirus group; however, the translation profiles of MCMV and southern bean mosaic virus are distinct.

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EFFECTS OF ULTRAVIOLET IRRADIATION ON INFECTION WITH TOBACCO MOSAIC VIRUS (TMV). M. Chessin and A. Mitra, Department of Botany, University of Montana, Missoula, MT 59812.

Short-wave UV (UV-C) reduces both lesion number and size when applied to Pinto bean and *Nicotiana glutinosa* leaves prior to TMV infection. This suggests that both virus establishment and replication are inhibited. Irradiation 24 hrs. after inoculation increases both lesion number and size in Pinto bean, but gives results similar to pre-infection irradiation in *N. glutinosa* and in Samsun NN tobacco. Lower doses of UV-C partially overcame local resistance to TMV induced by ethylene maleic anhydride (EMA), while higher doses enhanced EMA-induced resistance. Post-infectious irradiation of Pinto bean had no effect on systemic acquired resistance (SAR) to subsequent virus infection, indicating that localization and SAR are not based on identical mechanisms. UV-C enhanced virus titers in "green islands" of TMV-infected tobacco, but reduced virus concentration in intervening yellow-green tissue. The UV probe may be useful in studying other kinds of non-hypersensitive resistance.

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THE INTERACTION OF HOST AND VIRUS STRAIN ON THE STABILITY OF INCLUSIONS OF MAIZE DWARF MOSAIC VIRUS. S. B. Jensen, N. Van Pelt, M.K. Palomar, USDA, Univ. of Nebraska, Lincoln, NE 68583.

The stability of the cylindrical inclusion in maize dwarf mosaic virus (MDMV) infected plants varied with virus strain and host. Inclusions of MDMV I-188 (type culture of MDMV-B) could be seen by electron microscopy in corn tissue (*Zea mays* L.) fixed and embedded at room temperature, but they could be seen in sorghum tissue [*Sorghum bicolor* (L.) Moench.] only if fixed and embedded at 0 C. MDMV A inclusions and inclusions of other MDMV-B isolates were stable in both hosts. Various pHs, buffers, temperatures and fixatives were ineffective in stabilizing MDMV I-188 inclusion during extraction. Mixing infected or healthy sorghum with corn during extraction did not destroy the MDMV I-188 inclusion from the corn. Inclusions of MDMV A but not inclusions of MDMV I-188 were recovered from sorghum doubly infected with both viruses.

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MOVEMENT OF MAIZE DWARF MOSAIC VIRUS IN THREE CORN HYBRIDS Vera K. Varner and Robert T. Gudauskas, Department of Botany, Plant Pathology and Microbiology, Auburn University, AL 36849

Enzyme-linked immunosorbent assay and serologically-specific electron microscopy were used to monitor movement of maize dwarf mosaic virus (MDMV) for 32 days after inoculation in corn hybrids of varying resistance to MDMV. Virus was initially detected in the inoculated leaves (1st and 2nd) of seedlings of all hybrids at 2 days after inoculation and subsequently in the 3rd & 4th leaves and stem of the susceptible hybrid (H60 X C103) at 4 days post inoculation; it was not found until 6 days after inoculation in the intermediate (FRMol7 x T232) and resistant hybrids (FR2A X B68J(T232)). Virus was found in roots of all hybrids at 4-6 days post inoculation, but numbers of particles never reached levels detected in the leaves. Maximum levels of virus were detected in all hybrids at 10 days after inoculation. Numbers of virus particles in the susceptible hybrid were 9 to 16 fold higher than in the intermediate and resistant hybrids.

DOUBLE-STRANDED RNAs DETECTED IN CUCURBIT VARIETIES NOT INOCULATED WITH VIRUSES. S. T. Nemeih and J. A. Dodds, Dept. Plant Pathology, University of California, Riverside, CA 92521.

Some field grown cucurbit plants naturally infected with potyviruses (zucchini yellow mosaic virus, and watermelon mosaic virus 2) contained virus specific and non-virus specific dsRNAs. Forty of 50 greenhouse grown cucurbit varieties that were not inoculated with viruses contained readily detectable amounts of dsRNA (MW = 0.5 to 11.0 x 10<sup>6</sup>). Five patterns of novel dsRNAs have been detected. Species tested were *Cucumis melo*, *Cucurbita pepo*, *Cucumis sativus*, *Citrullus lanatus* and *Luffa* spp. Presence and type, or absence of dsRNA in a given variety was consistent, indicating possible seed transmission of these factors in dsRNA positive varieties. Two of the dsRNA patterns were not graft transmissible between melon and watermelon. Phenotypic abnormalities have not been observed in varieties that are dsRNA positive. Virus specific dsRNAs of known cucurbit viruses were detected and described in extracts from varieties known to lack dsRNA prior to inoculation.

CHARACTERIZATION OF AN ENDOGENOUS PROTEINACEOUS INHIBITOR OF PLANT VIRUS FROM *Datura stramonium* L. A. Zipf and M. Chessin, Department of Botany, University of Montana, Missoula, MT 59812.

The biological activity of a preformed inhibitor of plant virus isolated from *Datura stramonium* L. was further studied. The inhibitor does not precipitate virus yet its inhibitory effect is restricted to the treated surface. The inhibitor has no effect on viral replication- it is an inhibitor of infection only. The inhibitor is effective on hypersensitive hosts except *D. stramonium* and not on systemic tobaccos. The thermal inactivation point (TIP) was between 65-70 C. The inhibitor is more stable under low pH conditions than high pH. Though some of the physico-chemical characteristics of the inhibitor are similar to other plant and animal antiviral proteins, the biological activity is not.

BIOLOGICAL AND SEROLOGICAL COMPARISON OF TOMATO RINGSPOT VIRUS ISOLATES. M.W. Bitterlin and D. Gonsalves, Cornell University, NY State Ag Exp Sta, Dept of Plant Path, Geneva, NY 14456

Tomato ringspot virus (TmRSV), which is endemic in North America, causes serious diseases in many crops, but the level of disease severity varies according to geographic location. This study was done to determine the pathogenicity and the serological relationship within a wide range of TmRSV isolates. Antisera produced to four TmRSV isolates (Chickadee, Grape Yellow Vein, Peach Yellow Bud Mosaic and WV 18) from different areas of the U.S.A. were tested against 26 TmRSV isolates collected in various areas of the U.S.A. and abroad. The isolates could be separated into four serogroups by direct enzyme-linked immunosorbent assay (ELISA), but the distinctness was less prominent by indirect ELISA. Agar double diffusion tests showed that the isolates were related but distinct. A pronounced variation in virulence among the isolates was observed, especially on *Nicotiana benthamiana*.

OVERGROWTH OF FUSIFORM RUST GALLS IN GREENHOUSE AND FIELD. C. H. Walkinshaw, Southern Forest Experiment Station, Box 2008 GMP, Gulfport, MS 39505; and F. F. Jewell, Sr., School of Forestry, Louisiana Tech. Univ., Ruston, LA 71272

Overgrowth and girdling of slash pines (*Pinus elliottii* Engelm. var. *elliottii*) appear in stem infections caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*. Affected trees die after this growth develops in the greenhouse or field. Analysis of greenhouse data shows differences in percent of galls with overgrowth and in some cases for inoculum X family interaction. Percent abnormal galls in 9-month infections was 40, 69, and 87 for three resistant families inoculated with a highly pathogenic rust isolate. Histological observations showed cambial degradation and callus cell proliferation. Similar galls occur in progeny tests in Florida. After the fifth growing season, slash pine families had up to 34% abnormal galls and a mortality as high as 54 percent. Significant differences among families for overgrowth galls strongly suggest the genetic basis for disease severity.

HISTOPATHOLOGY OF FUSIFORM RUST-INFECTED PROGENY OF SHORTLEAF X SLASH HYBRIDS BACKCROSSED TO SHORTLEAF PINE. F. F. Jewell, Sr., School of Forestry, Louisiana Tech University, Ruston, LA, 71272; and C. H. Walkinshaw, Southern Forest Experiment Station, Gulfport, MS 39503.

Test progeny were from 4 shortleaf x slash hybrids backcrossed by bulked shortleaf pine pollen. Samples from progeny with stem swellings similar to galls of fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) were taken 1 yr. after artificial rust inoculation at 6 wks. of age. Check samples were from uninoculated progeny. Check samples exhibited normal anatomy for *Pinus*. Samples from swellings revealed anatomical reactions typical for fusiform rust galls. Five samples initiated resistance-zones that failed to contain the pathogen. Hyphae and haustoria typical of fusiform rust were abundant in affected tissues. Backcross progeny of the type studied, which theoretically contained 2/3 fusiform rust resistant shortleaf germ plasam, expressed typical rust-susceptible anatomical reactions.

FUNGI ASSOCIATED WITH THE PINE WOOD NEMATODE, *BURSAPHELENCHUS XYPHILUS*, AND CERAMBYCID BEETLES IN WISCONSIN. Michael J. Wingfield, Peter J. Bedker and Robert A. Blanchette, Department of Plant Pathology, University of Minnesota, St Paul, MN 55108.

Fungi in pupal chambers of *Monochamus* spp. (cerambycid beetles) in jack and red pine (*Pinus banksiana* and *P. resinosa*) infested with *Bursaphelenchus xylophilus* were identified. They included seven *Ceratocystis* spp., two nematode trapping fungi (*Dactylella superba* and *Arthobotrys cladodes*), a mycoparasite (*Gloeosporium roseum*) and other wood inhabiting hyphomycetes. Of these, only *C. minor*, *A. cladodes* and *G. roseum* were isolated from adult *Monochamus* spp. Isolations from *B. xylophilus* yielded the same nematode trapping species found in the pupal chambers. This is the

first report of nematode trapping fungi associated with *B. xylophilus* in the United States.

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PERIODS OF SPORULATION AND INFECTION FOR *CHRY SOMYXA WEIRII* ON *PICEA PUNGENS* IN VERMONT. D.R. Bergdahl, School of Natural Resources, The University of Vermont, Burlington, VT 05405.

Sporulation and infection by *Chrysomyxa weirii* Jackson was monitored on blue spruce (*Picea pungens*) in a nursery in north-western, Vermont during May and June 1983-84. Each week, 10 silicone coated microscope slides were randomly placed near diseased trees to trap teliospores and basidiospores. Ten healthy seedlings of blue spruce also were exposed (one near each slide) each week during 1984 to determine periods of natural infection. Precipitation events also were recorded. More teliospores than basidiospores were trapped; peak numbers of both occurred during the same period. Peak spore dissemination occurred between May 17 and June 3, each year and was associated with precipitation. Few spores were trapped prior to or following the peak period of dissemination. Highest levels of needle infection (about 25%) were recorded on seedlings exposed during the peak period of basidiospore dissemination.

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PATHOGENICITY OF FASTIDIOUS, XYLEM-INHABITING BACTERIA (FXIB) ON AMERICAN SYCAMORE. J.L. Sberald, S.J. Kostka and S.S. Hurtt, Center for Urban Ecology, NPS, Wash., DC 20242, Crop Genetics Intl., Dorsey, MD 21076, and USDA, Beltsville, MD 20705.

Sycamore seedlings 8-12 cm tall were stem injected in May, 1981 by hypodermic needle with sycamore leaf scorch (SLS), or elm leaf scorch (ELS), or Pierce's disease (PD) FXIB. By Sept., 1982, leaf scorch appeared in 15/23 SLS-, 1/24 ELS-, 0/27 PD-, and 0/27 buffer-injected trees. Bacteria resembling FXIB were observed by phase contrast microscopy in supplemented PW broth/wood chip cultures from 19/20 SLS-injected trees, but not from 10 each of ELS-, PD-, or buffer-injected trees. Buffer- and SLS-injected trees were relocated from the greenhouse to the nursery in Oct., 1983. By Oct., 1984, leaf scorch affected 22/23 SLS- and 0/27 buffer-injected trees. Branch dieback affected 22 SLS-injected trees but only 3 buffer-injected trees. Mean trunk caliper and height were significantly smaller ( $P = 0.05$ ) for SLS- trees (2.35 cm, 1.95 m) than for buffer-injected trees (2.72 cm, 2.88 m).

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DORMANT HYMENOMYCETES AND WOUNDING IN ADVANCED WHITE AND GRAND FIR REGENERATION. Gregory M. Filip, Forest Pest Management, P.O. Box 3623, Portland, OR 97208; Paul E. Aho, 223 N.W. 30th St., Corvallis, OR 97330-5136; and Frances F. Lombard, Center for Forest Mycology Research, U.S. Dept. Agr., Forest Products Laboratory, P.O. Box 5130, Madison, WI 53705.

A total of 464 living white and grand fir stems in 23 stands in Oregon and Washington were dissected to detect dormant hymenomycete infections in woody tissue. Hymenomycetes most frequently isolated were *Echinodontium tinctorium* and *Heterobasidium annosum*, both of which caused the most discoloration and decay. Over 46% of 300 trunk wounds on 248 trees yielded hymenomycetes of which *E. tinctorium* and *Pholiota imonella* were isolated most frequently. Discoloration associated with *E. tinctorium* was always within 30 cm of wounds which were as recent as one year and as small as 56 cm<sup>2</sup>. Nearly 20% of all hymenomycete isolations, particularly *Hericium abietis* and *E. tinctorium*, were from dormant infections in healthy-appearing tissue not associated with discolored or decayed wood.

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REACTION OF BIGTOOTH AND QUAKING ASPEN TO ELK DAMAGE AND CYTOSPORA IN MICHIGAN. J.H. Hart, Dept. Forestry, Michigan State Univ., E. Lansing, MI 48824.

Bigtooth aspen (BTA), *Populus gradidentata*, was browsed by elk 60% more than quaking aspen (QA), *P. tremuloides*. Of 149 BTA stems broken by elk 46% were dead one year later. Mortality of 82 elk-broken QA was 24%. *Cytospora chrysosperma* was always associated with the dead stems. Twenty BTA and 20 QA were broken in July 1983 to simulate elk injury. By June 1984 3 BTA had died and cankers (caused by *C. chrysosperma*) averaged 38cm in length; none of the QA had died and cankers averaged 14cm.

Thus BTA was more susceptible to both herbivory and pathogen attack. This differential sensitivity to predation could reduce the relative competitiveness of BTA compared to QA thereby reducing populations of BTA compared to QA. This is an example of population changes based on a herbivore-pathogen complex.

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RELATIONSHIP BETWEEN ASPEN, SEWAGE SLUDGE, FUNGI AND ELK IN MICHIGAN. J.H. Hart, J.B. Hart, P.V. Nguyen, H. Campa III, and J.B. Haufler, Dept. Forestry, Michigan State Univ., E. Lansing, MI 48824.

Sludge fertilization of a 10-year-old bigtooth aspen increased first-year mortality from 4% to 27%. All of 132 recently-killed stems were infected with *Cytospora chrysosperma*, 65 with *Armillaria mellea*. Treated plots were browsed by elk more than control plots. Stem mortality associated with *C. chrysosperma* was 25% by September 1984 for aspen given simulated elk injury in July 1983. High and low root starch levels were associated with stable and contracting clones, respectively. Sludge treatment led to increased elk damage which altered growth form and created wounds, predisposing clones to pathogenic but non-lethal fungi. *C. chrysosperma* reduced the number of energy-requiring stems and hence the probability of attack by a lethal pathogen, *A. mellea*. Protecting energy reserves in a competitive environment is paramount to survival and hence maximizes fitness.

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Inoculation of Blue Spruce with *Cytospora kunzei* and ten other *Cytospora* spp. isolates. Tyre Proffer and John H. Hart, Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824-1312

Intact or excised branches from mature Colorado Blue Spruce (*Picea pungens*) were inoculated with mycelial plugs of four *Cytospora kunzei* isolates or with mycelial plugs of *Cytospora* spp. isolates from ten non-spruce hosts. Inoculations with the *C. kunzei* isolates consistently caused canker initiation on intact and excised branches. Differences in isolate virulence were indicated. None of the *Cytospora* spp. from non-spruce hosts caused canker initiation in intact trees. However, *C. leucostoma* did cause an expanding discoloration of cortical tissues in excised branch segments in 14% of the test inoculations. Inoculating branch segments appears to be a reliable method for studying the pathogenicity and/or virulence of *Cytospora* spp. isolates on blue spruce. *Cytospora* spp. on non-spruce hosts do not appear to serve as a source of inoculum for *Cytospora* canker of spruce in the field.

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COLONIZATION OF CONIFER THINNING STUMPS BY ROOT DISEASE FUNGI IN SE ALASKA. Charles G. Shaw III, USDA Forest Service, PNW, Forestry Sciences Laboratory, PO Box 909 Juneau, Alaska, 99801.

Young Sitka spruce and western hemlock trees (5-13 cm dbh) were felled in 1978 and half of the stumps inoculated with cultures of *Fomes annosus*. All 340 stumps were sampled in 1979 and 1983 for colonization by *F. annosus* and *Armillaria* sp. In 1979, *F. annosus* occurred in 10% of the stumps, including some of both species, but remained in only one hemlock stump by 1983. Decay caused by *F. annosus* is rare in live, young trees of either species. *Armillaria* sp. occurred in 14% of the stumps in 1979 and 36% in 1983. No trees near infected stumps have been killed by *Armillaria* sp., nor has the fungus caused high levels of mortality in young stands, although isolates of *Armillaria* sp. obtained from thinning stumps have infected and killed inoculated spruce seedlings. Currently, neither organism appears to be a threat to young forests. In 1983, *Resinicium bicolor* occurred in 26% of these stumps, including some of both species. The damage potential of this root disease organism, previously unreported from forests in SE Alaska, is unknown.

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BLACKSTAIN ROOT DISEASE ON LODGEPOLE PINE IN BRITISH COLUMBIA. D.J. Morrison and R.S. Hunt, Pacific Forest Research Centre, 506 West Burnside Road, Victoria, B.C. V8Z 1M5 CANADA

Surveys were made for incidence and damage by blackstain root disease (*Verticillium wageneri*) on *Pinus contorta* in British Columbia. Incidence surveys indicated the hard pine pathotype to be largely restricted to the west Kootenays, north Okanagan and southern Cariboo. Damage surveys of 177 ha in 12 stands revealed severe damage in pure dense stands 80 years and older at elevations above 1000 M. In one-half the stands, *V. wageneri* was associated with *Armillaria ostoyae* and in one-third, some trees in *V. wageneri* centers were currently infested with *Dendroctonus ponderosae*. Most disease centers were small, but sometimes there were many per stand, so that up to 45% of a stand was affected. The pattern of disease centers suggested that unknown vectors were responsible for initiating disease centers. Isolations from excavated stumps indicated little survival of *V. wageneri* after 1 year and none after 2 years. Infested stands can be clear cut and regenerated to *P. contorta* without risk of infection from the previous stand.

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A PUTATIVE NEW RACE OF *CRONARTIUM RIBICOLA* IN BRITISH COLUMBIA. R.S. Hunt and M.D. Meagher, Pacific Forest Research Centre, 506 West Burnside Road, Victoria, B.C. V8Z 1M5 CANADA.

On Vancouver Island, British Columbia, three plantations of 11-year-old western white pine (*Pinus monticola*) from the Idaho breeding program were surveyed for blister rust (*Cronartium ribicola*). In Idaho, this F<sub>2</sub> stock is 60-70% resistant to blister rust (based mostly on early shedding of infected needles). One plantation on a dry site had 10% of 506 trees infested, while 21% of 78 local-stock control trees were infested. A second plantation, about 40 km south of the first and near a lake, had 42% of 80 trees killed or with stem cankers from blister rust. At the third plantation, 4 km farther south, 23% of 65 trees were killed and another 52% of the stems were cankered, while at a nearby control plantation of local-stock, 8% of 50 trees were killed and another 44% of the stems were cankered. The abnormally high incidence of mortality and stem cankers in the latter two plantations suggests that a new race of *Cronartium ribicola* is present. The low incidence of rust in the first plantation suggests that the putative new race has a limited geographic distribution.

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ADDITIONAL ALTERNATIVE GRASS HOSTS OF LEPTOSPHERA NODORUM. L. K. Khokhar and R. P. Pacumbaba, Dept. of Natural Resources and Environmental Studies, Alabama A&M University, Normal 35762

*Leptosphaeria nodorum* Müller (Septoria nodorum blotch of wheat) was isolated from various wheat sources, air, triticale, *Hordeum pusillum*, *Lolium perenne*, *Bromus inermis*, *Aegilops cylindrica*, *Agropyron repens*, and *Cynodon dactylon*. No isolate was obtained from 7 other grasses. On oxgall agar, isolates appeared as pale green to light yellow green fluorescing colonies under UV light in the dark. Symptoms of disease were induced when the isolates were inoculated on wheat cv. Callahan 513, *L. perenne*, *B. inermis*, *H. pusillum*, and *C. dactylon*. The rate of infection of the isolates from wheat straw on cv. Callahan 513 was 58% and varied 4-26% from *H. pusillum*, *L. perenne*, *B. inermis*, *A. cylindrica*, *A. repens*, *C. dactylon*, volunteer wheat, and air. *L. perenne* and *C. dactylon* were established as two additional alternative hosts of *L. nodorum*.

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EVALUATION OF TRIADIMENOL SEED TREATMENT FOR EARLY SEASON CONTROL OF TAN SPOT, POWDERY MILDEW, SPOT BLOTCH AND SEPTORIA NODORUM SPOT ON SPRING WHEAT. W. C. da Luz and G. C. Bergstrom, Plant Pathology Dept., Cornell University, Ithaca, NY 14853.

Triadimenol seed treatment (TST) was evaluated for control of early infections of spring wheat cultivars Max and Sinton by *Pyrenophora tritici-repentis* (tan spot), *Erysiphe graminis* f.

*sp. tritici* (powdery mildew), *Leptosphaeria nodorum* (Septoria nodorum spot), and *Cochliobolus sativus* (spot blotch). In controlled environment studies, TST controlled both tan spot and powdery mildew up to 20-30 days and 40-50 days after sowing, respectively, but provided no control of disease incited by inoculum of *L. nodorum* or *C. sativus*. In field experiments, TST controlled early development of tan spot and powdery mildew for 5 and 10 days longer, respectively, than in controlled environment studies at 22 C. Control of both diseases in Sinton and tan spot control in Max (resistant to powdery mildew) resulted in yield increases of approximately 20 and 15%, respectively, at two different field locations.

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TRANSMISSION OF *CORYNEBACTERIUM FLACCUMFACIENS* BY SOYBEAN SEED. J. M. Dunleavy, U.S. Dept. Agriculture, 417 Bessey Hall, Iowa State University, Ames, IA 50011

*Corynebacterium flaccumfaciens* causes bacterial tan spot of soybean (*Glycine max*). The bacterium is transmitted by bean (*Phaseolus vulgaris*) seed, but has not been reported to be seed transmitted by soybean. Fifty field-grown Clark 63 soybean plants naturally infected with *C. flaccumfaciens* and showing symptoms of tan spot before plants flowered were harvested as single plants. Ten seeds from each plant were sown in sterile soil (2 seeds per 12.5 cm pot) and germinated in a greenhouse at 30 C. Seeds from non-infected Clark 63 plants were sown in separate pots as a control. Tan spot symptoms appeared on 26% of the seedlings from seed of diseased plants. Of the original 50 plants tested, 90% transmitted the bacterium to some seedling progeny. The mean bacterium transmission per plant was 2.6%, and the range was 0-90%. No tan spot developed on seedlings derived from seed of non-infected plants.

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MEASUREMENT OF MOISTURE STRESS DIFFERENCES BETWEEN BROWN STEM ROT DISEASED AND HEALTHY SOYBEANS. H. Tachibana and J. D. Hatfield, USDA and Department of Plant Pathology, Seed and Weed Science, Iowa State University, Ames, IA 50011

To study the interactions of moisture stress (MS), brown stem rot (BSR), and soybean yields, different methods of monitoring MS had been tested previously but each method had limitations that restricted the studies. We used thermocouples in conjunction with the Campbell CR21x data logger and recorded soybean plant leaf temperatures minus ambient temperatures (Delta T) to determine relative MS of healthy and BSR diseased plants. The combination thermocouple and data logger method provided consistent and significant differences in Delta T values between BSR infected and uninfected soybeans.

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INFLUENCE OF APPLICATIONS OF THIABENDAZOLE ON THE ISOLATION OF DIAPORTE BIOTYPES CAUSING SOUTHERN STEM CANKER OF SOYBEAN. D. V. Phillips, T. W. Hobbs, J. D. Arnett and F. M. Shokes, Georgia Experiment Station, Experiment, GA 30212 and North Florida Research and Education Center, Quincy, FL 32351.

Soybeans (cultivar Bragg) were planted in a field where soybeans were damaged extensively by southern stem canker the previous season. Thiabendazole (257 g ai/187 L water/hectare) was applied at weekly intervals beginning 4 wks after planting. Plants were assayed for *Diaporthe phaseolorum* isolates causing stem cankers (DP) by surface-sterilizing 2 cm sections from petioles and plating on a selective medium. Twelve weeks after spraying began petioles from the middle 6 nodes were taken from plants which received 0, 3, 6, 9, or 11 weekly thiabendazole applications. DP was isolated from 4.4 to 47.5% of the petiole sections plated from each plant sampled. The mean percentage of isolation of DP was 28.3, 21.4, 20.5, 15.0 and 13.9 for plants receiving 0, 3, 6, 9, and 11 applications, respectively. Plants were defoliated by severe drought before extensive stem canker symptoms developed.

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LEAF SPOT DISEASES OF WHEAT RELATED TO STUBBLE HEIGHT. J. M. Krupinsky and A. Bauer, USDA, ARS, Northern Great Plains Research Center, P. O. Box 459, Mandan, ND 58554.

The effect of spring wheat (*Triticum aestivum*) stubble height on subsequent development of leaf spot diseases was evaluated in two eight-leaved winter wheat cultivars, Roughrider and

Mironovskaya, in 1984. Five flagleaf collections were made at weekly intervals beginning when plants reached stage 9 on the Haun scale. Leaves were rated for percent necrotic tissue and lesions per cm<sup>2</sup>. The stubble height and stubble X cultivar interaction factors were significant. Plants in the tall stubble plots had consistently more disease symptoms than those in the short stubble plots. Since the stubble X cultivar interaction was significant, data from the two cultivars were analyzed separately. The stubble factor was significant for Mironovskaya but non-significant for Roughrider. In the tall stubble, Mironovskaya had a higher level of leaf spot diseases than Roughrider. *Pyrenophora tritici-repentis* was the most common plant pathogen present in the field.

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EFFECT OF SOIL pH ON INFECTION OF WHEAT BY CEPHALOSPORIUM GRAMINEUM. C. S. Love, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430

Spring wheats Dirkin and Wampur, and winter wheats Stephens and line BRL80112, were grown in nonsterile field soil adjusted to five pH levels (4.5, 5.0, 6.0, 7.0, 8.0) in the greenhouse. Seedlings in the 7-leaf stage were inoculated with a suspension ( $7 \times 10^7$  / ml) of *C. gramineum* conidia applied as a soil drench. Disease severity was assessed by separating tillers into healthy and diseased classes, then calculating percent infected tillers. No disease developed at pH 8.0. Disease severity was greatest at pH levels of 4.5-5.0 for Wampur and at 4.5-6.0 for Stephens. The pH did not affect disease severity in Dirkin and BRL80112. The greatest difference was found at pH 4.5 between Stephens, which was the most susceptible, and BRL80112, which was the most resistant, with 60.4% and 6.7% infected tillers, respectively. In conclusion, there appears to be an increase in disease severity with decreasing soil pH, especially in the highly susceptible cultivars.

#### 148

FUSARIUM SCAB OF WHEAT IN CENTRAL WASHINGTON. C. A. Strausbaugh and O. C. Maloy, Washington State University, Department of Plant Pathology, Pullman, WA 99164-6430.

One hundred sixty-seven wheat fields in Columbia Basin region of Washington were surveyed for the presence of scab caused by *Fusarium* spp. *Fusarium graminearum*, *F. culmorum*, and *F. nivale* commonly occurred in the irrigated fields, but *F. graminearum* was most prevalent. *Fusarium graminearum* was more pathogenic than the other two *Fusarium* spp. in a greenhouse study. Perithecia of *Gibberella zeae* were formed on wheat heads in the field and stored in the laboratory. Sporulation occurred for at least 32 wk with maximum sporulation after 11 wk. In fields with scab, the percentage of infected heads ranged from a trace to 89%. Scab was more prevalent in the center pivot irrigated fields and most severe in the center of those fields. Of the fields that had scab, 71% had 4% or less of the heads infected; therefore, scab had little effect on wheat yield. Infected grain had a low test weight, low germination, and reduced seedling vigor, consequently the quality of the grain was reduced.

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WHEAT TAKE-ALL IN COLORADO HIGH COUNTRY IRRIGATED SPRING WHEAT. W.M. Brown, Jr., L.E. Perotti, and J.P. Hill. Dept. of Plant Path. and Weed Sci., Colo. State Univ., Ft. Collins, CO 80523.

In 1981 a disease new to spring wheat growing under center pivot irrigation was detected in southern Colorado's San Luis Valley (SLV), elevation 2288 m. Subsequent investigation found the causal agent to be *Uromyces graminis* var. *tritici*. This was the first confirmed report of take-all on wheat in Colorado. While take-all has since been observed in continuous wheat culture elsewhere in Colorado, it is most serious in the SLV where reduced tillage is necessary to control wind erosion. The increasing take-all incidence is jeopardizing the implementation of this vital erosion management tool.

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SURVIVAL AND INOCULUM BUILD-UP OF FUSARIUM MONILIFORME AND F. SUBGLUTINANS IN COLORADO. R. L. Gilbertson, W. M. Brown, Jr., E. G. Ruppel, and L. G. Skoglund. Dept. of Plant Pathology and Weed Science, and USDA-ARS, Colorado State University, Fort Collins 80523.

Field population densities of the stalk rot pathogens *Fusarium moniliforme* and *F. subglutinans* increased in the corn rhizosphere throughout the season, then declined over winter. The fungi were recovered from a small portion of organic debris recovered from soil, but were consistently isolated from corn stubble on the soil surface. Stubble was the major overwintering site for these fungi, and disking of stubble in spring resulted in a flush of fungal growth and an increase in their recovery from organic debris. In the greenhouse, population densities of these fungi increased in the rhizospheres of corn seedlings. The amount of colonization was influenced by seed treatment and cropping history of field soils used in the experiments.

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EFFECT OF HERBICIDE REGIMES ON BEAN ROOT ROT SEVERITY AND DRY BEAN YIELDS IN COLORADO. R. L. Gilbertson, E. G. Ruppel, and E. E. Schweizer. Dept. of Plant Pathology & Weed Science and USDA-ARS, Colorado State University, Fort Collins 80523.

Field studies were conducted at Windsor, CO, to determine possible herbicide effects on the severity of bean root rot caused by a complex of *Fusarium solani* f. sp. *phaseoli* and *Rhizoctonia solani*. Herbicide regimes included: W<sub>1</sub>, untreated control; W<sub>2</sub>, preplant incorporated (ppi) EPTC; W<sub>3</sub>, ppi EPTC plus trifluralin; W<sub>4</sub>, ppi alachlor plus trifluralin followed by EPTC incorporated layby. In 1982 and 1983, herbicide regimes had no significant effect on disease severity or bean yield. In 1984, disease severity was not significantly affected, but bean yield was significantly lower in the W<sub>4</sub> regime than in the W<sub>2</sub> and W<sub>3</sub> regimes. Yield from the W<sub>4</sub> regime also was not significantly different from control yield. In Colorado, with beans grown under furrow irrigation in a rotation system with nonhost crops, root rot was not significantly enhanced by the usual herbicide regimes employed by growers.

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EFFECT OF TILLAGE AND HERBICIDES ON FUSARIUM STALK ROT OF CORN. L. G. Skoglund, R. L. Gilbertson, W. M. Brown, Jr., and E. G. Ruppel, Dept. of Plant Pathology & Weed Science and USDA-ARS, Colorado State University, Fort Collins, CO 80523.

In 1984, three tillage regimes (reduced till, chisel till, and conventional till) and three herbicides (alachlor, atrazine, and EPTC) applied at recommended rates, were tested for their effects on corn rhizosphere population densities (PDs) of *Fusarium moniliforme* (FM) and *F. subglutinans* (FS) and on disease incidence of Fusarium stalk rot. PDs were assayed by the dilution plate technique on Komada's medium while FM and FS infections of corn stalks were assayed by plating nodal tissue cores on Komada's medium. There were no significant effects of the herbicides on the PDs of FM and FS or on disease incidence. Reduced till plots had significantly higher FM and FS PDs than chisel till and conventional till plots. Stalks from all treatments were 100% infected by FM and/or FS in September.

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FIELD MEASUREMENT OF WHITE MOLD DISEASE RESISTANCE AND AVOIDANCE. D.H. Casciano and H.F. Schwartz, Dept. of Pl. Path. & Weed Sci., CSU, Ft. Collins, CO 80523.

Genetic resistance and plant architectural characteristics can reduce damage caused by *Sclerotinia sclerotiorum* in dry beans. Twelve bean lines were compared in replicated field plots to measure the effects of inoculum production and disease incidence on yield. Entries were split into control and protected subplots. Protection consisted of three weekly foliar sprays of benomyl (2.24 kg/ha) after initiation of blossoming. The number of apothecia/m<sup>2</sup> varied from 0.37 - 3.27 on 14 August to 1.26 - 5.28 on 23 August. Generally, fewer apothecia were found in plots with upright plant types. The differential disease pressure created by the subplots distinguished entries with genetic resistance (P.I. 169787, Black Valentine) from those with upright (83 VEF HXA 222, A51) or prostrate (U.I. 114, Olathe) plant types on the basis of disease incidence and yield differences. These parameters were lowest for entries with genetic resistance and disease avoidance.

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UREDINIOSPORE YIELDS TO DEMONSTRATE AND MEASURE OAT CULTIVAR DIFFERENCES IN TOLERANCE TO PUCCINIA CORONATA. M. D. Simons, M.

Khan, and J. Sacks. ARS, USDA, Dept. of Plant Pathology; Dept. of Statistics; and ARS, USDA, Dept. of Statistics, respectively, ISU, Ames, IA 50011

Seven greenhouse grown, *P. coronata* susceptible oat cultivars were measured for urediniospore yield (an objective measure of disease intensity), grain yield and seed weight. All plants were heavily infected (urediniospore yields of 12.2 to 20.7 g/cultivar). Percentage reductions in yield and seed weight of inoculated plants compared to controls ranged from 11% to 59% and 10% to 34% respectively. These reductions reflected differences in percentage reduction in grain yield and seed weight/g of spore yield, and did not reflect differential levels of resistance since correlations between yield or seed weight and spore yield were small and statistically nonsignificant. The results imply cultivar differences in tolerance to *P. coronata*, and show substantial cultivar variation in ability to maintain yield under heavy rust conditions.

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DISEASE INCIDENCE AND SEVERITY, AND SOYBEAN SEED MYCOFLORA IN SIX TILLAGE SYSTEMS. Cavanaugh, K. J., Machado, C. C., Manandhar, J. B., and Sinclair, J. B. Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

Disease incidence and severity, and seed mycoflora were recorded from six different tillage systems: two rotations, two cultivars, two row spacings. Narrow-row spacing (25-cm) in rotation with corn had a higher incidence of seed mycoflora than seeds from 76-cm rows. The incidence of *Macrophomina phaseolina* (charcoal rot) and *Colletotrichum truncatum* (anthracnose) on stem pieces collected at harvest was recorded after desiccant herbicide treatment. *M. phaseolina* was highest in Corsoy 79 in 1983 and 1984; *C. truncatum* varied within year and tillage system. *C. truncatum* was higher in 25-cm than 75-cm rows. Disease incidence was highest in reduced tillage early in the season. Seedborne *Phomopsis* spp. and other fungi were highest in Cumberland and in 25-cm rows for both cultivars. Incidence of *Phomopsis* spp. and other fungi was negatively correlated with germination.

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EFFECT OF MATRIC AND OSMOTIC POTENTIAL, TEMPERATURE, AND pH ON THE GERMINATION OF *TILLETIA INDICA* TELIOSPORES. M. Dupler, J. L. Smilanick, and J. A. Hoffmann, USDA-ARS, Logan, UT 84322.

The effect of variable physical factors on *T. indica* teliospore germination on soil and synthetic media was determined. Water potential was osmotically adjusted with KCl, NaCl, or sucrose or matrically adjusted with polyethylene glycol 6000. Optimum germination on all osmoticants occurred at the highest potential tested (-1.4 bars); significant reduction occurred at lower potentials and germination did not occur below -10.5 bars with NaCl or KCl or below -14.5 bars with sucrose. Similarly, germination did not occur below -11.0 bars on matrically adjusted media. Optimal (15-20C) or suboptimal (10C) temperatures allowed greater germination at lowered potential compared with supraoptimal temperature (25C). Highest germination (55-60%) occurred at 15 - 20C on both soil and water agar. On pH adjusted soil or water agar, maximum germination occurred from pH 5.0 to pH 8.8 with significant reduction at less than pH 4.5.

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SOME FEATURES OF RHIZOMORPH MORPHOGENESIS IN *ARMILLARIA* SPP. J. J. Worrall, I. Chet\* and A. Huttenmann, Forstbotanisches Institut, Univ. Göttingen, 3400 Fed. Rep. Germany, and \*Dept. of Plant Path. and Microb., Hebrew Univ., Rehovot 76100, Israel

Rhizomorph (RM) induction in *Armillaria* spp. by ethanol required alcohol dehydrogenase. Acetaldehyde also functioned as an inducer. Induced cultures contained higher lipid levels than non-induced cultures, and suppression of fatty acid synthetase with cerulenin prevented growth of RMs but not of mycelium. Ethanol apparently provides acetyl-CoA necessary for lipid synthesis, thus allowing RM production. Following induction by any inducer, RM production is associated with laccase (PPO). Laccase activity appeared just prior to RMs, remained proportional to RM growth rate, was localized in RMs rather than mycelia, was correlated with RM production by a series of isolates, and its inhibition by various means suppressed RM formation. Thus laccase, thought to play a role in fruiting body formation by basidiomycetes, may also be important in morphogenesis of rhizomorphs.

## 158

Cultural characteristics and spot test reactions of *Endothia parasitica* on four agar media. D. F. Hindal, WVU Plant Path. and Ag. Micro., P.O. Box 6057, Morgantown, WV 26506-6057.

Cultural characteristics produced on four selected agar media and the reactions of spot test reagents (syringaldazine, naphthol and gum guaiac) were evaluated among *Endothia parasitica* strains. These evaluations were conducted after 10-days growth at 25C in darkness or in a 16:8 photoperiod. Generally, dark grown cultures exhibited reduced orange and brown pigmentation and sporulation and poor reactions to the spot test reagents. Although reduced radial growth was common, pigment formation and sporulation usually occurred at 16:8 on glucose-yeast extract, asparagine or arginine media, whereas, zonation, orange and purple pigmentation and modest sporulation occurred on tryptophane. Syringaldazine, naphthol, and gum guaiac reactions were strongest on the glucose-asparagine medium and weakest on tryptophane.

## 159

EFFECT OF CONTROLLED ATMOSPHERES ON PROPAGULE FORMATION BY *PYTHIUM APHANIDERMATUM* AND *TRICHODERMA HAMATUM* IN VITRO. P.A. Mauk and I.J. Misaghi, Dept. of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721.

*Pythium aphanidermatum* and an antagonist, *Trichoderma hamatum*, were grown individually and collectively on 5% V8 agar at 27C in the dark under the following percentages of CO<sub>2</sub>/O<sub>2</sub>: 0.03/20.9 (ambient atmosphere), 1/20, 4/17, 7/14, and 10/11. The numbers of oospores and sporangia of *P. aphanidermatum* decreased 50% in cultures exposed to CO<sub>2</sub> levels above 1%, compared to those exposed to the ambient atmosphere. However, the suppressive effect of CO<sub>2</sub> on oospore production was reduced as much as 33% when *P. aphanidermatum* was co-cultured with *T. hamatum*. Conidial formation by *T. hamatum* increased when levels of CO<sub>2</sub> were increased from ambient to 1%, but had a linear decrease when levels were raised from 1% to 10%. However, changes in % CO<sub>2</sub> had no influence on the numbers of chlamydospores formed. The radial growth rates of *T. hamatum* and *P. aphanidermatum* were not affected significantly with increases in CO<sub>2</sub> levels.

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CARBON DIOXIDE MODULATES SPORULATION OF *ALTERNARIA* SPECIES. P.J. Cotty, Dept. of Plant Pathology, Univ. of Arizona, Tucson 85721.

*Alternaria alternata*, *A. brassicae*, *A. carthami*, *A. citri*, *A. cucumerina*, *A. macrospora*, *A. porri*, *A. raphani*, and *A. tagetica* varied in their ability to sporulate in constant dark (27 C). Sporulation (27 C, 12 hr 11,000 lux cool-white fluorescent light daily) of all species was either partially or totally inhibited when culture plates were sealed. This inhibition was reversed when CO<sub>2</sub> was removed via absorption by KOH. A mutant of *A. tagetica* insensitive to plate sealing was generated. The mutant sporulated profusely in continuous dark and continuous light (20 C, 9,000 lux); the wildtype did not. Mutant spore yield was not increased by the diurnal light cycle compared to constant dark; constant light (9,000 lux) totally inhibited its sporulation at 27 C. Absorption of CO<sub>2</sub> induced species which typically do not sporulate in constant dark to do so. These results suggest that CO<sub>2</sub> may play an important role during light regulated sporulation of *Alternaria* species. The mutant was pathogenic and sporulated in liquid shaker cultures. Thus, similar mutants may be useful for inoculum production of mycoherbicides.

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INCORPORATION AND DISTRIBUTION OF <sup>3</sup>H-ARACHIDONIC ACID IN THE MYCELIAL LIPIDS OF *PHYTOPHTHORA INFESTANS*. R. M. Bostock, K. McCue and J. R. Creamer, Department of Plant Pathology, University of California, Davis, CA 95616

Arachidonic (AA) and eicosapentaenoic acids elicit sesquiterpene phytoalexin accumulation in potato tuber and occur primarily as esters in the lipids of *Phytophthora infestans*. Mycelial cultures of *P. infestans* race 0 growing on a synthetic medium at 20°C were supplied with 1-2 uCi of <sup>3</sup>H-AA 72 hr prior to extraction of the cultures with a mixture of chloroform and methanol (2:1, v/v). Lipids were fractionated and analyzed by thin-layer chromatography and liquid scintillation. Label was efficiently recovered in all the principal lipids which included in order of abundance: triglycerides, diglycerides, free fatty acids, phosphatidylethanolamine, monoglycerides, diphosphatidylglycerol, ceramide aminoethylphosphonate, phosphatidylinositol, lysophosphatidylethanolamine, phosphatidylcholine and steryl esters. The potential of this system for investigating possible effectors

of release of elicitor fatty acids from the mycelium and relevance to the host-pathogen interaction will be discussed.

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GLUCOSE EFFECTS ON NADP AND NAD GLUTAMATE DEHYDROGENASES IN *BIPOLARIS MAYDIS* RACE T. T. W. Bischoff and M. O. Carraway, Dept. of Plant Path., The Ohio State Univ., Columbus, OH 43210.

*Bipolaris maydis* race T was grown on a glucose (2 g/l) L-asparagine (4 g/l) mineral salts liquid medium. Activity of NADP-glutamate dehydrogenase (NADP-GDH) and NAD-glutamate dehydrogenase (NAD-GDH) was determined by measuring the change in absorbance at 340 nm of reduced NADP or NAD in a reaction mixture containing  $\text{NH}_4\text{Cl}$ ,  $\alpha$ -ketoglutarate and an extract of the fungus after 48, 72 or 96 hrs. NADP-GDH activity was highest after 48 hrs dropping 8 fold after 72 hr and remaining constant after 96 hrs. In contrast, NAD-GDH activity was low after 48 hrs and increased 4 fold after 72 hrs before returning to its original level. Glucose was detected in the culture medium after 48 hrs but not after 72 hrs. In contrast ammonium was not detected in cultures after 48 hrs but after 72 or 96 hrs increased to 10.6 and 23.0  $\mu\text{moles/ml}$ . Assimilation of ammonium by NADP-GDH and the suppression of ammonium production by NAD-GDH may be one way glucose acts to delay the accumulation of ammonium in cultures of *B. maydis* race T on L-asparagine.

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PURIFICATION AND PROPERTIES OF THE PHYTOXIN(S) PRODUCED BY *ALTERNARIA BRASSICAE*. P.S. Bains and J.P. Tewari, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

An alternative method for isolating the phytotoxin(s) produced by *Alternaria brassicae* was developed. Concentrated culture filtrate was passed through Sephadex G-50 and Sephadex G-25 columns. The active fractions thus obtained were adsorbed overnight on activated charcoal at 4 C, and desorbed from the charcoal by washing with ethyl acetate. Partially Purified Toxin (PPF) from the ethyl acetate washings was isolated by adsorption chromatography using salicylic acid. Application of PPF on scratched excised leaves of *Brassica napus* and *B. campestris*, the two natural hosts, caused chlorosis and necrosis within 48 h. Phytotoxic response to PPF was also observed on non-hosts such as wheat, barley, potato, and peas. Tomato and cowpea, however, were not affected.

## 164

INFLUENCE OF TEMPERATURE AND MOISTURE ON CONIDIAL GERMINATION OF *CERCOSPORA ARACHIDICOLA*. S. C. Alderman and M. K. Beute, Dept. Plant Pathology, North Carolina State University, Raleigh, N.C. 27695-7616.

Conidia of *Cercospora arachidicola* atomized onto peanut leaves and incubated in dew chambers at 16, 19, 22, 25, 28, or 31 C began germinating by 2-4 hr; maximum germination (85-95%) occurred by 24-48 hr at 16-25 C. At 28 and 31 C, only 50 and 30% of the conidia, respectively, had germinated by 48 hr. Germination and germ tube elongation were similar for conidia applied dry or in water suspension and dried onto leaves. Germination of conidia was poor in a water film on glass slides but was enhanced in water on leaf wax (extracted from leaves with chloroform and dried on glass slides) at 16, 20, 24, 28, or 32 C and was similar to that on leaves incubated in dew chambers. Germination on wax-coated slides was greatest at or near saturation but occurred at humidities as low as 94% by 48 hr. For humidity control, conidia on slides were positioned on screens in a 4 mm gap between two NaCl-amended agar slabs in sealed 9 cm diam petri plates.

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RELATIONSHIP OF FUNGAL CELL WALL REGENERATION TO CERCOSPORIN RESISTANCE. K. D. Gwinn and M. E. Daub, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

*Cercospora nicotianae* protoplasts were isolated to study the role of the fungal cell wall in the resistance of this fungus to the toxin, cercosporin. Approximately 90% of the protoplasts regenerated in medium containing 0.7 M NaCl. Osmotic sensitivity, which was confirmed by lysis in media lacking salt, remained high for 12 hours but then decreased linearly. Tinopal 5BM bound preferentially to glucans with both  $\beta$  1,3 and 1,6 linkages which are known to occur in hyphal walls. Only 1% of freshly isolated protoplasts fluoresced when treated with Tinopal 5BM, but by 4 hr, approximately 90% fluoresced. Tinopal 5BM binding did not affect protoplast regeneration. Freshly isolated fungal protoplasts

were killed by 10  $\mu\text{M}$  cercosporin, but at 1.0  $\mu\text{M}$  (a concentration lethal to plant cells) 50% of the protoplasts remained viable. Protoplasts began to regain resistance to cercosporin within 4 hours. Thus, cercosporin resistance appears to correlate with the regeneration of cell wall glucans, but precedes the regeneration of an intact cell wall.

## 166

THE ROLE OF CAROTENOIDS IN RESISTANCE OF FUNGI TO CERCOSPORIN. M. E. Daub and G. A. Payne. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Cercosporin is a photosensitizing toxin produced by *Cercospora* species which generates singlet oxygen and superoxide when activated by light. Plants, mice, and bacteria are all sensitive to cercosporin, but *Cercospora* spp. and several other fungi are highly resistant. Of the species tested, the Ascomycetes and Deuteromycetes were resistant to cercosporin whereas the Oomycetes were sensitive. Carotenoids, which are potent quenchers of singlet oxygen and of the activated state of photosensitizers, appear to play a role in resistance since mutants of *Neurospora crassa* blocked at 3 different loci for carotenoid production are more sensitive to cercosporin than the wild type strain. *Cercospora* spp. produce high concentrations of carotenoids, approximately 90% being  $\beta$ -carotene.  $\beta$ -carotene production is highest at the beginning of the growth cycle (approximately 12  $\mu\text{g}$   $\beta$ -carotene per g dry wt mycelium at 3 days) and then decreases. We have been unable to block carotenoid production in *C. nicotianae* by the use of 3 different carotenoid inhibitors.

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SCREENING FOR MONOCLONAL ANTIBODIES TO T-2 TOXIN AND OCHRATOXIN BY COMPETITIVE INDIRECT ENZYME IMMUNOASSAY. E. H. Gendloff, J. J. Pestka, and L. P. Hart. Michigan State University, East Lansing, MI 48824.

A hemisuccinate derivative of T-2 toxin (T-2HS), conjugated to polylysine or ovalbumin, was used in a competitive indirect enzyme immunoassay. In this assay, free toxin inhibits binding of anti-T-2 antibodies to the T-2HS conjugate adsorbed to microtiter plates. Bound antibody is quantitated with an enzyme labeled second antibody and enzyme substrate. The assay detected anti-T-2 mouse antibodies in hybridoma supernatants. The ovalbumin conjugate allowed more sensitive detection than the polylysine conjugate. By duplicate testing of supernatants with and without free toxin, binding of nonspecific antibody could be distinguished from specific binding. An equivalent assay for ochratoxin using polylysine behaved similarly, although some interference by serum was noted.

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DEGRADATION OF PHENOLIC CONSTITUENTS IN PEACH BARK BY *CYTOSPORA CINCTA*. Elke Endert-Kirkpatrick and David F. Ritchie, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Conidial suspensions of *Cytospora cincta* or sterile distilled water were inoculated onto propylene oxide-sterilized bark cores from peach (*Prunus persica* 'Redhaven'). Aqueous, filtered extracts of macerated bark discs were analyzed periodically from 0 to 5 wk. Total polyphenolic content was determined at peak UV absorbance in ethanol between 280-285 nm. Tannic acid and flavanol contents were quantified at 760 nm using Folin-Denis reagent and at 500 nm against vanillin- $\text{H}_2\text{SO}_4$ , respectively. Polyphenolic content in colonized bark decreased throughout the experiment as compared to uncolonized controls. Tannic acid levels were initially equivalent in both treatments but declined in colonized bark 4 wk after inoculation. Flavanol content declined after 7 da and remained lower with respect to controls. Flavanol and tannin decreases were concomitant with onset of visible colonization and pycnidial maturation, respectively.

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PHYTOXIN PRODUCTION BY VIRULENT AND AVIRULENT ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *MEDICAGINIS*. T.R. Knous, C.L. Hartman and J. Druin. Department of Plant Science, University of Nevada, Reno, NV 89557

Alfalfa plants resistant to *Fusarium oxysporum* f. sp. *medicaginis* were obtained from cell cultures selected for resistance to toxic components from virulent fungal cultures. Studies utilizing

susceptible cell cultures as a bioassay demonstrated that toxin production was maximum at 25C and remained relatively constant after 15 days in culture. Comparisons between chromatograms (HPLC) of extracts from virulent and avirulent cultures reflect the absence of apparent toxin from avirulent isolates of the fungus.

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Effect of smut caused by *Ustilago scitaminea* on yield of sugarcane in Louisiana. J. W. Hoy<sup>1</sup>, C. A. Mollier<sup>2</sup> and D. B. Fontenot<sup>2</sup>, Dept. of Plant Pathology and Crop Physiology, La. Ag. Exp. Station and <sup>2</sup> La. Coop. Extension Service, La. State Univ. Agricultural Center, LSU, Baton Rouge, LA 70803.

A highly significant negative correlation was found between levels of smut infection and sugarcane yield. Natural infection levels and yield estimates were determined in 25 single-row plots within four commercial fields, three plant-cane and one ratoon-cane, of a smut-susceptible cultivar, CP 73-351.  $R^2$  values for each field were 0.61, and 0.62, 0.74 and 0.79, respectively.  $R^2$  for a combined analysis was 0.74. The most significant effect of smut on yield was a reduction in number of healthy canes in diseased plots. Smut infection levels sufficient to cause appreciable yield losses have not been observed in moderately susceptible commercial cultivars, such as CP 65-357 and CP 74-383.

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PYRENOCHAETA LEAF BLOTCH DEVELOPMENT AND YIELD REDUCTION OF SOYBEANS IN ZAMBIA. L. E. Datnoff, J. B. Sinclair, Dept. of Plant Path., Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801, and D. M. Naik, I I T A, Ibadan, Nigeria.

Pyrenochaeta leaf blotch (PLB) of soybeans, caused by *Pyrenochaeta glycines* Stewart, is a potentially serious disease in Zambia. Development of PLB and the effects of PLB on soybean yield and 300-seed weight were studied in a 2 x 6 factorial experiment that included four replications. Main plots were weekly applications of fentin acetate sprays at 0.9 a. i. g/ha and unsprayed treatments. Six soybean cultivars were subplots. There was a significant ( $P=0.05$ ) treatment\*cultivar interaction for disease severity, PLB vertical progress, yield and 300-seed weight. Disease severity, PLB vertical progress, and AUDPC for the unsprayed cultivars ranged from 16.4 to 30.5 %, 90 to 100 %, and 653.9 to 1322.5, respectively. Yield and 300-seed weight losses between sprayed and unsprayed plots ranged from 6.6 to 37.1 % and 20.1 to 25.8 %, respectively. These results indicate that yield losses due to PLB occurred through a reduction in seed size, but differed for the six cultivars.

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CUCURBIT BLIGHT IN NORTHWESTERN IRAN. M. Babadoost, Dept. of Plant Pathology, College of Agric., Univ. of Tabriz, Tabriz 51664, Iran, and P. Asadi, Plant Pest and Disease Research Center, P. O. Box 237, Tabriz 51338, Iran.

In the summer of 1984, a severe blight occurred in cucumber, squash, melon, and watermelon fields in Azarbaijan and Zanjan, the northwestern provinces of Iran. Disease severity was assessed 77% causing an estimated yield loss of \$100 million. Plants showed a prominent yellow mosaic at first followed by necrosis and foliar distortion. Fruits on infected plants were small, distorted, and with glossy knobs, particularly on squashes. In addition to the above ground symptoms, most of the diseased plants showed root infection from which *Fusarium*, *Phytophthora*, and *Macrophoma* were isolated. Due to an unusual warmer weather in the winter and spring of 1984, there was an outbreak of aphids in the area. Therefore, it is speculated that the yellow mosaic virus was disseminated by aphids throughout the area and the virus disease interacted with root infection resulting in rapid destruction of the plants.

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IMPACT OF ANTHRACNOSE ON YIELD OF EIGHT ALFALFA CULTIVARS IN WISCONSIN, 1983. M. A. Hansen, Dept. of Plant Path., Physiol., and Weed Sci., VPI & SU, Blacksburg, VA 24061 and C. R. Grau, Dept. of Plant Path., Univ. of Wis., Madison, WI 53706.

Anthracnose of alfalfa, caused by *Colletotrichum trifolii*, has been recognized only recently as a problem in Wisconsin and other midwestern states. Severe outbreaks of the disease cause reductions in forage dry weight yield and plant density in the

southeast United States. Eight alfalfa cultivars were field tested in Wisconsin during a mild epiphytotic in 1983. Stem lesions and foliar chlorosis developed on the cultivars, but crown necrosis was absent. Disease severity was significantly greater in cvs. Thor and Phyltor than in cvs. Atlas, Vancor, Duke, Armor, and Valor during July and August. Reductions in forage dry weight but not plant density were attributed to anthracnose. Use of anthracnose-resistant cultivars would improve forage yields in Wisconsin even under conditions of low anthracnose disease pressure.

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QUANTITATIVE RELATIONSHIPS BETWEEN ALFALFA LEAF SPOT DISEASES AND YIELD. S. C. Broscious and H. W. Kirby, Department of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Different levels of alfalfa leaf spot disease epidemics were established in each of 8 trials using natural infection, fungicide applications, and inoculations. Several yield models were fitted by least-squares regression for each trial with area under disease-progress curve (AUC), defoliation index, or weekly disease severity assessments as the independent variable. More than one model adequately described the disease-yield relationship for each trial. Severity of leaf spot diseases at harvest ( $X_T$ ) and AUC were the simplest and most consistent predictors for all trials ( $r^2=0.33-.72$  and  $.24-.76$ , respectively). Intercepts from the AUC and  $X_T$  models were equivalent within trials and used to transform yields to percentage of maximum (Y) within trials. Regression of Y on AUC and  $X_T$  was conducted over locations. Six of 8 trials had homogenous slopes which combined to develop the prediction equations:  $Y = 99.14 - 0.12(AUC)$ , ( $r^2=.51$ )  
 $Y = 99.96 - 1.47(X_T)$ , ( $r^2=.52$ ).

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EFFECT OF TWO VIRUSES ON COMPONENTS OF YIELD OF CORN IN SOUTH CAROLINA. Graydon Kingsland and O. W. Barnett, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, S.C. 29631.

Maize dwarf mosaic virus strain A (MDMV) and maize chlorotic dwarf virus (MCDV) limit yields of corn (*Zea mays* L.) throughout South Carolina each year. An average of 42% of symptomless plants of 3 varieties produced 2 ears, compared with 6% of adjacent plants with MDMV and/or MCDV. Fewer kernels ( $P=0.05$ ) were produced on ears of Funk G4522 and Golden Harvest H2775A plants with MDMV (417 and 449, respectively) or MCDV (179 and 77, respectively) than on ears from adjacent symptomless plants (546 and 632, respectively). Average weight of grain was less ( $P=0.05$ ) from ears of Funk G4522 and Golden Harvest H2775A plants with MDMV (114 g and 105 g, respectively) or MCDV (15 g and 12 g, respectively) than from adjacent symptomless plants (168 g and 181 g, respectively). Infection of Golden Harvest H2775A by MCDV resulted in greater reduction of kernels per ear and weight of grain than infection by MDMV.

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THE ROLE OF WHEAT LEAVES IN GRAIN YIELD AND LEAF RUST LOSSES. M. Seck, P. S. Teng, and A. P. Roelfs, Dept. of Plant Pathology, and Cereal Rust Lab. USDA, ARS, University of Minnesota, St. Paul, MN 55108.

Inoculation with leaf rust, at the boot stage, of a leaf or combination of leaves was used in place of the usual clipping and shading. The regression of yield on average disease severity per tiller was significant with a slope of 0.47 and  $r^2 = 0.90$ . However equal disease severities resulted in different yield losses, since at later growth stages an increase of disease on the lower leaves resulted in little decrease in yield. The flag leaf and the two leaves below it contributed 26, 12, and 3% to the final grain weight, respectively. Disease severity (DS) on each leaf was weighted by the contribution (K) of that leaf (i) to obtain an effective severity (ES) per tiller, i.e.  $ES = \sum K_i \times DS_i$ . The regression between the effective severity and the yield loss was highly significant, with  $r^2$  of .96 and a slope of 1.0, which suggests a 1:1 relationship between yield loss and ES.

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DISEASE AND WEED EFFECTS ON YIELD COMPONENTS OF BARLEY. J. R. Burleigh, M. Tajani and M. Seck, Institut Agronomique et Veterinaire, Rabat, Morocco and Department of Plant Pathology, Univ. of Minn., St. Paul, MN 55108



The effects of net blotch (caused by *Pyrenophora teres*) and weeds on barley yield were studied. When area under the disease progress curve [AUDPC, (y axis = disease scale 0.0 to 1.0 and x axis = growth stage 0 to 100)] were under 1.0 from tillering to soft dough, grain yield and weight were positively correlated with AUDPC. When AUDPC was more than 6.0 it was positively correlated with total number of florets/spike and grain/spike. But negatively correlated with grain yield and weight. Therefore, low levels of disease severity normally encountered during spikelet formation might increase floret numbers and enhance their survival while severe disease during grain filling negatively affects grain filling and yield. Weeds explained a significant portion of the variability in grain yield and improved  $r^2$  values from 61% for disease alone to 78.6% for weeds and disease. Yield decreased linearly with disease increase but asymptotically with an increasing weed population.

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RELATIONSHIPS AMONG DISEASE, YIELD AND THE NUMBER OF SUSCEPTIBLE PLANTS IN A MIXTURE. I. S. Hoang, A. P. Roelfs, and P. S. Teng. Dept. of Plant Pathology, and Cereal Rust Lab., USDA, ARS, Univ. of Minnesota, St. Paul, MN 55108

Seeds of Alex (resistant) and Baart (susceptible) cultivars were mixed in ratios of 100:0, 99:1, 90:10, 80:20, 60:40, and 0:100, respectively, and planted in 33 x 36 m plots replicated twice. Each plot was infected with *Puccinia graminis* f. sp. *tritici*, race 15-TNM. A diseased and sprayed section were maintained in each plot. Four impaction spore traps were installed per plot and the number of spores trapped was obtained daily. Severities were estimated twice weekly at four sites in each plot section. Terminal disease severities were 18, 56, 74, 82, and 92% for the susceptible cultivar in mixtures of 1 to 100%, respectively. The log cumulative number of spores and log severities were closely correlated. Total grain yield and 1,000 kernel weight of the susceptible cultivar were related to the ratio of susceptible plants in the mixture. The disease and yield components were directly correlated.

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THE EFFECT OF INTERACTING PEST POPULATIONS ON POTATO YIELD. K.B. Johnson\*, E.B. Radcliffe\*\*, and P.S. Teng\*. Depts. of Plant Path.\* and Entomology\*\*, Univ. of Minn. St. Paul, MN 55108.

Yield reduction in potato caused by the potato leafhopper (*L*)(*Empoasca fabae*), early blight (*E*)(*Alternaria solani*), and Verticillium wilt (*V*)(*V. dahliae*) was studied on three cultivars (*C*) in factorial field experiments. Levels of *L*, *E*, and *V* were significant ( $p=0.05$ ). In addition, *CxL*, *CxE*, *LxE*, and *LxEV* interactions were significant ( $p=0.05$ ). Maximum yield reduction by each pest alone was 63, 37, and 16% for *L*, *E*, and *V*, respectively. Maximum yield loss by any combination of pests was 75%. Significant effects in 1984 were *C*, *L*, *E*, and *CxE*. Pest x *C* interactions were related to greater yield losses on late maturing cultivars compared to an early maturing cultivar. Pest x Pest interactions show that yield losses by solitary pests are not additive.

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ANALYSIS OF POTATO FOLIAGE LOSSES CAUSED BY MULTIPLE PESTS AND THE RELATIONSHIP TO YIELD. K.B. Johnson\*, P.S. Teng\* and E.B. Radcliffe\*\*, Depts. of Plant Path.\* and Entomology\*\*, Univ. of Minn., St. Paul, MN 55108.

In a factorial field experiment with potato infested with leafhoppers (*L*)(*Empoasca fabae*), early blight (*E*)(*Alternaria solani*), and Verticillium wilt (*V*)(*V. dahliae*), weekly readings of % hopper burn (HB), % early blight (EB) and % defoliation (DEF), were made and area under curves (AUC) determined. Principal component analysis of AUC's and yield revealed two components that explained 89% of the variation in the data. Factor loadings in the first component were .36, .09, .96, and -.79 for AUBC, AUEBC, AUDEFC, and yield (respectively) indicating the relationship between defoliation and yield. The second component contrasted AUBC and AUEBC suggesting competition for sites. In an ANOVA of AUDEFC, significant effects included *L*, *E*, *V*, *LxE*, *LxV*, *ExV*, and *LxEV* ( $p=0.05$ ). Regression of yield on the integral  $((1-DEF/100) \times (1-(EB+HB)/100))$  was significant ( $R^2=61$ ). Because all three pests can cause defoliation, it is difficult to partition yield loss among pests.

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ASSESSMENT OF DOWNY MILDEW YIELD LOSS WITH NEAR-ISOGONIC SORGHUM POPULATIONS. J. Craig and G. N. Odvody, USDA-ARS, P.O. Box EC, College Station, 77841, and Texas A&M Res. and Ext. Center, Corpus Christi, 78410.

Three sorghum populations differing in susceptibility to sorghum downy mildew (SDM) were produced with  $F_3$  progenies derived from a cross of resistant and susceptible sorghum inbreds. The  $F_3$  families were tested for reaction to SDM and those with similar reactions were combined to form resistant, intermediate, and susceptible populations. No significant differences in yields were found among the populations when protected from SDM by treatment with metalaxyl. In an unprotected trial, incidences of SDM were 2%, 17%, and 28% for resistant, intermediate, and susceptible populations, respectively. Differences among populations for disease incidences and grain yields were statistically significant. Correlation coefficient for yield and SDM incidence was -0.99 ( $P=0.05$ ). A linear regression model described the relationship between SDM severity and yield reduction.

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EFFICIENCY OF CHEMICAL CONTROL OF MUNGBEAN DISEASES PLANTED AFTER RICE. F.A. Elazegui & T.W. Mew. The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

The fungicide use pattern of rainfed rice farmers was studied at two sites, Pangasinan and Iloilo. The experiments were carried out in two villages at Pangasinan and three at Iloilo. Efficiency in disease control was analyzed on the basis of marginal benefit-cost ratio. In one village at Pangasinan, where the grain yield was higher, the marginal-cost ratio was 4.2 with thiophanate methyl (70% WP) as compared to benomyl (50% WP) at 3.0 and 3.8 for two rates of application. In the second village, the ratio was 3.3 for thiophanate methyl and 1.7 for benomyl. Benomyl was more efficacious in controlling powdery mildew of mungbean. In Iloilo, where the yield was low due to poor crop establishment, the marginal benefit-cost ratio was not different even though differences in disease index were significant.

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SUPPRESSION OF SEPTORIA TRITICI AND PUCCINIA RECONDITA INFECTION ON WHEAT SEEDLING LEAVES BY FLUORESCENT PSEUDOMONADS. E. Levy<sup>1</sup>, Z. Eyal<sup>1</sup>, and I. Chet<sup>2</sup>. <sup>1</sup>Dept. of Botany, Tel Aviv Univ., Tel Aviv 69978 and <sup>2</sup>Dept. of Plant Pathology and Microbiology, The Hebrew University, Rehovot 76100, Israel.

Two isolates of fluorescing pseudomonads (LEC 1 and LEC 2) secured from soil exhibit suppression of *S. tritici* and *P. recondita* infection on wheat seedlings. The isolates expressed inhibition toward *Geotrichum candidum*, *Rhizoctonia solani*, *S. tritici*, *Sclerotium rolfsii*, and towards *Aerobacter aerogenes*, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus* spp., and *Proteus vulgaris* but was ineffective towards *Serratia marcescens*, on defined media. Inhibition of *S. tritici* development was manifested on silica gel pre-coated thin layer chromatography (TLC) plates by several fractions soluble in organic solvents. Some of these fractions also inhibited *S. tritici* growth on defined media as well. In addition, inhibition of *S. tritici* growth on defined media was also expressed by ammonia-like gaseous compounds produced by these pseudomonads on King's B medium plates.

DIFFERENTIAL EFFECTS OF VERTICILLIUM WILT RESISTANT AND SUSCEPTIBLE POTATO GENOTYPES ON POPULATIONS OF RHIZOSPHERE BACTERIA. H. R. Azad, J. R. Davis, W. C. Schnathorst, and C. I. Kado. University of Idaho, Moscow and Aberdeen, and University of California, Davis, CA 95616.

A two-year field study in Idaho provided the first evidence in potato research that verticillium wilt resistant A66107-51 and susceptible Russet Burbank potato genotypes differentially influence populations of rhizosphere bacteria. In field plots bacterial populations were logarithmically higher in the rhizosphere of the resistant clone. These differences included increases in bacteria that exhibited *in vitro* antagonism to *Verticillium dahliae* RB-5 and the ability to fix nitrogen. Bacteria antagonistic to *V. dahliae* were predominantly *Bacillus* spp. Other antagonists were spp. of *Pseudomonas*, *Gluconobacter*, *Flavobacterium*, and *Streptomyces*. Nitrogen-fixing bacteria were *Azotobacter* and *Azomonas* spp. Suppression of disease in Russet Burbank in the growing season following the planting of A66107-51, may in part be explained by these findings.

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BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF POME FRUITS. W. J. Janisiewicz, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, West Virginia, 25430.

Antagonists against *Penicillium expansum* (incitant of blue-mold of pome fruits) and *Botrytis cinerea* (incitant of grey-mold) were isolated throughout the season from apple fruit, leaves, and orchard soil. The majority of these antagonists consisted of bacteria and yeast. *In vitro* antagonistic activity was demonstrated on NYDA medium in Petri plates by inhibition of mycelial growth in the case of *B. cinerea* and by spore germination in case of *P. expansum*. Tests were also conducted in water suspension where fungal spore germination was determined. When tested *in vivo* on apples and pears, few antagonistic isolates applied in water suspension at concentrations of approximately  $5 \times 10^7$  cells/ml gave total control of the rot on fruit wounded 3 mm deep and challenged with *P. expansum* spore suspension of  $1 \times 10^5$ /ml. The best performing antagonists were those isolated from apple fruit and leaves. Attempts are being made to determine which antagonist is most active and best adapted to storage conditions.

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FIELD PRODUCTION OF ORRINA PHYLLOBLIA FOR THE BIOLOGICAL CONTROL OF SILVERLEAF NIGHTSHADE. P. E. Parker and E. Rivas, USDA-APHIS-PPQ, Biological Control Laboratory, Edinburg, TX 78539.

The use of an endemic, foliar, gall forming nematode as a biological control organism against silverleaf nightshade, *Solanum elaeagnifolium* Cav., is dependent in part upon development of reliable and economical methods of mass producing the nematode. Field plots of the weed were established by transplanting four month old potted seedlings at a rate of 17,000 plants per acre. Plots were inoculated with galls at a rate of 10 lbs/acre by three different methods; soil incorporation prior to plant emergence, spray suspension of nematodes to emerged plants, and broadcasting of dried nematode inoculum over the tops of emerged plants. Soil incorporation prior to plant emergence produced the highest total amount of inoculum; up to 155 pounds per acre of dried nematode galls per acre. Following the first harvest, plots were disked, reinoculated and irrigated. This technique allows two harvests of nematode galls per growing season.

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EFFECT OF *Rhizobium* spp. ON BEAN ROOT ROT PATHOGENS. A.J. Buonassisi, R.J. Copeman and H.S. Pepin. Crop Protection Branch, B.C. Min. of Agr. and Food, 17720-57th Ave., Surrey, B.C. V3S 4P9; Plant Science Dept., #248-2357 Main Mall, University of B.C., Vancouver, B.C. V6T 2A2; and Agriculture Canada Research Station, 6660 N.W. Marine Drive, Vancouver, B.C. V6T 1X2.

In dual culture plate assays indigenous *Rhizobium* strains isolated from nodules obtained from commercial snap bean fields in the Lower Fraser Valley of British Columbia inhibited the radial growth of most strains of *Fusarium moniliforme*, *F. oxysporum* and *F. solani* f.sp. *phaseoli*. Growth of all *Pythium sylvaticum*, *P. ultimum* and *Rhizoctonia solani* strains tested was unaffected by *Rhizobium*. *Rhizobium* strains causing growth inhibition *in vitro* also caused a significant reduction in the

severity of *Fusarium* root rot in both growth pouch and greenhouse pot experiments. These data suggest that the potential exists for reducing *Fusarium* root rot of bean by employing nodulating *Rhizobium* strains which are also highly antagonistic to *F. solani* f.sp. *phaseoli*.

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AFFECTS OF WHEAT RESIDUE AND TILLAGE ON *PYTHIUM ULTIMUM* POPULATIONS IN A DRYLAND PEA-WHEAT ROTATION. Charles M. Rush, R. E. Ramig, and John M. Kraft, USDA/ARS, IAREC, P.O. Box 30, Prosser, WA 99350.

A field study was conducted at two sites to evaluate the effects of wheat residue and tillage on *Pythium ultimum* populations. At each site, chaff and straw were collected from 12x12-m plot areas as it came from a commercial combine and either removed (no chaff treatment) or placed back on the center 6 m of each plot at a rate of approximately 357 g/m<sup>2</sup> (1X chaff treatment) or 715 g/m<sup>2</sup> (2X chaff treatment). Six wks after wheat harvest, one-half of each plot was lightly disked and one-half moldboard plowed. At 10 and 22 wks after plowing, there was no significant difference in *Pythium* populations between any of the residue plots, nor were there any population differences between plowed and disked treatments. An evaluation of chaff colonization indicated that 3% or less of the chaff was colonized by *P. ultimum* at either site, however over 90% was colonized by *Penicillium hordei*, suggesting a natural biocontrol.

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SUPPRESSION OF *PYTHIUM ULTIMUM* POPULATION BUILDUP IN WHEAT CHAFF AMENDED SOILS BY FUNGAL AND BACTERIAL BIOCONTROL AGENTS. Charles M. Rush and John M. Kraft, USDA/ARS, IAREC, P.O. Box 30, Prosser, WA 99350.

Sterile wheat chaff was treated with *Trichoderma reesei*, *T. viride* (T-1-R4, T-1-R9, 4208-75), *Glucocladium virens*, *Penicillium oxalicum*, *P. hordei*, *Coniothyrium minitans*, *Pseudomonas fluorescens* (Qz30-8, Qz-30-1, Qa-72-4, Rz-B-2, Ry-5-5), and *Bacillus subtilis*. Non-treated chaff and soil without chaff were included as controls. Chaff colonization by *P. ultimum* was correlated ( $r=.84$ ) to final *Pythium* populations. *Pythium* populations were increased to 183 and 641 cfu/g soil in the no chaff CK and chaff CK treatments, respectively. *Penicillium oxalicum* and T-1-R4 maintained *Pythium* populations at the base density of 50 cfu/g soil, and T-1-R9, *T. reesei*, and *G. virens* reduced populations to 16 cfu/g soil. Generally, the bacterial treatments were ineffective in preventing population buildups with the exception of *B. subtilis* and Ry 5-5.

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RELATIONSHIP OF *IN VITRO* INHIBITION OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* AND *IN VIVO* SUPPRESSION OF TAKE-ALL BY FLUORESCENT PSEUDOMONADS. D. M. Weller, W. J. Howie, and R. J. Cook. USDA-ARS, WSU, Pullman, Washington 99164

Nine fluorescent pseudomonads were isolated from roots of wheat grown in a take-all suppressive soil. These strains were highly inhibitory to *G. graminis* var. *tritici* (Ggt) on King's medium B and potato dextrose agar and in general more suppressive of take-all *in vivo* than were 18 pseudomonads from two conducive soils that were slightly or not inhibitory to Ggt *in vitro*. Eight mutants selected from suppressive strains 2-79 and 11a-80 for reduced or lack of inhibition of Ggt, were still able to colonize roots but were less suppressive of take-all than the parents. When FeEDTA was added to the soil, the ability of strains 11a-80, 2-79, and L30b-80 to suppress take-all was, respectively, unchanged, reduced, and completely eliminated. It appears that strain 11a-80 suppresses take-all by antibiotic production, strain L30b-80 by siderophore production, and strain 2-79 by both kinds of compounds.

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BIOCONTROL OF CUCUMBER AND CARROT POWDERY MILDEW BY *AMPELOMYCES QUISQUALIS*. Abraham Szejtberg and Shlomit Mazar. The Hebrew University of Jerusalem, Department of Plant Pathology and Microbiology, The Faculty of Agriculture, Rehovot 76100, Israel.

Effective biocontrol of cucumber and carrot powdery mildew (PM) was obtained through hyperparasitism by *Ampelomyces quisqualis* (AQ), applied as a bio-fungicide suspension of 1-2 million spores/ml. In a trial of greenhouse-grown cucumbers heavily naturally infected with PM, (18 plants per treatment),

no yield was obtained in the untreated plants. Combined alternate treatments of two sprays with AQ and one fungicide spray at a low concentration (pyrazophos 0.05%), yielded 3.6 Kg/plant. Plants treated with AQ alone yielded 2.5 Kg/plant and with the fungicide alone, 2.8Kg/plant. Although, the combined treatment gave ca 27% yield increase over treatment with AQ alone or fungicide alone, it was not statistically significant. In an "organic" carrot field at Sde-Eliyahu treated weekly (x7) with AQ, complete parasitism of the PM was achieved. Plants with hyperparasitized PM remained more vital than those with unparasitized PM, although both had the same PM coverage.

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USE OF *TRICHODERMA* IN PROPAGATION MEDIA USED FOR BEDDING PLANTS. M. Windham, Y. Elad, and R. Baker. Dept. of Plant Pathology and Weed Sci., Colorado State Univ., Fort Collins, CO 80523

The feasibility of using *Trichoderma* spp. for enhanced plant growth in propagative media for bedding plants was investigated. *T. harzianum* (T-12) was added to seedling or transplant media used for marigolds; rate of flowering and number of flowers per plant were stimulated by T-12. Six *Trichoderma* isolates were screened for the ability to enhance tomato growth in peat pellets. Plant growth effects were isolate dependent and ranged from a significant 162% increase in tomato dry weight at 6 wk after planting to a nonsignificant 4% decrease. Initial T-12 population densities of  $1 \times 10^5$  and  $1 \times 10^6$  colony forming units (cfu) (added as peat bran culture) /g of peat (from peat pellets) increased leaf area of eggplant significantly over controls. Plants grown in pellets with initial T-12 population densities of  $1 \times 10^4$  and  $1 \times 10^7$  cfu/g of peat did not differ in leaf area from controls. Initial population densities of  $1 \times 10^8$  were detrimental to eggplant growth.

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ENHANCED PLANT GROWTH INDUCED BY *TRICHODERMA* AMENDMENTS. M. Windham, Y. Elad, and R. Baker. Dept. of Plant Pathology and Weed Science, Colorado State University, Fort Collins CO 80523

Enhanced plant growth induced by *Trichoderma* added to soil was investigated to determine if observed increased plant growth was due to a direct effect of *Trichoderma* or a secondary effect due to control of plant pathogens. *Trichoderma* increased emergence of tomato and tobacco seedlings in autoclaved soil over that of controls. Microflora populations in soil with and without *Trichoderma* amendments did not differ qualitatively or quantitatively except for *Trichoderma* populations. Increased soil fertility corresponded with increased *Trichoderma* growth enhancement of tomato. Radish plants, grown under gnotobiotic conditions with *T. koningii*, were significantly larger than plants grown without the fungus. Rate of seed germination of corn, tomato, and tobacco was stimulated by *Trichoderma* separated from the seed by a cellophane membrane.

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ENHANCED SAPROPHYTIC DEVELOPMENT OF *TRICHODERMA HARZIANUM* ISOLATE T95 IN SOIL. R. Clevestine, and R. Baker. Dept. of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

In a series of experiments, the effects of substrate amendment, soil pH, matric potential, and temperature were studied on washed conidia of *Trichoderma harzianum* isolate T95 added to a sandy loam ( $10^6$  conidia/g) and incubated 1 week. In this soil system, the greatest population densities were achieved in soil amended with cellulose (2.5% w/w) and  $\text{NH}_4\text{NO}_3$  (C/N = 20), at pH 5.0 and a matric potential of -1.0 bar. Densities attained under these conditions at 17, 25, and 33 C were  $3.2 \times 10^6$ ,  $1.7 \times 10^7$ , and  $6.8 \times 10^6$  cfu/g, respectively.

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

The rhizosphere competence of *Trichoderma harzianum* was measured for roots of bean, cucumber, maize, radish and tomato. When seeds were coated with conidia of the wild type under constant matric potential with no additional  $\text{H}_2\text{O}$  added, the fungus was not detected in the rhizospheres from 1-8 cm depths of roots after 8 days. Mutants tolerant to benomyl, however, were rhizosphere competent when 10 ug benomyl per g. of soil was added. These mutants were also rhizosphere

competent even if benomyl was not added. Degree of colonization of rhizospheres by mutants of *T. harzianum* was influenced by soil temperature and pH.

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EPIDEMIOLOGY OF PUCCINIA HETEROSPORA ON ANODA CRISTATA. W. H. Ridings, P. H. Hoyer, and L. E. Schimmel, Dept. of Plant Pathology & Physiology, Clemson University, Clemson, SC 29631.

*Puccinia heterospora*, a microcyclic rust, was studied for its potential use in biological control of spurred anoda (*A. cristata*). Basidiospores were released from teliospores in telia that were incubated for 4 hr at 95-99% RH at 15, 20, or 25 C. Germination and germ tube elongation occurred best at 15 and 20 C. Basidiospore release and germination were none to very poor at 30 C. Basidiospores were produced equally well by teliospores from telia collected at 7 and 33 days following initiation of symptoms. An average of 20 telia per leaf developed after 5-7 days on *A. cristata* seedlings treated with  $8 \times 10^3$  teliospores per ml and incubated at 20 C and 95-99% RH for 48 hr in a growth chamber. Basidiospore inoculum resulted in good infection (21 telia/leaf) after 4 hr incubation at 20 C and 95-99% RH. No symptoms developed on inoculated plants incubated at 30 C.

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RESORCINOL-5-HEPTADECENYL, AN ANTIFUNGAL COMPOUND POSSIBLY INVOLVED IN THE LATENCY OF ALTERNARIA ALTERNATA IN UNRIPE MANGO FRUITS. S. Droby, D. Prusky, B. Jacoby and A. Goodman. Department of Fruit and Vegetable Storage, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

A preformed antifungal compound was isolated from unripe mango fruits and identified as resorcinol-5-heptadecenyl. Concentration of the compound in peel of unripe fruits was 200  $\mu\text{g/g}$  f.wt. tissue, but decreased during ripening to 100  $\mu\text{g/g}$  f.wt. and was accompanied by an increase in the rate of symptom expression of *A. alternata*.  $\text{ED}_{50}$  for growth of germinated conidia of *A. alternata* was 120  $\mu\text{g/ml}$ . Ethylene treatment enhanced the decrease of the antifungal compound and shortened the period for symptom expression. Storage under hypobaric pressure delayed symptom expression and the decrease in the antifungal compound concentration. In fully ripe 'Kitt' fruits the antifungal compound did not decrease and no symptoms of decay were observed. The results suggested a possible involvement of the antifungal compound in the latency of *A. alternata* in unripe mango fruits.

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REGULATION OF LIPOXYGENASE ACTIVITY AND ITS POSSIBLE RELATION TO LATENCY OF COLLETOTRICHUM GLOEOSPORIOIDES ON AVOCADO FRUITS. D. Prusky, B. Jacoby, J.J. Sims and S.L. Midland. Department of Fruit and Vegetable Storage, The Volcani Center, Bet Dagan 50250, Israel and Department of Plant Pathology, University of California, Riverside, Ca 92521, U.S.A.

A natural inhibitor of avocado lipoxygenase was isolated from peel of unripe avocado fruits and identified as epicatechin. Epicatechin concentration in unripe fruits was 514  $\mu\text{g/g}$  f. wt. and decreased during ripening to 8  $\mu\text{g/g}$  f. wt., before symptoms of *Colletotrichum* were expressed. Concentration of the inhibitor decreased differentially in ripening fruits of two avocado cultivars on which *Colletotrichum* symptoms developed at different rates. Ethylene treatment enhanced the decrease of the lipoxygenase inhibitor on avocado fruits and shortened the period before disease symptoms were expressed. The evidence supports the hypothesis that the lipoxygenase, which catalyzes metabolism of 1-actoxy-2-hydroxy-4-oxo-hexacos-12,15-diene involved in the latency of *C. gloeosporioides* (Prusky et al., 1982, *Phytopathology* 72: 1578) is affected by epicatechin.

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CHARACTERIZATION OF THE HOST-SELECTIVE TOXINS FROM COCHLITOBOLUS VICTORIAE: STRUCTURE OF DEGRADATION PRODUCTS. T.J. Wolpert, V. Macko, Boyce Thompson Inst., Cornell Univ., Ithaca, NY 14853; W. Acklin, B. Jaun, J. Seibl, J. Meili, D. Arigoni, Org. Chem., Swiss Federal Inst. of Technology, CH-8092 Zurich. Investigation of the host-selective toxin complex (victorin) from *C. victoriae* has led to the isolation of several selectively toxic compounds. Three of these compounds, designated B, C, and D, inhibited root growth of susceptible oat seedlings by 50% at 80-120pg/ml, and were completely characterized. FAB measurements indicated that the major compound, C, has an apparent

molecular weight of 796 and contains 3 Cl atoms. Acid hydrolysis of this compound yielded a number of fragments including glyoxylic acid, 5,5-dichloroleucine (Cl<sub>2</sub>leu), threo-β-hydroxylysine (OHlys), erythro-β-hydroxylysine (OHleu), and 2-alanyl-3,5-dihydroxy-Δ<sup>2</sup>-cyclopenten-one-1 (victala). Extended analysis of the UV and NMR spectroscopic data of the toxin eventually disclosed the additional presence of an α-amino-β-chloro-acrylic acid (αClaa) residue. Thus the apparent molecular weight of the toxin must correspond to the composition C<sub>21</sub>H<sub>43</sub>O<sub>12</sub>N<sub>6</sub>Cl<sub>3</sub>.

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STRUCTURE OF THE HOST-SELECTIVE TOXINS PRODUCED BY COCHLIOBOLUS VICTORIAE. T.J. Wolpert, V. Macko, Boyce Thompson Inst., Cornell Univ., Ithaca, NY 14853; W. Acklin, B. Jaun, J. Seibl, J. Meili, D. Arigoni, Swiss Federal Inst. of Technology, CH-8092 Zürich. The previous abstract describes the structure of the residues from which the major toxin (C) of the title organism is assembled. Partial hydrolysis of the toxin yielded a set of degradation products from which the sequence of the residues could be unambiguously determined as: N-glyoxylyl-Cl<sub>2</sub>leu-OHlys-OHleu-αClaa-victala. In addition, these experiments revealed the presence of an acid labile oxygen bridge which is part of a vinylogous lactone group linking the hydroxyl group of OHleu with the chromophore of the victala residue. According to the NMR data the amide bound glyoxylic acid moiety must be present as a hemiacetal or more probably as the hydrated form. In the latter case the composition of the toxin would have to include one H<sub>2</sub>O unit in excess of the apparent molecular weight of 796. Closely related structures could be derived for toxins B and D by comparison of the spectroscopic data. In contrast to toxin C, toxin B contains a monochloroleucine residue while toxin D lacks the 5-hydroxyl group of the victala moiety.

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DIFFERENTIAL PHYTOTOXICITY OF PEPTIDES FROM VERTICILLIUM DAHLIAE RACES 1 AND 2. A. Nachlas, V. Buchner, L. Tsrer, Y. Burstein and N. T. Keen. Volcani Institute, Gilat, Israel; Dept. of Organic Chemistry, Weizmann Institute, Rehovot, Israel; and Dept. of Plant Pathology, Univ. of Calif., Riverside, CA 92521.

Resistance to Verticillium wilt of tomato caused by race 1 of *V. dahliae* is conferred by the *Ve* gene. However, race 2 isolates pathogenic on *Ve* tomato cultivars have recently been isolated. A race 1 isolate of *V. dahliae* from potato was previously shown to produce a phytotoxic peptide that is associated with wilt symptoms and root damage in potato. The race 1 peptide also caused disease symptoms on tomato cultivars lacking the *Ve* gene but not on *Ve* cultivars. The analogous peptide from culture fluids of *V. dahliae* race 2 had a different amino acid composition and was equally phytotoxic to tomato cultivars carrying or lacking the *Ve* gene. We therefore suggest that the pathogenicity of *V. dahliae* race 2 is related to the production of an altered phytotoxic peptide that is able to overcome the tolerance of *Ve* gene tomatoes to *V. dahliae* race 1 and its phytotoxic peptide.

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EFFECT OF CHITOSAN ON THE VIABILITY OF PLANT AND FUNGAL CELLS IN THE PEA-FUSARIUM SOLANI SYSTEM. L. A. Hadwiger and D. F. Kendra, Dept. of Plant Pathology, Wash. State Univ., Pullman, WA 99164-6430.

Changes in pea cell viabilities were compared after treatment with either *Fusarium solani* or chitosan. Pea endocarp cells retain their viability as measured by fluorescein diacetate (FDA) staining, through the first 6 hr after treatment with chitosan (1 mg/ml), *F. solani* f. sp. *pisi*, or *F. solani* f. sp. *phaseoli*. Within 15 hr, viability is lost in pea cells directly in contact with f. sp. *phaseoli* spores, while a larger area is involved with f. sp. *pisi* spores. Chitosan treatments reduce cell viability within 12 hr. Since resistance is expressed within 4-6 hr following treatment, it is apparent that resistance is not associated with host cell death. FDA staining of f. sp. *phaseoli* and f. sp. *pisi* is reduced within 12 hr and is regained in f. sp. *pisi* as active growth resumes. Chitosan (1 mg/ml) reduces the viability of f. sp. *phaseoli* and f. sp. *pisi* at 1 hr and 3 hr, respectively.

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RELATIVE EFFECT OF CRAB SHELL AND FUNGAL WALL CHITOSAN ON FUSARIUM GROWTH AND INDUCED RESISTANCE. D. F. Kendra and L. A. Hadwiger, Dept. of Plant Pathology, Wash. State Univ., Pullman, WA 99164-6430

Immunochemical studies indicate that some compounds released during the pea-*Fusarium* interaction contain hexosamine determinants. In this study *F. solani* fungal cell wall and crab shell chitosans (hexosamine polymers) were evaluated for their ability to: suppress fungal growth, induce phytoalexin formation and disease resistance in pea. Fungal wall chitosan inhibited *F. solani* f. sp. *phaseoli* and *F. solani* f. sp. *pisi* at 16 µg/ml and 32 µg/ml, respectively, and induced pisatin formation in pea tissue at levels similar to those induced by crab shell chitosan. Fungal wall chitosan (500 µg/ml), added to pea endocarp tissue at 0, 4, 8 and 12 hr prior to challenge with f. sp. *pisi* induced resistance. These results confirm the hypothesis that fungal chitosan activity is indistinguishable from that of commercially prepared crab shell chitosan and that it functions as an elicitor in the pea-*Fusarium* interaction.

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HISTOPATHOLOGY OF MACROPHOMINA PHASEOLINA IN SOYBEAN SEEDS AND SEEDLINGS. Indra K. Kunwar, Singh, T., Machado, C. G., Sinclair, J. B., Dept. of Plant Path., 1102 S. Goodwin Ave., Univ. of Illinois, Urbana, IL 61801.

This is the first report of *M. phaseolina* infection of soybean seeds in Illinois. Microtome sections (10-20 µm) of soybean seeds were stained with safranin and light green and mounted in Canada balsam. Histopathologic studies on symptomatic seeds showed that hyphae and sclerotia were present ecto- and endophytically. Hyphae were inter- and intracellular in seed coat layers and in the embryo. Seed coats of severely infected seeds were fragmented. About 4% of the asymptomatic seeds produced sclerotia after 2 days incubation on PDA. The sclerotia were formed in or adjacent to the seed coat hypodermis and endosperm. This would suggest that *M. phaseolina* can penetrate and colonize seeds without producing symptoms and subsequently form sclerotia when conditions are favorable for seed germination. After 3-4 days post germination of infected, asymptomatic seeds, sclerotia were formed in the cotyledons and after 4-5 days in the hypocotyl-radicle axis.

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CYTOCHEMISTRY OF THE EXTRAHAUSTORIAL MATRIX IN RUST FUNGI. D.E. Harder, J. Chong and R. Rohringer, Agriculture Canada Research Station, 195 Dafoe Rd., Winnipeg, Canada R3T 2M9.

The extrahaustorial matrix of mature haustoria of *Puccinia graminis tritici* and *P. coronata* was investigated cytochemically. The tests and their results were: 1) Periodate-thiocarbohydrazide-silver proteinate (PA-TCH-SP) for glycosubstances with vicinal hydroxyls - positive staining; 2) Cellulase and protease - reduced staining by uranyl acetate/lead citrate (UA/PbC) and PA-TCH-SP (cellulase data only for *P. coronata*); 3) Lipid solvents (*P. coronata* only) - reduced staining by UA/PbC and PA-TCH-SP; 4) Gold-conjugated lectin probes: Concanavalin A for α-linked bound sugars - strong affinity, unaffected by protease; wheat germ lectin for bound N-acetylglucosamine (chitin) - no affinity; β-lectin for β-linked bound sugars - no affinity except after protease treatment. The results indicate that the extrahaustorial matrix contains lipids, bound carbohydrates and protein, probably in complex forms.

Loss of ethylene dibromide (EDB) fumigant nematicide leaves many farmers without an adequate means for controlling nematodes on cotton. The alternative fumigant, Telone II (1,3-D), requires a 7-14 day waiting period between application and planting. It is also more costly than EDB and the alternative non-fumigants. Lower rates of 1,3-D injected at planting are being evaluated and are different rates and application methods of non-fumigants such as NemaCur, Temik and Furadan. Field trials indicate no advantage of incorporating these on 12-inch bands versus 6-inch bands across the seed furrow. Other means of controlling nematodes must be used in management schemes in order to compensate for loss of EDB. These will include rotation, stalk plowup, trap crops, tolerant varieties, etc. which allow the crop to either tolerate or escape nematode damage.

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DIFFERENTIAL CHEMICAL CONTROL OF HETERODERA SCHACHTII ON SUGARBEET. G. D. Griffin, USDA-ARS, Crops Res. Lab., Utah State Univ., Logan, UT 84322

1,3-dichloropropene (1,3-D), aldicarb, and terbufos were applied at rates of 224 kg/ha, 4.5 a.i. kg/ha, and 8.0 a.i. kg/ha, respectively, to *Heterodera schachtii* infested soils with soil population densities of 3.9, 7.7, 12.8, and 16.6 J2/cm<sup>3</sup> soil. Sugarbeet seeds were planted into chemical treated and nontreated plots at soil planting temperatures of 6, 12, 18, and 24C. All chemicals effectively controlled *H. schachtii* at an initial nematode population density of 3.9 J2/cm<sup>3</sup> soil. There was, however, differential control, between the chemicals at nematode population densities of 7.7 J2/cm<sup>3</sup>/soil and above, and 1,3-D gave the best control. Planting made at 24C in soil with an initial nematode population density of 16.6 J2/cm<sup>3</sup> soil resulted in sugarbeet yields of 41, 24, 17, and 6 metric tons/ha in 1,3-D, aldicarb, terbufos, and nontreated control plots, respectively.

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DIFFERENTIAL REDUCTION OF XIPHINEMA AND PRATYLENCHUS POPULATIONS IN A BEARING APPLE ORCHARD WITH CARBOFURAN, CARBOSULFAN, AND PHENAMIPHOS. D. A. Rosenberger and F. W. Meyer, New York State (Geneva) Agricultural Experiment Station, Hudson Valley Laboratory, P.O. Box 727, Highland, NY 12528.

Carbofuran and carbosulfan at 11.2 kg/ha and phenamiphos at 16.8 kg/ha were applied 7 May 1982 and 29 April 1983 to replicated plots of Delicious/MM.106 apples planted in 1974. Application was to bare ground in the herbicide strip beneath trees and extended into the sodded areas between tree rows. Nematode counts were made from samples collected 19 July 1982 and 7 October 1983. Within the herbicide strip, phenamiphos reduced mean *Pratylenchus* populations from 45 to 2 per 100 cc soil. Carbofuran and carbosulfan both reduced mean *Xiphinema* populations from 56 to less than 2. Phenamiphos had no effect on *Xiphinema* populations, and neither carbofuran nor carbosulfan was effective against *Pratylenchus*. In the sodded areas, however, both phenamiphos and carbofuran controlled both *Pratylenchus* and *Xiphinema*. None of the treatments resulted in a significant increase in total apple production from 1982 through 1984.

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RELATIONSHIPS BETWEEN PRE-SEASON NUMBERS OF MELOIDOGYNE INCOGNITA AND YIELD LOSSES IN CHILE PEPPER CULTIVARS. S. H. Thomas and M. Cardenas, Dept. of Entomology and Plant Pathology and Dept. of Experimental Statistics, New Mexico State University, Las Cruces, NM 88003.

Yield losses in chile pepper (*Capsicum annuum* L.) cultivars 'Jalapeño', 'New Mexico 6-4' and 'Sandia,' resulting from parasitism by the southern root-knot nematode *Meloidogyne incognita* race 3 were evaluated in microplots during two growing seasons. Pre-plant inoculum densities (0, 50, 100, 200 and 500 *M. incognita* eggs and larvae/500 cm<sup>3</sup> soil) were arranged in a factorial design with five replications within each cultivar. NM 6-4 fruit weight was reduced 40% by an initial density of 50 nematodes and damage thresholds for Jalapeño and Sandia fluctuated between 100 and 200 *M. incognita*/500 cm<sup>3</sup> soil during the two seasons.

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COMPARISON OF TWO GEOGRAPHICAL ISOLATES OF THE LESPEDEZA CYST. N. H. Fagbenle<sup>1</sup>, D. I. Edwards<sup>2</sup>, and R. B. Malek<sup>2</sup>. <sup>1</sup>Dept. of Plant Pathology, Okla. State Univ., Stillwater, OK 74078; and <sup>2</sup>Dept. of Plant Path., Univ. of Illinois, Urbana, IL 61801

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DIFFERENTIAL CROSS PROTECTION OF WATERMELON TO FUSARIUM WILT BY RELATED FORMAE SPECIALES. R. D. Martyn, Dept. of Plant Pathology & Micro., Texas Agric. Exp. Stn., College Stn. 77843

Two closely related cucurbit wilt fusaria, *F. oxysporum* f. sp. *cucumerinum* (FOC) pathogenic to cucumber, and *F. oxysporum* f. sp. *melonis* (FOM) pathogenic to muskmelon were evaluated for their ability to protect watermelon from *F. oxysporum* f. sp. *niveum* (FON). FOC protected 2-wk-old Jubilee seedlings, but FOM did not, when seedlings were root-dipped in 2.5 x 10<sup>7</sup> microconidia/ml and challenged with FON 5 days later. No wilt was observed in FOC-treated seedlings 8 wk after challenge, while FOM and water-treated seedlings had 33% and 50% wilt, respectively. When watermelon was inoculated with FOC or FOM alone, each colonized only tap roots and caused no external visible symptoms. When FON followed FOC, FON could not be recovered from any tissue. When FON followed FOM, FON was recovered from lateral and tap roots as well as from stem tissue. Based on percentage of colonized or occluded vessels, there was no evidence that protection by FOC was due to prior tissue colonization or tyloses formation.

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TRANSFER OF INDUCED SYSTEMIC RESISTANCE IN TOBACCO TO BLUE MOLD (PERONOSPORA TABACINA ADAM) VIA CALLUS. S. Tuzun and J. Kud, Dept. of Plant Pathology, Univ. of Kentucky, Lexington, KY 40546.

Injecting sporangia of *P. tabacina* (5x10<sup>5</sup> sporangia/ml) into tobacco (Burley Ky 14) stem tissue external to the xylem systemically protected foliage against blue mold. Plants derived via callus from leaves and leaf midribs of parents injected with *P. tabacina* were also systemically protected against the disease. Protection was expressed as a reduction in sporulation on younger plants, whereas a reduction in symptom severity as well as sporulation were observed on older plants. It is possible that injecting stems with *P. tabacina* induced changes in the genome or the expression of the genome of tobacco which were carried over to offspring generated via callus.

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HISTOPATHOLOGY OF A SUSCEPTIBLE ALFALFA CLONE INFECTED WITH VERTICILLIUM ALBO-ATRUM. B.W. Pennypacker and K.T. Leath, Dept. of Plant Pathology, Penn State University, and USDA-ARS, U.S. Regional Pasture Research Lab., University Park, PA 16802.

Alfalfa plants, cloned from a *Verticillium* wilt susceptible plant, were stubble inoculated with *Verticillium albo-atrum* and sampled 6 times at weekly intervals. Stem samples were embedded in paraffin and examined microscopically. Infected plants had intermittent alterations in xylem vessel element development characterized by atypically narrow metaxylem vessel elements and hyperplastic cambial derivatives. Lack of cambial derivative differentiation caused mature xylem vessels to be interrupted by groups of immature cells. Xylem vessel elements frequently had plugged lumens and pit chambers, cell wall deposits, and occasional cell wall deterioration. Protoxylem and some metaxylem vessel elements were obliterated by hypertrophied xylem parenchyma. Disruption of xylem vessel element differentiation is an indication that *V. albo-atrum* may cause an alteration in hormone levels in susceptible plants.

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CONTROLLING NEMATODES ON COTTON WITHOUT EDB. J. L. Crawford, University of Georgia Extension Service, P. O. Box 1209, Tifton, GA 31793; R. E. Mottsinger, University of Georgia Extension Service, Athens, GA 30602; W. M. Powell, Plant Pathology and Genetics, University of Georgia, Athens, GA 30602.

Geographical isolates of *Heterodera lespedezae* from North Carolina (NC) and Illinois (IL) were compared on the basis of reproduction on selected hosts and effects of temperature on larval emergence, penetration, cyst production and pathogenicity. Larval penetration of striate lespedeza roots by NC was greater at 30 and 35 C than by IL. Cyst production on selected hosts differed between isolates. Tolerance to soil temperature (14-36 C at 4 C increments) varied with the isolate and the host plant. Pathogenicity and cyst production at selected temperatures varied with the isolate. Only IL produced males. A few cysts were produced by NC on soybean while none were produced by IL. Significant differences in pathogenicity, tolerance to temperature ranges, cyst production on soybean and development of males demonstrate that the IL and NC populations of *H. lespedezae* represent distinct biotypes.

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INTERACTION BETWEEN *PRATYLENCHUS THORNEI* AND *FUSARIUM ROSEUM* 'CULMORUM' ON WINTER WHEAT. D.J. Eschen and H.S. Fenwick, Dept. Plt., Soil, & Ent. Sci., Univ. of ID., Moscow, ID. 83843.

Twenty-five g of air dried, 16 mesh screened field soil were placed in each of 75 25x150 mm test tubes. *Triticum aestivum* L. cv. Stephens was disinfested in ETOH, -Cl, incubated in H<sub>2</sub>O 24 hrs., and 1 seed planted 4 cm deep in each tube. Treatments (trs) of 15 reps each were as follows: 1) Ck, 2) *Pratylenchus thornei* (Sher & Allen) (P.t.) alone, 3) *Fusarium roseum* f. sp. cerealis 'culmorum' (Syn. & Hansen) (F.r.) alone, 4) F.r. + root wounding, and 5) P.t.+F.r. Inoculum consisted of 50 P.t. /g soil and 400 F.r. conidia/g soil. Tubes were held 4 wks. at 15% soil moisture at 25C. Disease rankings were significantly higher for tr 5 compared to trs 4, 1, or 2. Plant hts. of trs 5, 3, and 4 were significantly reduced from tr 1 and were 70.4, 84.7, and 84.0% of tr 1, respectively. Top wts. of tr 5 were significantly reduced (38.5% of tr 1), while trs 3, 4, and 2 were 63.5, 76.6, and 93.5% of tr 1, respectively. F.r. was isolated from 33% more crowns in tr 5 than in trs 3 or 4.

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EFFECT OF *PRATYLENCHUS PENETRANS* ON PLANT WATER RELATIONS IN SIX POTATO CULTIVARS AND CORRELATION WITH HOST TOLERANCE. J. B. Kotcon and R. Loria, Dept. of Plant Pathology, Cornell Univ., Long Island Horticultural Research Lab, Riverhead, NY 11901

Potato plants (cv. Chippewa, Hudson, Katahdin, Onaway, Russet Burbank, and Superior) were inoculated with *Pratylenchus penetrans* at initial densities (Pi) of 1, 265, or 5081 per plant in a greenhouse trial. Transpiration, leaf stomatal conductance, leaf water potential, hydraulic conductivity of the root system (HC), and shoot, root, and tuber dry weights were determined on five plants per treatment at 30 and 45 days after transplanting. Compared to plants with low Pi, HC in Chippewa and Katahdin plants with high Pi was reduced 47 and 48%, respectively (P=0.05); shoot dry weight of Katahdin was reduced 38%; and root, shoot, and tuber dry weights of Chippewa were reduced 27, 27, and 41%, respectively. Transpiration, stomatal conductance, and leaf water potential were not affected by Pi in any cultivar. Tolerance of potato cultivars to *P. penetrans* was negatively correlated with the effect of Pi on HC.

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POPULATION DENSITIES AND DISTRIBUTION OF *PRATYLENCHUS PENETRANS* AND *P. CRENATUS* IN LONG ISLAND, NY POTATO FIELDS. D. A. Florini, J. B. Kotcon, and R. Loria, Department of Plant Pathology, Cornell University, Long Island Horticultural Research Lab, Riverhead, NY 11901

*Pratylenchus* spp. populations were assessed in 56 potato fields on the north fork (NF) and south fork (SF) of Long Island during early summer 1984. Nematodes were extracted from both root and soil samples. Twenty *Pratylenchus* females from root samples from each of 28 fields were identified to species. Number of *Pratylenchus* spp. per gram of root was higher (P=0.05) on the SF (mean 749; range 3-2749) than on the NF (mean 302; range 1-1167). Results from soil samples were similar. Field location also affected species distribution: 83% of NF samples and 12% of SF samples contained only *P. penetrans*, 8% of NF samples and 69% of SF samples contained only *P. crenatus*, while 8% of NF samples and 19% of SF samples had both species. Neither crop rotation nor soil edaphic factors significantly influenced population densities.

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EMERGENCE OF *GLOBODERA ROSTOCHIENSIS* JUVENILES FROM ROOTS OF SUSCEPTIBLE AND RESISTANT POTATO. B. Mullin and B. B.

Brodie, USDA, ARS, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Emigration of *Globodera rostochiensis* juveniles (J2s) from resistant (cv. 'Rosa') and susceptible (cv. 'Katahdin') potato roots was assessed. Twenty plants of each cultivar were exposed to ca. 1130 J2s for 24 hrs and then transferred to liquid culture. Culture solutions were screened daily for 14 days to collect exiting J2s. Percent invasion was 27% in both cultivars. Nematodes emigrated from all plants, but over twice as many exited from 'Rosa' ( $\bar{X}=493 \pm 93$  SD) as did from 'Katahdin' ( $\bar{X}=226 \pm 89$  SD) (P=0.0001). When J2s that had emerged from 'Katahdin' or 'Rosa' were applied to new 'Katahdin' roots for 3 weeks, invasion efficiencies were 80% and 14%, respectively. Host resistance increases emergence of *G. rostochiensis* J2s from roots in liquid culture, and also renders emerged J2s less able to infect susceptible host roots.

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RESISTANCE OF BERMUDAGRASS TO ROOT-KNOT NEMATODES. D. E. Bertrand, A. W. Johnson and G. W. Burton. USDA, ARS, Univ. of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

The resistance of six hay and pasture bermudagrasses to *Meloidogyne incognita* and a mixed population of *M. javanica*, *M. arenaria* and *M. hapla* was evaluated in the greenhouse. Cultivars were Tifton 44, Tifton 68, Tifton 78, Tifton 79-16, Coastal (resistant control) and Coastcross-1 (resistant control). Stem cuttings were planted into pots containing steamed soil or soil infested with *M. incognita* or a mixed population of *M. javanica*, *M. arenaria* and *M. hapla*. Coastal, Coastcross-1 and Tifton 44 were resistant to *M. incognita* and the mixed population of *Meloidogyne* spp. Tifton 68 and Tifton 79-16 were susceptible to both *M. incognita* and the mixed population of *Meloidogyne* spp. Tifton 78 was resistant to the mixed population of *Meloidogyne* spp. and exhibited some resistance to *M. incognita*.

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SOYBEAN CULTIVARS IN FIELDS WITH MIXTURES OF CYST AND ROOT-KNOT NEMATODES. R. Rodriguez-Kabana, D. Weaver, and P. S. King. Depts. of Botany, Plant Pathology, and Microbiology, and Agronomy, Ala. Agric. Expt. Sta., Auburn University, AL 36849.

Soybean (*Glycine max*) cultivars Braxton, Ransom, Kirby, Leflore, Foster, Forrest, Gordon, Coker 368, Terravig 606, and S69-96 were planted in a field infested with a mixture of *Heterodera glycines* (race 3) and *Meloidogyne incognita* to study their tolerance to the nematodes. Breeding lines N81-1756 and F77-7142 were also included in the study. Each cultivar or line was planted in soil fumigated with 16.8 L EDB/ha and in untreated soil. All cultivars and lines sustained significant populations of both nematodes and evidenced significant yield responses to fumigation. Kirby, Foster, F77-7142, and Leflore were the highest yielding soybeans in untreated soil and showed the smallest degree of response to fumigation. Ransom, N81-1756, and S69-96 were the soybeans with the lowest yields and the greatest response to fumigation.

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THREE FUNGI ASSOCIATED WITH A LEAF AND STEM ROT OF AZALEA CUTTINGS WHEN EXPOSED TO FOGGING. B. C. Raju, J. C. Trolinger, C. R. Semer, IV. Technical Division, Yoder Bros., Inc., Alva, FL 33920.

Severe leaf and stem rot symptoms were observed on unrooted cuttings of azalea (*Rhododendron* sp.) propagated in a fogging system. Initial symptoms were small black lesions on lower leaves which extended over the entire leaves in 3-4 days. Stem rot was observed in several cases after complete defoliation of the cutting. *Colletotrichum gloeosporioides*, *Cylindrocladium scoparium*, and *Pestalotia* sp. were isolated consistently from affected leaves, but not healthy-appearing leaves. *Colletotrichum gloeosporioides* was most frequently isolated. In pathogenicity tests using each organism separately and in combination, typical leaf spot and leaf and stem rot developed on the inoculated cuttings with each pathogen and each combination. The symptoms were severe when the three pathogens were combined. Of the fungicides tested, mancozeb provided some control at 2.4g/l.

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VARIATION IN SENSITIVITY TO *PHYTHIUM ULTIMUM* IN SELECTED SEED-PROPAGATED HYBRID GERANIUMS (*PELARGONIUM* X *HORTORUM*). M.K. Hausbeck, C.T. Stephens\* and R.D. Heins, Departments of

Sensitivity to crown and root rot disease caused by *Pythium ultimum* varied among cultivars as measured by plant mortality, plant size, and days to flower. Immunity to *P. ultimum* was not identified in the 37 seed-propagated hybrid geranium cultivars screened. Foliar applications of silver thiosulphate applied to prevent premature flower petal abscission greatly increased plant mortality due to *P. ultimum* in all cultivars when grown in a *P. ultimum*-infested medium. Geraniums not treated with silver thiosulphate but grown in *P. ultimum*-infested medium were significantly smaller than control plants but otherwise appeared healthy. *P. ultimum* was consistently isolated from the root tissue of stunted plants. Cultivars most tolerant to *P. ultimum* may be useful in breeding programs as a means of increasing tolerance to *P. ultimum*.

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A LEAF, PETIOLE AND STEM LESION DISEASE OF POINSETTIA INCITED BY *ALTERNARIA* SP. Arthur W. Engelhard and J. E. Jones, IFAS, U. of FL., Gulf Coast Res. & Edu. Center, Bradenton, FL 34203.

An *Alternaria* sp. incited lesions on leaves, petioles and stems of poinsettia plants in Florida. Leaf lesions on naturally infected plants occur as 1-2 mm tan spots, usually with a chlorotic halo. Spots enlarge, become angular, irregular shaped, and coalesce to form large necrotic areas. Chlorotic areas develop where multiple spots occur, or the entire leaf becomes chlorotic and abscises. A single lesion on one side of a leaf may cause unilateral chlorosis on the affected side causing the leaf to develop a curved growth pattern. Elongate, dark, sunken lesions form on mid- and lateral veins and are especially visible on the lower leaf surfaces. Similar lesions also develop on petioles and stems, and if they enlarge they can cause the death of the affected leaf or terminal. Stem infection on a young plant causes stunting of the plant and death of the terminal. This disease was most severe on V-14 cvs (Glory, White, and Jingle Bells). Serious losses have occurred under commercial growing conditions.

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INFLUENCE OF HEAT STRESS ON ROOT SUSCEPTIBILITY TO PHYTOPHTHORA INFECTION. J. D. MacDonald and J. D. Shapiro. Department of Plant Pathology, University of California, Davis, CA, 95616.

Solar radiation in outdoor nurseries can heat portions of the root systems of container-grown plants to temperatures as high as 50-55 C during the summer months. When rooted cuttings of *Chrysanthemum morifolium* were immersed for 30 min in aerated solutions ranging from 25-50 C, a sharp increase in root rot severity caused by *Phytophthora cryptogea* was consistently observed in roots exposed to temperatures >40 C. The increase was most pronounced in roots inoculated immediately following heat exposure, but also occurred when roots were exposed to heat 4 or 8 hours after inoculation. Zoospore attraction to *Chrysanthemum* or *Juniperus sabina* 'Tamariscifolia' root exudates was stimulated by exposure of roots to temperatures >40 C. While prolonged exposure of *P. cryptogea* to temperatures >35 C reduced sporangium formation and viability, mycelium in established root infections may be more tolerant of heat. The results suggest that in nurseries, heat stress may significantly increase the severity of *Phytophthora* root rots in container-grown plants.

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FUSARIUM WILT OF *EUSTOMA GRANDIFLORA*. Robert D. Raabe, Department of Plant Pathology, University of California, Berkeley, CA 94720.

*Eustoma grandiflora* is a plant native to the south central plain states and Mexico. Recently, the Japanese have improved the plants by selection and hybridization. These are being grown and sold as potted plants and bedding plants in California under the name *Lisianthus* (*Lisianthus russellianus*). Some of the potted plants have been found to show severe wilt symptoms. From some of these, a form of *Fusarium oxysporum* was isolated. *Eustoma* plants were inoculated by dipping bare rooted seedlings in a suspension of spores produced on potato dextrose agar. Such plants developed typical wilt symptoms and from such plants, the fungus was reisolated. Inasmuch as this is a new host for the wilt type *Fusaria*, the fungus is named *Fusarium oxysporum* f. sp. *eustomae*.

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FUSARIUM SPOROTRICHOIDES AND PHOMOPSIS SP., ASSOCIATED WITH

BASAL CANKER OF NURSERY-GROWN EASTERN HEMLOCK. R. L. Wick, Suburban Exp. Sta., Univ. of MA, Waltham, MA 02254, D. B. Schroeder Dept. of Renewable Natural Resources, Univ. of CT., Storrs, CT 06268, and K. K. Rane, Suburban Exp. Sta., Univ. of MA, Waltham MA 02254.

In the 1970's hemlock canker was identified in a number of Connecticut nurseries. In one nursery, mortality of more than 80% was attributed to this disease. More recently, a planting in Massachusetts was found to have a 42% incidence of canker. The cankers, which may become 20 cm or more in length, begin at the basal portion of the main stem at or below ground level. Pitch exudate and dead branches originating at the canker face are the most conspicuous field symptoms. Isolations from cankers in MA and CT yielded *Fusarium sporotrichioides* (in CT, previously identified as *Fusarium tricinctum*) and *Phomopsis* sp. *Phomopsis* and *Fusarium* isolates from both MA and CT consistently produced cankers when inoculated into eastern hemlock. *Fusarium sporotrichioides* produced larger cankers (2 cm/wk) than *Phomopsis* sp.

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REDUCED SYMPTOMS OF FOLIAR NECROSIS OF CHRYSANTHEMUM MORIFOLIUM CULTIVAR PINK MARBLE BY CONTINUOUS HYDROPONIC CULTURE WITH TETRACYCLINES. R. J. McGovern and R. K. Horst, Plant Path. Dept., Cornell University, Ithaca, N.Y. 14853.

Symptom expression of the foliar necrosis disease of *Chrysanthemum morifolium* can include flecking, veinal necrosis, yellowing and desiccation of lower leaves. Since this disease is hypothesized to be induced by a fastidious prokaryote, the suspected pathogen was further characterized based on its sensitivity to various antibiotics. Infected cultivar Pink Marble was grown in the greenhouse in a hydroponic solution of 0.3 g/l Peters Hydrosol and 0.2 g/l  $\text{Ca}(\text{NO}_3)_2$ , which was renewed and amended weekly with 12.5 ppm tetracycline-HCl, 25 ppm oxytetracycline, or 250 ppm penicillin-G. Disease severity, percentage of affected leaves and plant height were reduced after 2 months by tetracycline or oxytetracycline, but not by penicillin. Disease remission also resulted from hydroponic application of 10 ppm tetracycline in controlled environment chambers at constant temperatures of 15.6 and 32.2 but not at 23.9 C.

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CONIDIAL PRODUCTION, GERMINATION, AND PATHOGENICITY OF *ENTOMOSPORIUM MESPILLI* PRODUCED ON CARROT JUICE AGAR. L. G. Brown and L. W. Baxter, Jr. Dept. of Plant Pathology and Physiology, Clemson University, Clemson, SC 29631.

Carrot juice agar (CJA), malt extract agar (MA), potato-dextrose agar, made from fresh potatoes, (PDA) and dehydrated BBL potato-dextrose agar (BBL-PDA) were compared for optimum sporulation and germination of *E. mespili* (= *E. maculatum*) at 21, 28, and 35 days after seeding. Conidial production was equivalent between two isolates of *E. mespili* from *Photinia x fraseri*. After 35 days in the dark at 21 C, more conidia ( $P=0.05$ ) were produced on CJA,  $5.1 \times 10^6$  conidia per plate; than on PDA,  $6.8 \times 10^5$  conidia; MA,  $3.5 \times 10^6$  conidia; or BBL-PDA, very few conidia. At all dates more conidia were produced on CJA than on MA, PDA, or BBL-PDA ( $P=0.05$ ). Germination was higher ( $P=0.05$ ) on CJA (59%) than on MA (28%) or PDA (24%), and germination % was highest after 35 days. Conidia from isolates maintained on CJA for 133 days (transferred twice) had a 100% infection rate on *P. x fraseri* when applied at  $4 \times 10^4$  conidia/ml.

USE OF GLIOCLADIUM VIRENS FOR CONTROL OF RHIZOCTONIA SOLANI ON SNAPDRAGON, CHRYSANTHEMUM, AND POINSETTIA CROPS IN THE GREENHOUSE. J. C. Locke and R. D. Lumsden, USDA, ARS, Beltsville, MD 20705.

Greenhouse produced floral crops such as snapdragon, chrysanthemum, and poinsettia are susceptible to root and stem rot caused by *Rhizoctonia solani* during part or all of their production cycles. Traditionally, pasteurization and sanitation practices have been used to minimize losses. Evaluation of a *Gliocladium virens* isolate, which had previously shown biological control potential against *R. solani*, was carried out in three cropping systems. Introduction of *G. virens* into steam-pasteurized growing medium prior to transplanting snapdragon seedlings resulted in nearly 100% suppression of disease. In a similar manner, chrysanthemum transplant losses to *R. solani* were reduced to nearly zero and basal stem lesions were reduced by 50%. With poinsettias, reduction in stem lesions and plant death was only achieved following a six-day pre-incubation period of *G. virens* in the *R. solani* infested medium, but control was excellent over a range of inoculum levels.

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A LEAF SPOT OF RHAPIS PALM CAUSED BY *CERCOSPORA RHAPISICOLA*. M. A. Yoshimura, N. M. Nagata, and J. Y. Uchida. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

A serious leaf spotting disease of Rhapsis palm [*Rhapis excelsa* (Thunb.) A. Henry] was found on several commercial nurseries in Hawaii. Leaf spots ranged from minute, raised flecks to large elliptical spots, frequently up to 7 mm but sometimes more than 30 mm in length. Individual black necrotic spots had tan to brown centers and were surrounded by chlorotic borders. A fungus, tentatively identified as *Cercospora rhapsisicola* Tomimaga was readily isolated. A single-spore isolate 1037 was used as the test organism in pathogenicity studies. Conidia taken from this isolate measured  $120.9 \pm 21.5 \times 2.8 \pm 0.2$   $\mu\text{m}$  with  $12.9 \pm 4.1$  septa. Approximately 13% of the conidia had a lateral appendage near the apex or the base, measuring up to 20  $\mu\text{m}$  long. Healthy plants inoculated with a conidial suspension showed early symptoms in 3 to 4 weeks and typical disease symptoms appeared in 2 to 3 months.

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FUNGICIDE DELIVERY AND SEED PERFORMANCE IN HYDRATED GEL CAPSULES. C. E. Nelsen, R. H. Davis, and R. E. McLennan, Plant Genetics, Inc., 1930 Fifth Street, Davis, CA 95616.

Hydrated gel capsules are being developed to deliver cell-culture produced, somatic embryos (synthetic seeds) and as seed treatments for botanic seeds. Seeds of tomato (*Lycopersicon esculentum* Mill., cv. UC-82) were encapsulated in gel capsules with either of the fungicides metalaxyl or captan or with no fungicide and tested against infection by the pathogen *Pythium ultimum* (2 isolates). At recommended or reduced rates of the fungicides, encapsulated seeds were protected as well as or better than fungicide-treated raw seeds as measured by % live seedlings 14 days after planting. Gel capsules alone, in the absence of any fungicides, protected the seed from the pathogen, and encapsulated seed emerged to a significantly higher final % emergence than did untreated raw seed ( $51.8 \pm 11.1\%$  versus  $22.5 \pm 7.2\%$ ; mean % live seedlings  $\pm$  S.E. of 5 exp.). Delivery of fungicides in hydrated gel capsules is effective and may improve performance of seeds and/or seed protectant compounds.

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EFFECTS OF SPRAY METHODS ON CONTROL OF FOLIAR DISEASES OF ONIONS AND PEPPERS. T. A. Kucharek, R. E. Cullen, and R. E. Stall. Plant Pathology Dept., University of Florida, Gainesville, Fla. 32611.

Purple blotch and blast of onions were reduced with mancozeb by as much as 68 & 86%, respectively by combining two opposing hollow cone or flat fan nozzles/row, angled at  $45^\circ$  from the horizontal, with a spreader-sticker. The use of a single, downward discharging, hollow cone nozzle/row without a spreader-sticker reduced purple blotch by 34-46% and blast by 37-64% depending on the assessment date. Treatments without a spreader-sticker and with two nozzles/row of either type were never significantly different from the single hollow cone nozzle/row. Pepper leaves with bacterial spot were reduced by more than 90% on all assessment dates if maneb was added to cupric hydroxide and if spray intervals were twice/wk rather than once/wk regardless of nozzle type. As in the onion test, hollow cone and flat fan nozzles did not differ significantly; but in the pepper test, reduction of nozzle number/row from three to one did not significantly reduce control. Spray pressure and volume and fungicide rate were constant within tests.

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THE CHEMICAL AND FUNGICIDAL CHARACTERISTICS OF MYCLOBUTANIL. H. E. Carley, W. S. Hurt, and J. W. Long, Rohm and Haas Company, Independence Mall West, Philadelphia, PA 19105.

Myclobutanil is the proposed generic name for the fungicide  $\alpha$ -butyl- $\alpha$ -(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile. The systemic ergosterol biosynthesis inhibitor, coded RH-3866, is under extensive worldwide development. Myclobutanil possesses a unique balance of systemic curative and protectant properties which provides effective control of economically important treefruit, vine, and vegetable crop diseases.

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PERFORMANCE OF MYCLOBUTANIL FOR THE CONTROL OF GRAPE POWDERY MILDEW. J.T. Schlesselman, Rohm and Haas Company, Reedley, CA 93654.

Studies conducted during 1983-1984 have shown myclobutanil (RH-3866) to be highly effective for the control of grape powdery mildew (*Uncinula necator*). Myclobutanil use rates ranged from 0.3-2.7 ounces ai/A during 1983 and from 0.5-2.0 ounces ai/A during 1984. Application timing consisted of 2 or 3 sprays per season. The 3-spray schedule began at prebloom (12-24 inch canes), a bloom spray (50% bloom), and a postbloom spray (1/4-1/2 inch berries). The 2-spray schedule consisted of a pre- and postbloom application. Triadimefon (2 ounces ai/A) was used as the standard treatment in all experiments. The optimum myclobutanil use rate was 1 ounce ai/A. The 2 and 3-spray schedules achieved 93% and 99% powdery mildew control, respectively. No adverse effects to grape foliage or berries was observed when myclobutanil was applied 6 times at 2 ounces ai/A.

## 238

GUAZATINE A CONTACT FUNGICIDE, FOR POSTHARVEST USE ON CITRUS AND OTHER CROPS by H.J. Kaplan and R.N. Ryno, Penwalt Corporation, 1713 S. California Avenue, Monrovia, CA 91016

Guazatine is a contact fungicide containing acetates of guanidated amines (GTA); it will be marketed in the U.S. under the trademark KENOPEL, a 40% a.i. liquid that is completely soluble in water. The most outstanding feature of this new fungicide is its ability to control sour rot (*Geotrichum candidum*), a disease of citrus now causing serious market losses due to the absence of any effective control measures. Lemons, mandarins and late season grapefruit are particularly susceptible to sour rot. Sold in Australia as Panocrine 400, it has also demonstrated control of both green (*Penicillium digitatum*) and blue mold (*P. italicum*) of citrus, especially against strains of *Penicillium* that are resistant to benzimidazole fungicides. Kenopel has outstanding performance against *Mucor* species on tomatoes and pears. It has the ability to control most decay causing organisms of cantaloupe.

## 239

DPX-H6573: A BROAD-SPECTRUM FUNGICIDE FOR THE CONTROL OF FIELD CROP DISEASES. J. A. Bruhn, T. M. Fort, and S. J. Denis E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898.

DPX-H6573, bis(4-fluorophenyl)methyl (1H-1,2,4-triazol-1-yl)methyl silane, is a new broad spectrum ergosterol biosynthesis-inhibiting fungicide. Greenhouse and field tests have demonstrated that DPX-H6573 effectively controls early and late peanut leafspots (*Cercospora arachidicola*, *Cercosporidium personatum*), wheat footrot (*Pseudocercospora herpotrioides*), cereals powdery mildew (*Erysiphe graminis*), wheat leaf rust (*Puccinia recondita*) and leaf and glume blotch of wheat (*Septoria* spp.). Greenhouse tests also have shown DPX-H6573 to possess preventive, curative, and systemic activities against these pathogens.

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DPX-H6573: A BROAD-SPECTRUM FUNGICIDE FOR THE CONTROL OF TREE FRUIT DISEASES. A. E. Davis, T. M. Fort, S. J. Denis, and M. J. Henry. E. I. du Pont de Nemours & Co., Inc., Stine-Haskell, Elkton Road, Newark, DE 19711.

DPX-H6573, [bis(4-fluorophenyl)methyl(1H-1,2,4-triazol-1-yl)methyl] silane, a new ergosterol biosynthesis-inhibiting fungicide, is highly effective against several important tree fruit diseases. Among pome fruit diseases DPX-H6573 provides excellent control of apple scab (*Venturia inaequalis*), apple powdery mildew (*Podosphaera leucotricha*), and cedar-apple



rust (*Gymnosporangium juniperi-virginianae*). Stone fruit diseases effectively controlled by DPX-H6573 include brown rot (*Monilina fructicola*) and cherry leafspot (*Coccomyces hiemalis*). In both greenhouse and field tests DPX-H6573 has demonstrated superior curative and residual disease control properties when compared with other ergosterol biosynthesis-inhibiting fungicides. Mode of action studies have shown that DPX-H6573 inhibits 14 $\alpha$ -demethylation in ergosterol biosynthesis.

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**MYCLOBUTANIL - A PROTECTANT/CURATIVE APPLE FUNGICIDE.** D. L. Loughner and S. E. Crane, Rohm and Haas Company, 727 Norristown Road, Spring House, PA 19477.

The efficacy of a new experimental apple fungicide, myclobutanil (RH-3866), was evaluated during the 1984 season by University and Rohm and Haas field research personnel across the United States. The results indicate that when applied on a protectant or postinfection spray schedule at rates from .5-2.0 oz. ai/100 gal, myclobutanil is highly active on the major apple diseases *Venturia inaequalis* (scab), *Podosphaera leucotricha* (powdery mildew), and *Gymnosporangium juniperi-virginianae* (cedar-apple rust). The high level of residual and postinfection activity of the fungicide lends itself to early season precover sprays.

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**CHEMICAL CONTROL OF ASCOCHYTA RABIEI IN INFECTED CHICKPEA SEEDS.** W. J. Kaiser and R. M. Hannan, ARS, USDA, Regional Plant Introduction Station, Washington State University, Pullman, WA 99164

In 1984, blight caused by *Ascochyta rabiei* was observed for the first time in commercial chickpea (*Cicer arietinum*) plantings in eastern Washington and northern Idaho. An effective method of preventing further introduction of the blight pathogen into new plantings was needed. The efficacy of different fungicides in controlling the seedborne phase of the disease in naturally infected or artificially infested chickpea seeds was tested on agar media in the laboratory and in sterile potting medium and nonsterile field soil in the greenhouse. The most effective seed treatment chemicals of nine tested singly or in various combinations were benomyl and thiabendazole (TBZ). After treatment with these two chemicals, *A. rabiei* was not detected in seeds plated on agar media or on plants from treated seeds which were >60% naturally infected or 100% artificially infested with the pathogen. These fungicides combined with captan controlled *A. rabiei* on infected seeds and preemergence damping-off by *Pythium ultimum* when planted in natural field soil.

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**CONTROL OF RHIZOCTONIA ROOT ROT IN SUGARBEET WITH SINGLE PRE-LAYBY APPLICATIONS OF EXPERIMENTAL FUNGICIDES.** E. G. Ruppel, and R. J. Hecker, USDA-ARS Crops Research Laboratory, Colorado State University, Fort Collins 80523.

Quasi-commercial conditions were established in a field heavily infested with *Rhizoctonia solani* (AG-2) to determine if pre-layby applications of experimental fungicides could control *Rhizoctonia* root rot of mature sugarbeet. Beets were planted 19 April 1984, and fungicides were banded in crowns at the cotyledon stage, the four- to six-leaf stage, or just before plants covered the furrows. Roots were harvested and evaluated for rot and yield on 13 October. Single applications of pencycuron 75WP (NTN 19701; 40 g ai/305 m row), triadimefon 50WP (14 g ai/305 m row), and triadimenol 25DF (14 g ai/305 m row) reduced disease severity 16-54%, and increased root yield 15-77% and sucrose content 11-26% in a susceptible but not a resistant commercial cultivar. Disease control resulted in a gross return of \$220-593/ha. None of the fungicides currently is registered for control of *Rhizoctonia* root rot in sugarbeet.

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**COMPETITIVENESS OF BENZIMIDAZOLE-TOLERANT AND SENSITIVE STRAINS OF CERATOCYSTIS ULMI.** L. R. Schreiber and J. W. Peacock, USDA-ARS, Nursery Crops Research Laboratory and USDA, FS, Forestry Sciences Laboratory, 359 Main Rd., Delaware, OH 43015

We determined the competitiveness of tolerant (T) and sensitive (S) strains of *Ceratocystis ulmi* by comparing the frequency of their isolation from bodies of smaller European elm bark beetles, *Scolytus multistriatus* that emerged from American elms. To isolate and identify fungus strains, beetle progeny were reared from elm bolts, killed by freezing, and plated onto elm extract agar. Isolates were subcultured onto benzimidazole-amended or

unamended PDA. When T and S-inoculated elms were infested with uncontaminated beetles, the percentages of T and S-contaminated progeny were 3.4 and 6.5, respectively. When T-contaminated beetles were placed on S-infected bolts, 86.4% of contaminated progeny carried the T strain; when S-contaminated beetles were placed on T-infected bolts, 89.0% of contaminated progeny carried the T strain. When healthy bolts were infested with beetles contaminated simultaneously with S and T strains, 40.0% of contaminated progeny carried the T strain.

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**FIELD EVALUATION OF SEVEN FUNGICIDES FOR THE CONTROL OF SCAB DISEASE AND ASCOCHYTA BLIGHT ON COWPEA IN RWANDA.** Mazo Price & David Cishahayo, B.P. 629, Kigali Rwanda (East Africa).

Cowpea (*Vigna unguiculata*) has an excellent potential in the subhumid and semiarid high altitude regions of East Africa. However, some of the most serious constraints to cowpea production in these regions are scab and ascochyta blight which are believed to be seed-borne. Chemical control may be required as an intermediate step before breeding resistant varieties. Therefore, seven fungicides were evaluated in a trial conducted for two seasons of 1984 at Karama Station in Rwanda. Of the fungicides used, Bavistin and Brestan were the most effective in controlling the two diseases on the most susceptible cowpea varieties Mwanza x and Cross 1-6E-2. The disease ratings were decreased by three applications of these two fungicides, thereby resulting in highest grain yield.

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**CONTROL OF ANTHURIUM BACTERIAL BLIGHT.** H. T. Nishijima and D. K. Fujiyama, Dept. of Plant Pathology and Coop. Ext. Serv., Univ. of Hawaii at Manoa, Hilo, HI 96720.

The foliar phase of anthurium bacterial blight caused by *Xanthomonas campestris* pv. *dieffenbachiae* (Xcd) was effectively controlled by a combination of streptomycin sulfate (SS) sprays and field sanitation. Six to 8 weekly SS sprays at 0.24 or 0.48 kg/ha, each preceded by field sanitation involving the removal of all visibly infected leaves, provided control for at least 2 months. Oxytetracycline (OT) at 0.19 kg/ha with field sanitation also controlled anthurium bacterial blight but was not as residual as SS. Copper fungicides when used alone or in combination with mancozeb were phytotoxic to anthurium leaves and flowers and appeared to increase disease incidence. SS or OT resistant strains of Xcd have not yet developed or been selected for as a result of this control program.

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**GROWTH AND SPORULATION OF PYRENOPHORA TRITICI-REPENTIS ON FUNGICIDE AMENDED MEDIA.** R. M. Hunger, Plant Pathology Dept., Oklahoma State University, Stillwater, OK 74078.

The effect of propiconazole (Tilt; Ciba-Geigy), BAY HWG 1608 (Mobay), and chlorothalonil (Bravo 500; SDS Biotech) on growth and sporulation of four single ascospore isolates of *P. tritici-repentis* (PTR) was investigated. Nine cm petri dishes containing clarified V-8 juice agar amended with 0, .01, .1, 1, 10, and 100  $\mu$ g ai of fungicide/ml were inoculated with 10 mm plugs from 5 day old PTR cultures growing on potato dextrose agar. Growth (hyphal extension) was measured after 5 days incubation in the dark at 24 C. Exposure to 12 hr light (2900 lux) followed by 12 hr of darkness induced conidia formation. Sporulation density (conidia/mm<sup>2</sup>) was determined by counting conidial suspensions in a nematode counting dish. The EC<sub>50</sub>'s for growth inhibition by propiconazole, BAY HWG 1608, and chlorothalonil were near .1, .1, and 10  $\mu$ g/ml, respectively. The EC<sub>50</sub>'s for inhibition of sporulation by propiconazole, and BAY HWG 1608 were near .1  $\mu$ g/ml. No sporulation occurred on plates amended with chlorothalonil.

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**SOME FUSARIUM INDUCED mRNAs ARE ALSO EXPRESSED AT HIGH LEVELS IN PSEUDOMONAS - PISUM RACE SPECIFIC INTERACTIONS.** Catherine Daniels, B. Frstensky, Wendy Wagoner and L. A. Hadwiger, Washington State University, Pullman, WA 99164-6430.

Alaska peas express a non-host resistance response against *F. solani* f. sp. *phaseoli* (Fsp) but are susceptible to *F. solani* f. sp. *pisi*. Five other pea varieties express differential race specific resistance to races 1, 2 and 3 of *P. syringae* pv. *pisi*

(Psp). cDNA clones from the Alaska pea-Fsp interaction were hybridized to total RNA from Psp challenged pea pods of the six pea lines. Enhanced accumulation of RNA homologous to three cDNA clones was temporally correlated with non-host resistance. Northern blots indicate that phenotypic resistance correlates with specific RNA accumulation and susceptibility correlates with depressed levels of, or absence of, a specific RNA species. Not all race-pea variety Northernblots were representative of the plant expressed resistance phenotype. We conclude that some commonly possessed genes are activated in plants expressing either a general or specific resistance.

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COMPARISON OF *TILLETIA INDICA* ISOLATES FROM INDIA AND MEXICO BY ISOZYME ANALYSIS. M. R. BONDE<sup>a</sup>, G. L. PETERSON<sup>a</sup>, W. M. DOWLER<sup>a</sup>, AND B. MAY<sup>b</sup>. <sup>a</sup>USDA, ARS, Plant Disease Res. Lab., Frederick, MD 21701; <sup>b</sup>Cornell Univ., Ithaca, NY 14853.

Field collections of *T. indica* (Mitra) Mundkur were obtained from four areas of the Punjab region of India and four areas in the state of Sonora, Mexico. Shake cultures established from single teliospores or single basidiospores were tested for the presence of 48 enzymes by starch gel electrophoresis. Activity for 29 enzymes was detected in high concentrations. Isozyme banding patterns obtained with 40 cultures were interpreted as representing 31 presumed genetic loci; 16 were polymorphic (genetic variation present) and 15 were monomorphic (no variation). These results demonstrate that *T. indica* has considerable genetic variability. Of the 40 alleles scored, 39 were present in at least one culture from India and 37 in at least one culture from Mexico indicating a high degree of similarity between the pathogen populations from the two countries.

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GENETICS OF *GIBBERELLA FUJIKUROI*. IX. VEGETATIVE COMPATIBILITY GROUPS. B.S. Sidhu, Dept. of Plant Path., Univ. of NE, Lincoln, NE 68583-0722.

A large number of *Gibberella fujikuroi* Group A cultures were collected from naturally-infected corn and sorghum fields. Two complementary *nit* mutants (deficient in nitrate reductase) were recovered from each culture. The *nit* mutants, recovered from 67 cultures, were paired in all possible combinations to differentiate vegetative compatibility groups (VCGs). Fungal cultures isoelectric at all heterokaryon loci belong to the same VCG. A total of 38 different VCGs were categorized. Heterokaryotic nuclear ratios from different VCGs were determined and compared. Qualitatively robust heterokaryons gave relatively balanced nuclear ratios as compared to weak heterokaryons. VCGs can be used for estimating the frequency of individual fungal cultures in the diseased tissue and in monitoring pathogenic cultures in disease complexes.

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VEGETATIVE COMPATIBILITY GROUPS AMONG ISOLATES OF THE FUNGUS CAUSING SOYBEAN STEM CANKER IN THE SOUTHEASTERN UNITED STATES. R.C. Ploetz and F.M. Shokes, North Florida R.E.C., Route 3, Box 4370, Quincy 32351.

Twenty-eight vegetative compatibility groups were

identified among 214 isolates of the fungus causing soybean stem canker in the Southeastern United States ("southern" *Diaporthe phaseolorum*). Barrage zones formed between isolates from different compatibility groups. Zones were pigmented brown with sparse mycelial growth when isolates were grown on Difco potato dextrose agar, but were indistinct or did not form when grown on Difco water agar, malt agar, Czapek-Dox agar, or a mineral salts medium. The utility of these groups when used as markers in identifying isolates used in epidemiological studies or when comparing this fungus with the one responsible for soybean stem canker in the Midwestern United States (*D. phaseolorum* var. *caulivora*) is discussed.

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INHERITANCE OF RESISTANCE TO DOWNY MILDEW IN SOYBEAN. S. M. Lim and R. L. Bernard, USDA-ARS, Department of Plant Pathology and Department of Agronomy, University of Illinois, Urbana, 61801.

Three soybean cultivars; Fayette, previously identified as resistant to races 2 and 33 of *Peronospora manshurica*, Union, resistant to race 2 but susceptible to race 33, and Williams 82, susceptible to both races, were used in crosses to determine the inheritance of resistance in Fayette. Union carries the gene *Rpm* which conferred resistance to all known races of *P. manshurica* prior to the occurrence of race 33 in 1981. Inoculated F<sub>2</sub> plants from the cross Williams 82 x Fayette segregated in a ratio of 3 resistant to both races : 1 susceptible to both races, indicating that a single gene in Fayette confers resistance to both races. The F<sub>2</sub> plants of Union x Fayette segregated 3 resistant : 1 susceptible when inoculated with race 33 and 15 resistant : 1 susceptible when inoculated with race 2. This indicates that the gene for resistance in Fayette segregates independently from the *Rpm* gene of Union.

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ISOLATION OF CUTIN INDUCIBLE DNA SEQUENCES FROM *COLLETOTRICHUM GLOEOSPORIOIDES* BY DIFFERENTIAL PLAQUE HYBRIDIZATION. Martin B. Dickman and Suresh S. Patil, Dept. of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Cutinase secreted by *Colletotrichum gloeosporioides* causal agent of papaya anthracnose is a pathogenicity determinant for this disease. In order to study the gene encoding the cutinase, genomic libraries of the fungus have been constructed in the bacteriophage 1059 and two derivatives EMBL3 and EMBL4. For library screening, total RNA from *Colletotrichum* was isolated from cultures grown on either potato dextrose broth, serving as a control or on purified papaya cutin as the sole carbon source, to induce cutinase production. From both the control and induced RNA populations, poly (A) RNA was obtained by chromatography with oligo dT-cellulose. Probes were constructed by synthesizing cDNA copies of mRNA using oligo dT (12-18) as a primer, reverse transcriptase and <sup>32</sup>P-dCTP. The mRNA in the hybrid molecule was hydrolyzed and the single stranded cDNA was used to screen the library, yielding sequences unique to the cutin grown culture.

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ESTABLISHMENT AND CHARACTERIZATION OF SINGLE-OOSPORE CULTURES FROM SELFED A1 ISOLATES OF *PHYTOPHTHORA INFESTANS*. R. C. Shattock, P. W. Tooley, and W. E. Fry. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Oospores in five A1 isolates of *Phytophthora infestans* were induced by an A2 isolate of *P. infestans*, when hyphal contact was prevented by a polycarbonate membrane. Oospores from two isolates germinated after being fed to water snails and ten single-oospore cultures were established. All the progeny were A1 compatibility type, but differed from the parents in growth rate and sporulation *in vitro*. Two of the ten single-oospore cultures sporulated sufficiently to test their abilities to overcome specific R genes, and these abilities were identical to the parent. However, two of nine single-oospore isolates from one parent were homozygous for the dimeric enzyme peptidase, whereas the parent and the other seven were heterozygous. This segregation indicates that progeny from selfed oospores are the products of meiosis rather than apomixis.

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DETERMINATION OF RECOMBINATION, SEGREGATION AND SELFING IN

SINGLE-OOSPORE CULTURES OF PHYTOPHTHORA INFESTANS BY ISOZYME ANALYSIS. R. C. Shattock, P. W. Tooley, and W. E. Fry, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Oospores from 10-28-day-old cultures of A1 x A2 Mexican isolates of *Phytophthora infestans* readily germinated on distilled water agar after being fed to water snails. The electrophoretic isozyme patterns of two dimeric enzymes, glucosephosphate isomerase-1 (GPI-1) and peptidase (PEP), were identified in 685 single oospore cultures from seven crosses. Hybridization was confirmed by recombination for isozyme phenotype and compatibility type. Progeny from matings between parents heterozygous at the GPI-1 or PEP loci segregated 1:2:1 as expected for a diploid organism. Fewer than 1% of the single-oospore cultures appeared to be selfs. However, a large number of progeny were self-fertile.

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GENETICS OF RESISTANCE TO EIGHT RACES OF *UROMYCES APPENDICULATUS* IN *PHASEOLUS VULGARIS* CULTIVAR MEXICO 235. J.R. Stavelly and K.F. Grafton, ARS, USDA, BARC-W, Beltsville, MD 20705, and Dept. of Agronomy, North Dakota State Univ., Fargo, ND 58105.

*P. vulgaris* 'Mexico 235' (M235) and 'B-190' have small uredinium resistance (R) or necrotic hypersensitive resistance (HR) to most races of *U. appendiculatus* (Plant Disease 68:95-99). F<sub>2</sub> segregation from susceptible (S) 'Fiesta' x M235 indicated a single dominant pleiotropic gene or tightly linked genes control HR of M235 to races 40, 52, 53, and 54. These genes are epistatic to the R genes for these races in B-190. M235 also contained a second, independent group of apparently linked single dominant genes for R to races 40, 45, and 48 and for HR to 49 and 50. The genes for HR to races 49 and 50 in M235 were apparently influenced by modifier genes, environment, or both so that their expression varied from HR to R in the F<sub>2</sub>. The F<sub>2</sub> of B-190 x M235 indicated allelism of their R genes for races 40 and 48, but one in 64 was S to race 45, indicating triplicate dominant epistasis.

## 258

THE INHERITANCE OF RESISTANCE IN TWO WHITE SEEDED DRY BEAN CULTIVARS TO SEVEN BEAN RUST ISOLATES. M.K. Kardin and J.V. Groth. Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

Simultaneous inoculations of the F<sub>1</sub> and F<sub>2</sub> progenies from crosses of dry edible beans Aurora x Pinto 111, Fleetwood x Pinto 111, and Aurora x Fleetwood with seven bean rust (*Uromyces appendiculatus*) isolates indicated that the resistance of Aurora and Fleetwood to each isolate was controlled by a single dominant gene. Aurora contains at least two resistance genes. Since individual F<sub>2</sub> progeny of Aurora x Pinto 111 always reacted the same to the first six isolates of bean rust, we hypothesize that the same resistance allele and locus in cv. Aurora is conditioning incompatibility to all six isolates. The small fleck gene in Aurora was epistatic to that in Fleetwood, a minute uredium. The gene in Aurora for resistance to the seventh isolate segregated independently from that which conditioned resistance to the other six isolates, and was independent of and epistatic to a third gene, in cv. Pinto 111, that gave a mesothetic reaction to this isolate.

## 259

LOGI IN *COCHLIOBOLUS HETEROSTROPHUS* WHICH CONDITION PERITHECIAL NUMBER. J.A. Kolmer and K.J. Leonard, North Carolina State Univ. Raleigh N.C. 27695-7616

A full-sib mating design was used in selecting for increased numbers of perithecia in matings between sexually compatible pairs of ascospore progeny of the fungus *Cochliobolus heterostrophus* through three generations. In the third generation half of the sexually compatible pairs produced abnormally low numbers of perithecia. Isolates could be grouped according to putative secondary incompatibility alleles such that perithecial number was high when the paired sexually compatible isolates had different alleles at the locus, and low when identical alleles were present. Expected segregations at the same locus were obtained in three related full-sib families. Two additional full-sib families also segregated at a single locus but in matings between families all sexually compatible pairs were fertile, indicating the families segregated at two and probably three different loci for perithecial number. This appears to be a unique case of homogenic incompatibility, independent of the mating type locus in fungi.

## 260

INHERITANCE OF RESISTANCE TO THREE PATHOTYPES OF *PERONOSCLEROSPORA SORGHII*. J. Sifuentes and R. A. Frederiksen. Dept. of Plant Pathology & Microbiology, Tx. Agric. Exp. Stn., College Station, TX 77843.

The resistant sorghum lines QL3 India and SC0414-12 were crossed to three susceptible lines and to three lines (SC0170-6-17, Tx430, and CS3541) with differential reaction to *P. sorghii* pathotypes one, two, and three. The parental lines and their F<sub>1</sub> and F<sub>2</sub> progenies were tested for local lesion reaction. Analysis of F<sub>1</sub> reciprocal crosses failed to detect cytoplasmic inheritance for resistance to any pathotype and indicated the dominance of resistance over susceptibility. Segregation of F<sub>2</sub> progenies of crosses involving SC0414-12 and QL3 India indicated the existence of one and two resistance genes in those lines, respectively. A 63:1 segregation in the F<sub>2</sub> population of the cross between the two resistant lines suggested that the gene in SC0414-12 is different from the two in QL3 India.

## 261

INHERITANCE ESTIMATES OF RESISTANCE IN MAIZE (*ZEA MAYS*) TO GRAY LEAF SPOT (*CERCOSPORA ZEA-MAYDIS*). R. R. Bergquist, Pfister Hybrid Corn Co., El Paso, IL 61738; G. A. Payne, Plant Pathology Dept., D. L. Thompson and D. T. Bowman, Crop Science Dept., N.C. State Univ., Raleigh, NC 27695.

Inbred parents, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and backcross generations were rated for reaction to gray leaf spot just prior to leaf death on a scale of 1 (resistant) to 5 (susceptible) in a natural epiphytotic near Marion, NC in 1984. In two generation mean experiments, parent NC250 was rated 1.7 and parents B73 and B73rhm were rated 4.5 and 4.6 in their respective experiments. Other generations were intermediate. In a diallel experiment, inbreds H99, NC250, B73ARB, B73, B73rhm and N28rhm had ratings of 1.8, 1.7, 2.3, 4.5, 4.6 and 4.8 as parents per se and had mean ratings of 2.9, 3.1, 3.1, 3.6, 3.9 and 3.8 in crosses, respectively. Estimates were large for pooled additive effects and general combining ability indicating that resistance involved loci which were primarily additive. Therefore, evaluation of inbreds per se would be an effective breeding procedure for selecting resistant genotypes.

## 262

EFFECTS OF THREE TOBACCO ETCH VIRUS ISOLATES ON DIFFERENT VIRUS RESISTANT CAPSICUM GENOTYPES. Benigno Villalon, Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, Texas 78596

Viral diseases of peppers reduce yield in many pepper production areas throughout the world. The virology program at Weslaco, Texas has yielded *Capsicum* breeding lines resistant to tobacco etch virus (TEV), pepper mottle virus, potato virus Y, and tobacco mosaic. Resistant lines exhibited virus-like symptoms in production areas of Texas and California. TEV was identified from some of these plants. It was believed that different strains of TEV might exist. TEV isolates from Texas, California, and Sinaloa, Mexico were collected and their reaction on several genotypes studied. Differences in growth and virus symptoms were observed in 'TAM MILD JALAPENO-1', 'TAMBEL-1' and 'TAM MILD CHILE-1' when inoculated with 3 TEV isolates. Reaction to the Sinaloa isolate was most severe on bell and chile types while the California isolate was most severe on jalapeno. The Texas isolate did not affect the resistant lines significantly.

## 263

VIRUS DISEASES OF CHICKPEA IN IDAHO AND WASHINGTON. W. J. Kaiser\* and S. D. Wyatt\*\*, \*ARS, USDA, Regional Plant Introduction Station and \*\*Department of Plant Pathology, Washington State University, Pullman, WA 99164

Chickpeas (*Cicer arietinum*) are a new cash crop in the dryland areas of eastern Washington and northern Idaho where they are grown in rotation with wheat, barley, peas, and lentils. Since 1979, several viruses have been isolated from naturally infected chickpeas in commercial and experimental plantings. Stunting, wilting, chlorosis, leaf deformation, mosaic, and phloem discoloration are some of the symptoms associated with virus infection. Techniques used in virus identification have included symptomatology, host range studies, serology, electron microscopy, and/or vector transmission. The following virus diseases of chickpea were found: Alfalfa mosaic, bean yellow mosaic, pea enation mosaic (PEMV), pea leaf roll, and pea streak. The most important virus disease was caused by PEMV.

All are transmitted by aphids, but none appear to be seedborne in chickpea. Important reservoir and overwintering hosts are alfalfa, white sweet clover, and hairy vetch.

#### 264

REDUCTION IN SPREAD OF NECROTIC RUSTY MOTTLE WITH REMOVAL OF AFFECTED TREES. H. R. Cameron and D. L. Moore, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331

In the late 1970's, two 18 hectare virus-indexed cherry (*Prunus avium* L.) orchards were planted about 16km apart in Western Oregon. Both orchards were bounded on at least three sides by native vegetation. In 1978, three trees of the cultivar Corum in one orchard, and in 1979, 17 trees in the second orchard expressed symptoms of necrotic rusty mottle (NRM). The first trees to show symptoms were in the outside rows of the orchard followed by secondary infections in the next adjacent Corum trees. In the first orchard trees were removed when symptoms were first evident. In the second orchard trees were removed as they were dying. Spread of NRM was greatly reduced (4.1%) in the first orchard compared to the other orchard (18.4%).

#### 265

ULTRASTRUCTURAL CYTOLOGY OF PEANUT INFECTED WITH PEANUT STRIPE VIRUS. N. A. Rechcigl, S. A. Tolin, G. R. Hooper, and R. L. Grayson. Dept. of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA 24061

Two strains of peanut stripe virus from Virginia were compared in peanut (*Arachis hypogaea* 'Florigiant') at several stages of leaf development. Mature leaves on plants infected with the stripe strain exhibited dark green vein-banding and oak-leaf patterns, whereas leaves affected by the blotch strain developed large, dark green spots. Ultrathin sections of young leaf tissue infected with either strain revealed pinwheel inclusions near plasmodesmata. In more mature leaves infected with either strain, pinwheel and scroll inclusions occurred in the cytoplasm in association with mitochondria. Virus particles were observed free in the cytoplasm as well as concentrated in linear arrays along the inner surface of the tonoplast. Cells in light green areas of mature leaves had prominent virus-packed cytoplasmic strands. In dark green areas, strands were less prominent and contained few if any virus particles. Membrane and organelle degradation was evident in cells infected with either strain.

#### 266

INOCULATION OF ADAXIAL LEAF SURFACES IN CORN WITH MAIZE DWARF MOSAIC VIRUS (MDMV) INCREASES INFECTION. Eugen Rosenkranz and G. E. Scott. USDA, ARS, Depts. of Plant Pathology & Weed Science and Agronomy, Mississippi State Univ., Miss. State, MS 39762

In the field, inoculation of corn with MDMV is done on the abaxial leaf surfaces where the virus is exposed to solar radiation. To determine if this exposure has a detrimental effect on infection, corn plants in the 5-leaf stage were inoculated with an artist's air brush on the upper vs. lower leaf surfaces on the same sunny day. In all 3 corn genotypes (Ab28A x T226, Mo12 x Tx601, T226 x T232) tested, plants inoculated on the lower leaf surfaces had significantly more diseased plants than those inoculated on the upper surfaces. The difference in disease incidence was highly significant ( $P = 0.01$ ) up to 20 days after inoculation and significant ( $P = 0.05$ ) thereafter. Of 72 comparisons (3 hybrids x 4 reps x 6 evaluation days), 67 had higher disease incidence for lower-leaf surface inoculation than upper-leaf surface inoculation. The disease incidence was 15-140% higher in plants inoculated on the adaxial leaf surfaces than in plants inoculated on the abaxial surfaces.

#### 267

DIFFERENCES IN RESPONSE TO MAIZE DWARF MOSAIC VIRUS AMONG PLANTS WITHIN CORN INBREDS HAVE NO GENETIC BASIS. G.E. Scott and Eugen Rosenkranz. USDA, ARS, Depts. of Agronomy and Plant Pathology & Weed Science, Mississippi State Univ., Miss. State, MS 39762

Corn inbreds resistant to maize dwarf mosaic virus (MDMV) produce some diseased plants when mechanically inoculated in the field. To determine if these diseased plants differ genetically from symptomless plants for response to MDMV, 8 plants with and 8 plants without symptoms in each of 3 inbreds (Tx601, Mo18W, T220A) were self-pollinated and the 24 progenies from symptomless plants were compared with 23 progenies from diseased plants for their reaction to MDMV. The comparison was made in two field tests (early and late planting) and one greenhouse test during the winter. Under the three different environmental con-

ditions the average disease incidences were 5, 12, and 68% for the 24 progenies from symptomless plants and 5, 12, and 71% for the 23 progenies from diseased plants. Thus, whether a plant of an inbred develops symptoms or remains symptomless is not conditioned by genetic variability within that inbred and therefore these differences must be attributed to factors other than genetics.

#### 268

SURVEY OF PEPPER VIRUSES IN CALIFORNIA BY THE ELISA TECHNIQUE. O. A. Abdalla, P. R. Desjardins, and J. A. Dodds, Dept. of Plant Pathology University of California, Riverside, CA 92521.

Virus diseases are still a primary limiting factor in pepper production in California in spite of past surveys. The enzyme-linked immunosorbent assay (ELISA) technique was used to detect the following viruses: potato Y (PVY), tobacco etch (TEV), cucumber mosaic (CMV), alfalfa mosaic (AMV), potato X (PVX), tobacco mosaic (TMV) and pepper mottle (PeMV). The pepper samples, collected late in the season, were from San Joaquin, Ventura and Imperial counties. All seven viruses were detected in samples from one or more of the locations, and their relative frequencies are as follows: San Joaquin: PVY (69%), TEV (99%), CMV (89%), AMV (19%), PVX (14%), TMV (0%) and PeMV (61%); Ventura: PVY (49%), TEV (59%), CMV (65%), AMV (5%), PVX (1%), TMV (0.6%) and PeMV (46%); and Imperial: PVY (80%), TEV (98%), CMV (25%), AMV (5%), PVX (0%), TMV (80%) and PeMV (70%). This is the first confirmed report of PeMV in California. The ELISA technique was found to be practical and reliable for surveys of pepper viruses in the field.

#### 269

SEROLOGICAL TITER OF CITRUS TRISTEZA VIRUS (CTV) ISOLATES OF VARYING SEVERITY IN DIFFERENT CITRUS HOSTS. S. M. Garnsey, R. F. Lee\*, R. H. Brlansky\*, and R. K. Yokomi, U.S. Horticultural Research Laboratory, ARS, U.S. Department of Agriculture, Orlando, FL 32803; and \*Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL 33850

Seventy-five different citrus host-CTV isolate combinations were prepared using 25 hosts and 5 virus isolates. Uniform young tissue samples from greenhouse-grown plants were extracted, freeze-dried and tested by ELISA. Titers were similar in most combinations of 12 hosts infected with each of the 5 CTV isolates. Titers were positively correlated with isolate severity and host reaction. *Citrus hystrix* had the highest average titer. Especially low titers were found in several mandarins (*C. reticulata*) tested with a single isolate. Significant variation in titer was detected between comparable shoots of the same plant and between replications of the same combinations. Accurate comparison of CTV titers was difficult when all interacting variables were considered.

#### 270

HYPERSENSITIVE REACTION IN ALFALFA CLONES CAUSED BY ALFALFA MOSAIC VIRUS. Z. Pesic and C. Hiruki, Dept. of Plant Science, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2P5.

Twenty eight alfalfa clones from the cv. Beaver were assayed for resistance to one isolate of alfalfa mosaic virus (AMV) from Alberta. Virus infection in clonal pieces was detected by direct ELISA 4 and 8 weeks after inoculation with purified AMV. Sixteen clones were systemically infected, developing mosaic symptoms. However, in twelve clones hypersensitive reaction was observed 3 to 4 days after inoculation and the infection was restricted to the area of necrotic local lesions. When the clones were inoculated with AMV in crude sap, distinct symptoms were not observed and the virus was detected only in inoculated leaves. Using purified AMV inoculum, a higher percentage of infection was obtained for all clones. These results indicated that purified AMV inoculum is more efficient than crude sap for screening of alfalfa clones for resistance to AMV.

electrophoresis. A similar preparation of citrus variegation virus (CVV), an Ilarvirus, was used for comparison. A band was observed 1.2 cm from the origin from CRSV-infected but not healthy tissue after electrophoresis for 4 h at 50 V in 0.5% gels (pH 7.5, 5 C) and staining with ethidium bromide. CVV formed a band 0.8 cm from the origin under similar conditions.

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DEVELOPMENT OF APPLE UNION NECROSIS AND DECLINE IN APPLE TREES INOCULATED WITH TOMATO RINGSPOT VIRUS. D. A. Rosenberger, J. N. Cummins, and D. Gonsalves, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

Rootstocks of 31 Delicious/MM.106 apple trees were inoculated with bark patches from tomato ringspot virus (TmRSV)-infected rootstocks of similar trees showing distinct graft-union symptoms of apple union necrosis and decline (AUND). Two inoculated trees showed graft-union symptoms after 2 yr and 26 additional trees developed symptoms the third year. TmRSV was detected serologically in rootstocks of symptomatic trees. Thirty-two control trees similarly inoculated with bark from the TmRSV-free scions of the diseased inoculum trees did not develop AUND and remained TmRSV-free. In earlier experiments, Delicious/MM.106 trees inoculated with the "Chicadee" isolate of TmRSV failed to develop any symptoms of AUND after 6 yr despite the presence of TmRSV in the stocks. These experiments provide the first evidence that AUND can be reproduced by inoculating trees with TmRSV-infected bark. They also suggest that the Chicadee isolate of TmRSV may not be capable of causing AUND.

#### 277

ACTIVITIES OF ARACHIDONIC ACID, FATTY ACIDS AND GLUCANS FROM PHYTOPHTHORA INFESTANS IN HYPERSENSITIVITY EXPRESSION IN POTATO TUBER. R. M. Bostock, D. Schaeffer and \*R. Hammerschmidt. Departments of Plant Pathology, University of California, Davis, CA 95616 and \*Michigan State University, E. Lansing, MI 48824.

The hypersensitive response of potato tuber to incompatible races of *Phytophthora infestans* is characterized in part by membrane lipid peroxidation and accumulation of sesquiterpene phytoalexins and lignin. Arachidonic (AA) and eicosapentaenoic acids from the mycelial lipids of *P. infestans* are specific elicitors of sesquiterpene accumulation in potato tuber and their activities are markedly enhanced by  $\beta$ -glucans extracted from the fungus. AA, linoleic acid (LA) and mycelial glucans but not saturated fatty acids induced lignification in tuber slices within 24 hr after treatment. AA and LA but not glucans or saturated fatty acids elicited the production of ethylene and ethane. Glucans stimulated AA- and LA-induced lignification and ethane production. The effect of AA on terpenoid metabolism and the stimulation of AA activity by glucans appears to be highly specific whereas lignin, ethylene and ethane also can be induced by other mycelial components.

#### 273

THE VIRUSES INVOLVED IN RHIZOMANIA DISEASE OF SUGARBEET IN CALIFORNIA. Heing-Yeh Liu and James E. Duffus, USDA-ARS, 1636 East Alisal St., Salinas, CA 93905

Rhizomania, one of the most destructive diseases of sugarbeet in Europe and Japan, was found in several of the important sugarbeet growing areas of California in 1983. Rhizomania is reported to be caused by beet necrotic yellow vein virus (BNYVV) vectored by a soil fungus, *Polymyxa betae* Keskin. In California, this disease was identified by the presence of BNYVV and *P. betae* in the roots of affected sugarbeet plants. Several distinct isolates of BNYVV have been found in California. All isolates reacted in ELISA tests with antiserum to Japanese and French isolates of the virus. Other virus entities different in symptomatology and virus particles have been found. The relationship of these entities to Rhizomania disease of sugarbeet is not yet known.

#### 274

MELON LEAF CURL VIRUS--A NEW GEMINI VIRUS WITH HOST AND SEROLOGICAL VARIATIONS FROM SQUASH LEAF CURL VIRUS. James E. Duffus, Heing-Yeh Liu, and Matthew R. Johns, USDA-ARS, 1636 East Alisal St., Salinas, CA 93905

A new whitefly-transmitted gemini virus causing leaf curl symptoms on melons has been isolated from the Imperial Valley, California. The infectious agent, melon leaf curl virus (MLCV), which affects melon, watermelon, cucumber, cantaloupe, pumpkin, squash and bean is transmitted by *Bemisia tabaci* as well as being mechanically transmitted. MLCV virions appeared identical to squash leaf curl virus (SLCV) on the basis of particle morphology and ELISA tests. However, SLCV does not affect melon, watermelon and cucumber; also, SLCV antiserum did not react with MLCV in agar double diffusion tests.

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COMPARISON OF CITRUS RINGSPOT AND CITRUS VARIEGATION VIRUSES BY AGAROSE GEL ELECTROPHORESIS. K.S. Derrick, Dept. of Plant Pathology and Crop Physiology, La. Agric. Exp. Sta., La. State Univ. Agr. Ctr., Baton Rouge, La 70803 and R.H. Briansky, R.F. Lee, and L.W. Timmer, Univ. of Fla., Citrus Res. and Ed. Ctr. Lake Alfred, Fl 33850.

Citrus ringspot virus (CRSV) is readily transmitted to many herbaceous plants by sap inoculation and produces local lesions on *Chenopodium quinoa*, but the infectivity in extracts is unstable, and the presumed virus has not been characterized. Extracts of *C. quinoa* from healthy and CRSV-inoculated leaves with numerous chlorotic lesions were clarified with Freon, precipitated with PEG 6000 and analyzed by agarose gel

#### 278

WATER FLOW AND XYLEM PLUGGING IN DECLINING AND APPARENTLY HEALTHY CITRUS TREES IN FLORIDA AND ARGENTINA. L. W. Timmer, R. H. Briansky, J. H. Graham, and H. A. Sandler, Univ. of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850

Citrus blight (CB) in Florida and declinamiento (CD) in Argentina are diseases of unknown etiology with xylem dysfunction. Water flow and plugging was measured in cores from trunks at 4 distances from the cambium. In healthy trees, water flow declined slightly with distance from the cambium; amorphous plugs were rare. In declining trees, there was little water flow beyond the 0-1 cm segment; amorphous plugs were numerous. Apparently healthy trees in Argentina on several rootstocks had low water flow beyond the 0-1 cm segment and many amorphous plugs. By scanning electron microscopy, these plugs were typical of those in CB and CD trees; thus, even some healthy appearing trees in Argentina could be affected by the disease.

#### 279

GREGATIN A - A METABOLITE PRODUCED BY PHIALOPHORA GREGATA, THE CAUSE OF SOYBEAN BROWN STEM ROT. L. E. Gray, R. E. Peterson, and Scott Taylor. USDA-ARS, Department of Plant Pathology, Univ. of Illinois, Urbana, IL 61801; USDA-ARS, Northern Reg. Res. Centr., Peoria, IL 61604 respectively.

Gregatin A has been separated from culture filtrates of

Phialophora gregata by HPLC. Preliminary experiments have shown that maximum Gregatin A production on Rice medium occurs at 23 C with lower production at 19 C and only a trace produced at 27 C after 4 weeks. Chlamydomonas reinhardtii Dangeard (a motile alga) has been used to bioassay purified Gregatin A and also to bioassay culture filtrates of various isolates of P. gregata. Cells of C. reinhardtii were expressed to 5 ug/ml of purified Gregatin A for 5 min in the light and oxygen evolution of illuminated cells was reduced compared to controls. When Century soybean seedlings were placed in solutions of 50 and 150 g/ml Gregatin A, typical vascular browning of stem and soybean leaf vascular tissue developed after 2 days at 25 C. Studies are currently underway to determine the role of Gregatin A in leaf symptom development of soybean plants infected with P. gregata.

## 280

ALTERATION OF NUCLEAR RNA POLYMERASE II IN A COMPATIBLE MAIZE-INTERACTION. CATHY H. WU, H.L. WARREN and C.Y. TSAI. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

Alterations of nuclear RNA polymerase II, the key enzyme for transcription of mRNAs, were determined in a compatible maize - Bipolaris maydis interaction. The enzyme was purified from healthy and B. maydis-inoculated leaves of W64A inbred and enzymatic properties and subunit structures compared. RNA polymerase II from healthy and diseased tissues differed in  $\alpha$ -amanitin sensitivity, Mn preference, heat sensitivity and template preference; although pH optimum and kinetics of UMP incorporation were similar. Analysis on SDS-Polyacrylamide gels indicated variations in M.W. of subunits. These alterations, especially the changes of template preference and subunit structure may account for observed changes in the transcription in a compatible maize - B. maydis interaction.

## 281

EFFECT OF TEMPERATURE ON LIGNIFICATION AND SUBERTIZATION OF ALMOND BARK WOUNDS AND ON WOUND RESISTANCE TO PHYTOPHTHORA SYRINGAE. M. A. Doster and R. M. Bostock. Department of Plant Pathology, University of California, Davis, CA 95616

Pruning wounds on almond trees made in the fall and winter in California gradually develop resistance to Phytophthora syringae, a causal agent of branch cankers. The effect of temperature on the formation of zones of lignin and suberin in bark tissues adjacent to pruning wounds was investigated in mature orchard trees throughout fall and winter and in excised branch segments and 1-year-old potted trees in growth chambers. In orchard trees, the proportion of bark tissue staining for lignin 2 weeks after wounding and the proportion staining for suberin 4 weeks after wounding was correlated with the cumulative degree days for that period ( $r \geq 0.70$ ) and most 4-week-old wounds and many 2-week-old wounds were immune to infection. Lignification in wounds on excised branches or on potted trees 3 weeks after wounding was completely inhibited at temperatures < 13 C and rate of lignification increased with temperature above 13 C. In all experiments, lignin was detected prior to suberin.

## 282

PURIFICATION AND ACTIVITY OF TWO ANALOGS OF THE SELECTIVE TOXIN FROM HELMINTHOSPORIUM CARBONUM. J.B. Rasmussen, R.P. Scheffer, Dept. of Botany and Plant Pathology, B.A. Horenstein, and S.P. Tanis, Dept. of Chemistry, Michigan State University, East Lansing, MI 48824.

A new and simpler purification scheme was devised for HC toxin. The preparations contained the previously described major toxin (toxin I) and two analogs (toxins II and III); all had host-selective activity. There was more of toxin II than of III, but the yield of each was <5% of toxin I. Chromatography on silica gel and octadecylsilane indicated that toxin III is the most and toxin I is the least polar compound. The  $ED_{50}$  values in bioassays were approximately 0.2, 0.4, and 2.0 ug/ml for toxins I, II, and III, respectively. Spectral data (see abstract by Horenstein et al.) indicate that both analogs contain 2-amino-9,10-epoxy-8-oxodecanoic acid. Brief exposure of toxins I and II to 0.1% trifluoroacetic acid resulted in production of more polar, apparently non-toxic compounds by hydrolysis of the epoxide to a diol.

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STRUCTURE DETERMINATION OF TWO NEW HOST-SPECIFIC TOXINS PRODUCED

BY HELMINTHOSPORIUM CARBONUM. Steven P. Tanis,<sup>1</sup> Benjamin Horenstein,<sup>2</sup> Robert P. Scheffer,<sup>2</sup> and Jack B. Rasmussen,<sup>2</sup> Michigan State University, (1)Department of Chemistry, (2)Department of Botany and Plant Pathology, East Lansing, Michigan 48824

The fungus Helminthosporium carbonum (Ullstrup) race 1 produces a host-specific toxin (HC-toxin-1) whose structure has previously been established. We have examined culture filtrates of the fungus and have isolated two additional toxic compounds.

The structures of HC-toxins-2 and -3 were elucidated through the use of a number of spectroscopic techniques including 2D-<sup>1</sup>H-Spin correlated spectroscopy, <sup>1</sup>H-nuclear Overhauser Difference Spectroscopy, <sup>13</sup>C-NMR, FAB-Mass spectroscopy and FI-Mass spectroscopy. These data suggest that HC-toxins-2 and -3 are cyclic tetrapeptides composed of 2-alanine,  $\beta$ -hydroxy proline, 2-amino-8-oxo-9,10-epoxydecanoic acid (AOE) and alanine, glycine, proline, AOE, respectively. The order of connection of the amino acid units will be discussed.

## 284

EVIDENCE THAT AN ALBICIDIN-LIKE TOXIN INDUCES CHLOROSIS IN SUGARCANE LEAF SCALD. Robert G. Birch & Suresh S. Patil, Department of Plant Pathology, University of Hawaii, Honolulu, Hawaii 96822.

Chlorosis-inducing isolates of Xanthomonas albilineans produce a family of antimicrobial compounds, including albicidin which inhibits prokaryote DNA synthesis. However, albicidin up to 1 mg/ml did not induce chlorosis in sugarcane leaves when injected in spindle tissues. Results with mutants and revertants in albicidin production showed a strong correlation between albicidin production and ability to cause chlorosis. Albicidin partially blocked DNA synthesis in isolated sugarcane proplastids but not in chloroplasts. The results indicate that albicidin fails to penetrate the target site in intact sugarcane tissues and that another member of the albicidin family causes chlorosis in sugarcane invaded by X. albilineans.

## 285

OCCURRENCE OF PHYTOFERRITIN AND ITS RELATIONSHIP TO THE EFFECTIVENESS OF SOYBEAN NODULES. M. P. Ko, P. Y. Huang, J. S. Huang, and K. R. Barker. Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695-7616

The occurrence and role of phytoferritin in soybean nodules induced by various (effective, ineffective, or mutant) strains of R. japonicum were investigated. Polyacrylamide gel electrophoresis and/or electron microscopy of nodules indicated that the presence of phytoferritin was dependent on the Rhizobium strain-soybean cultivar interaction, the stage of nodule development, or the physiological status of the soybean plant. Nodules with a notable amount of phytoferritin were characterized by a low leg-hemoglobin content and a low nitrogen-fixation rate (as measured by acetylene reduction). In particular, large amounts of phytoferritin, different from horse-spleen ferritin in molecular size and electrophoretic mobility, accumulated in nodules produced by a mutant strain of R. japonicum. Such nodules may become a useful system for studying the developmental or functional roles of phytoferritin from nodules of healthy or diseased soybean plants.

## 287

PAPILLA FORMATION IN ISOLATED ROOT-CAP CELLS OF MAIZE

INOCULATED WITH COLLETOTRICHUM GRAMINICOLA. R.T. SHERWOOD, ARS-USDA, U.S. Regional Pasture Research Laboratory, University Park, PA 16802.

A system was sought for studying papilla formation in single cells. *Zea mays* seedlings were germinated aseptically on water agar. At 2-3 days naturally detached root-cap cells were collected, washed to remove polysaccharide slime, plated on 1% glucose-Pfeffer salts agar, and inoculated with conidia of *C. graminicola*. Less than 25% of contacts between hyphae and the corn cells showed indications of attempted penetration. About 40-80% of penetration attempts resulted in formation of a papilla without penetration or cytoplasmic collapse. Cells that were penetrated usually had collapsed cytoplasm and lacked papillae. Some penetrations occurred through papillae. Differences in frequency of papilla formation and penetration were found among corn lines.

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Isolation and structure of the toxins specific for rough lemon J. M. Gardner and Y. Kono, Univ. of Florida, IFAS, Citrus Res. and Educ. Ctr., 700 Experiment Station Road, Lake Alfred, FL 33850; The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

A pathotype of *Alternaria citri* that infects rough lemon plants produces several related toxins in culture. The major toxin produced, Toxin I, was the most potent compound (ED<sub>50</sub> = 30 ng/ml). Five other minor toxins were active at ED<sub>50</sub> levels > 1 g/ml. On the basis of MS, <sup>1</sup>H and <sup>13</sup>C-NMR spectra, and decoupling studies of Toxin I and derivatives, Toxin I is a 19-carbon polyalcohol with an dihydro- $\alpha$ -pyrone ring. The minor toxins vary in chainlength and contain an  $\alpha$ -pyrone ring. Culture filtrates of *A. citri* also contained a biologically inactive, partially analogous, component possessing a tetrahydropyran ring. It probably arises from decarboxylation of Toxin I. Toxin I was highly specific and did not affect nonhost plants (except for Cleo mandarin) at 10,000 times concentrations affecting rough lemon.

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Host specificity and bioassay of the *Alternaria citri* pathotoxins affecting rough lemon and mandarins. J. M. Gardner and J. L. Chandler, Univ. of Florida, IFAS, Citrus Res. and Ed. Ctr., 700 Experiment Station Road, Lake Alfred, FL 33850

Two host-specific strains of *Alternaria citri* and their toxins were examined for host range and specificity. Toxins were bioassayed by 4 methods. The strain of *A. citri* pathogenic for rough lemon and Rangpur lime produced a very specific and potent toxin (ACRL toxin I) which was characterized as a polyalcohol-dihydropyrene structure. In 2 of the 4 bioassay methods, however, ACRL toxin I was active on Cleopatra mandarin (*C. resnii* Hort.) a nonhost of the fungus. The tangerine strain of *A. citri* infected many mandarin cultivars (*C. reticulata*) and, under optimal conditions, grapefruit cultivars. Isolated ACTG toxins had striking effects on nonhost plants depending on the bioassay method. In assays based on the incorporation of proline into protein, ACTG toxins were semi-specific. Resistance to ACTG toxin may be related to the ability of toxins to permeate tissues.

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GENE-SPECIFIC RESPONSE OF SORGHUM TO THE TOXIN FROM PERICONIA CINCLINATA. E.A. Taylor and L.D. Dunkle. USDA-ARS, Dept. of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907.

Milo disease symptoms in a susceptible (S) genotype of sorghum (cv 'Colby') treated with the pathotoxin from *Periconia cinclinata* are associated with an increased rate of synthesis of a group of proteins, each with a mol wt of 16 kD. The isogenic resistant (R) line is not affected by the toxin. To examine the correlation between symptoms (toxin-sensitivity) and the toxin-enhanced synthesis of the proteins, in vivo labeled proteins in sorghums with diverse genetic backgrounds were analyzed. In all S-sorghums, including varieties from China and Nigeria and S-selections of shattercane, toxin increased the rate of synthesis of the 16 kD proteins. In all R-sorghums, including spontaneous R-mutants of CV Colby and R-selections of shattercane, protein synthesis was not altered. Synthesis of the 16 kD proteins increased in the F<sub>1</sub> progeny from a cross of R x S isolines, but the rate was less than that in toxin-treated S-genotypes. The results indicate that the enhanced synthesis of specific proteins is strictly correlated with disease symptoms and suggest that this selective response is due to the involvement of the *Pc* allele.

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ANASTOMOSIS GROUP OF ISOLATES OF RHIZOCTONIA SOLANI FROM ALFALFA PLANTS WITH BLACK ROOT CANKER. W.C. Kronland and M.E. Stanghellini, Dept. of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721.

Black root canker is a disease of alfalfa roots that occurs during the hot summer months in the irrigated desert areas of Arizona and California. *Rhizoctonia solani* was previously identified as the causal organism. Root canker was found to occur in 5 out of 25 fields surveyed in 5 production areas in Arizona and California. Fourteen isolates were collected from lesions and feeder roots. The nuclear condition and the anastomosis group of the isolates were determined by growing the fungus on clean glass slides and staining with Safranin O. Pathogenicity was confirmed by inoculation of alfalfa seedlings and mature plants. All but one isolate (a binucleate *Rhizoctonia* sp.) anastomosed with an AG-4 tester isolate. Although all AG-4 isolates were pathogenic on alfalfa seedlings, not all isolates caused cankers on mature roots. Our survey indicated that black root canker occurred in only two of the five production areas surveyed, and was severe in only certain fields in these areas.

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SIZE AND SHAPE OF UREDINIOSPORES UNDER NATURAL ATMOSPHERIC CONDITIONS. W. K. Schimming and L. J. Littlefield, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Urediniospores of 21 rust species were examined under natural and artificial conditions to determine their size and shape when hydrated or dehydrated. Under room conditions spores typically dehydrated to 70-80% of their hydrated size and were indented or collapsed. Shapes of dry spores varied among species depending on several factors, primarily distribution and number of germ pores. Spores of 3 rust fungi were manipulated experimentally to determine the relative humidity (RH) at which they would assume either their expanded or collapsed shape. *Puccinia graminis* spores remained expanded down to 92.0% RH and were completely collapsed at 75.8% RH. *P. recondita* spores remained expanded at 92.0% RH and above but were collapsed at 80.3% RH. Spores of *Uromyces appendiculatus* were expanded at 96.9% RH and above, partially collapsed at 92.0% RH, and totally collapsed at 80.3% RH. Identification of rust fungi by scanning electron microscopy must take this information into account.

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*Pyrenophora tritici-repentis* on *Bromus inermis*. J. M. Krupinsky, USDA, ARS, Northern Great Plains Research Center, P. O. Box 459, Mandan, ND 58554.

Smooth brome grass, *Bromus inermis* is widespread in the Northern Great Plains and can be found in close proximity to fields of cereal crops. From 1981 through 1984, 125 leaf samples were collected in North Dakota, 32 in South Dakota, 24 in Minnesota, and 27 in Montana. Of the 208 samples collected, 59% were infected with *Pyrenophora* spp. Of the 71 isolates of *Pyrenophora* spp. obtained, 73% were identified as *P. tritici-repentis* and 27% as *P. bromi*. *P. tritici-repentis*, a pathogen of wheat, was widely distributed throughout the Northern Great Plains on smooth brome grass, an alternative host. Cultural studies were undertaken to aid in the separation of isolates of *P. tritici-repentis* from isolates of *P. bromi*. In general, the comparison of mycelium growth rate on sucrose proline agar (Shoemaker, R.A. 1962. Can. J. Bot. 40:809-836) and the comparison of spore production on lima bean agar aid in the separation of *P. tritici-repentis* from *P. bromi*.

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TWO POTENTIALLY DAMAGING DISEASES OF RABBITEYE BLUEBERRIES IN GEORGIA. Paul Bertrand, The University of Georgia Cooperative Extension Service, P.O. Box 1209, Tifton, Georgia 31793.

Rabbiteye blueberries (*Vaccinium ashei* Reade) have been cultivated in Georgia since the late 1950's. Two diseases have been recently discovered which caused heavy losses in some fields. In 1983 an epiphytotic of Botrytis flower blight (*Botrytis cinerea* Pers. x Fr.) occurred in several fields in the Bacon County area where most of Georgia's rabbiteye blueberries are grown. Loss of flowers was severe in many low lying fields and/or fields surrounded by woods. In 1984 three fields in this same area suffered severe losses from a mummy berry-like disease. Symptoms first developed as a severe shoot blight. The blighted shoots showed abundant sporodochia and conidia characteristic of *Monilinia vaccinii-corymbosi* (Reade) Honey.

Some rotting of flowers also occurred. At harvest, there were numerous slightly shriveled pinkish berries resembling the mummy berries on highbush blueberries. Apothecia have not been found preventing positive identification of the causal fungus.

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ANILINE BLUE POSITIVE DEPOSITS IN CELL WALLS OF *APIUM* ROOTS SUSCEPTIBLE OR RESISTANT TO *FUSARIUM OXYSPORUM* F. SP. *APII*. RACE 2. C. M. Jordan and R. M. Endo, Department of Plant Pathology, University of California, Riverside, CA 92521.

Cell walls of roots of seedlings of three yellows-susceptible or resistant lines of celery and celeriac (*Apium* spp.) inoculated with race 2 of *F. oxysporum* f. sp. *apii* developed aniline blue-positive (AB+) deposits 3-6 hr after inoculation. The AB+ material fluoresced yellow and bright blue under UV-light. Healthy controls, in the presence or absence of aniline blue stain, displayed little or no fluorescence. Periodic acid Schiff treatment or digestion assay with endo- $\beta$ -glucanase prior to staining with aniline blue eliminated the fluorescence, suggesting the presence of callose. AB+ deposits were detected initially in the epidermis, then in the cortex, and finally in the vascular parenchyma and xylem. In susceptible lines, AB+ material appeared to be deposited later, and less intense and more diffuse than in the resistant line.

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EFFECT OF LIGHT, TEMPERATURE, AND METHOD OF CULTURE PROPAGATION ON CULTURAL VARIABILITY IN *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2. R. T. Awuah and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Production of mycelial cultures of *Fusarium oxysporum* f. sp. *apii* race 2 was inhibited, and pionnotal cultures favored, by incubation at 27-30 C with either a 12 or 24 hr photoperiod (fluorescent cool white light; 3,875 lux) and at 15 and 20 C with a 12 hr photoperiod (2,475 lux). Incubation in darkness or diffuse sunlight favored production of mycelial cultures. The light effect was localized in the actively growing portions of mycelial cultures. The longer a mycelial culture was in the dark prior to incubation with light, the greater the size of the mycelial zone and the smaller the size of the pionnotal zone in the final dual culture. Single spore transfer (SST) followed by dark incubation reduced cultural variability more than either SST with light incubation or mycelial transfer with dark incubation.

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USE OF DOT-BLOT HYBRIDIZATION TO DETECT *BOTRYTIS CINEREA* IN GRAPE BERRIES. M. F. Conde\* and J. J. Marois, Department of Plant Pathology, University of California, Davis, CA 95616 and \*The Upjohn Company, Kalamazoo, MI 49001.

Bunch rot of grapes, caused by latent infections of *B. cinerea*, is a severe disease affecting California grape production. Current control strategies include fungicidal sprays at bloom, even though the level of infection cannot be consistently determined through isolation of the pathogen. To better understand the epidemiology of the disease and development of subsequent disease control, it is necessary to develop a method to determine the presence of *B. cinerea* during grape berry development. Dot-blot hybridization, used to identify the presence of viruses in plant tissue, was used to detect *B. cinerea* gene sequences in infected grape berries. DNA isolated from *B. cinerea* was ligated into PBR322 and used to transform *E. coli* JA221 cells; insert sizes ranged from 0.6 to 1.85 kb. The specificity of these clones indicate they may be useful in the detection of the pathogen from berries infected only with *B. cinerea* or berries colonized by *B. cinerea* and other fungi.

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ABSENCE OF INTERACTION BETWEEN *BOTRYTIS SQUAMOSA* AND *B. CINEREA* IN BLIGHTING LEAVES OF ONION PLANTS. D. L. Rist and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Leaves of onion plants inoculated with *Botrytis squamosa* blighted rapidly, while those on uninoculated control plants remained comparatively healthy. By contrast, the percentage blighting of leaves (limited or nil) and the rate at which that percentage increased on onion plants inoculated with *B. cinerea* never differed significantly from that on control plants. The percentage blighting of leaves and the rate at

which that percentage increased on onion plants inoculated with both *B. squamosa* and *B. cinerea* were sometimes more and sometimes less than that percentage and rate on plants inoculated with *B. squamosa* only, but such differences were never statistically significant. Thus, no interaction between *B. squamosa* and *B. cinerea* in blighting onion leaves was detected within the limits of the study.

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HEAT-INDUCED INCREASE IN RECOVERY OF *PHYTOPHTHORA PARASITICA* COLONIES FROM CITRUS SOILS DURING WINTER. A. Lutz and J. Menge, Department of Plant Pathology, University of California, Riverside, CA 92521.

Until now, recovery of *P. parasitica* from citrus groves during winter has been very difficult. To stimulate recovery of *P. parasitica*, rhizosphere soils taken from citrus groves during winter months were exposed to temperature treatments. Soil samples were pre-incubated at 8, 24 or 34 C for 48 h. Soil dilutions from each pre-incubated sample were then plated on a selective medium, post-incubated at 20, 24, 28, 31, and 34 C for 72 h, and the number of *P. parasitica* colonies counted. *P. parasitica* recovery was significantly higher when soils received combination heat treatments when compared to control field soils (8°C) incubated at room temperature. Between 3-4 times as many colonies grew when either heat treatment was 28 C or higher when compared to the control, except when both incubation temperatures were 34 C. These data suggest that recovery of *P. parasitica* colonies from soils collected in winter is enhanced by heat treatments, and that no threshold temperature exists.

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VARIATION IN '*PHYTOPHTHORA PALMIVORA*' MF4 (*P. CAPSICI*) ISOLATES FROM BLACK PEPPER IN INDIA, INDONESIA, AND MALAYSIA. Peter H. Tsao<sup>1</sup>, Y. R. Sarma<sup>2</sup>, R. Kasim<sup>3</sup>, I. Mustika<sup>3</sup>, and T. K. Kueh<sup>4</sup>. <sup>1</sup>Dept. Plant Pathol., Univ. California, Riverside, CA 92521; <sup>2</sup>C.P.C.R.I., Calicut, India; <sup>3</sup>Res. Sta. Industrial Crops, Natar and Bangka, Indonesia; <sup>4</sup>Agr. Res. Centre, Kuching, Malaysia.

Ten *Phytophthora* isolates from black pepper (*Piper nigrum*) from India, five from Indonesia, and six from Malaysia were all morphologically similar to one another and similar to many black pepper isolates from other parts of the world. All 21 isolates were identifiable as '*P. palmivora*' MF4. Based on the revision of *P. capsici* proposed by Alizadeh and Tsao, all can be identified as *P. capsici*. Considerable variation existed among these isolates in the ability to form sporangia on agar media in the light, to form chlamydospores, to grow at 35 C, and in the morphology of sporangia, sex organs, and colony growth. All produced caducous sporangia with long pedicels; average pedicel lengths ranged 36-126  $\mu$ m and sporangium L/B ratios ranged 1.4 - 2.8 among the isolates. All were self-sterile (heterothallic), the majority being the A<sup>1</sup> mating type.

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Factors Influencing Oospore Germination in *Phytophthora megasperma* f. sp. *glycinea*. S. E. Schechter and L. E. Gray, Dept. of Plant Pathology and USDA-ARS, University of Illinois.

*Phytophthora megasperma* f. sp. *glycinea* race 1 oospores from 4-week-old cultures grown on lima bean agar at 23 C in the dark were plated on water agar. Percent germination was determined at various intervals for two weeks to study the influence of temperature, light, storage temperature, and plating density. Germination was most rapid at 27 C, reaching 80 percent within 14 days but at 18 and 31 C germination was rare. The rate of germination in light was higher than in dark with germination at 14 days of 86 and 95 percent respectively. Oospores were stored at -10, 4 and 23 C for 24 hr prior to plating. Exposure to -10 C reduced total germination to 20 percent. Oospores stored at 4 C germinated at a lower rate than those stored at 10 C but within 14 days both treatments reached 80 percent germination. Oospore density had no effect on germination.

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VIRULENCE OF AN ENDEMIC ISOLATE OF *PERONOSPORA TABACINA* FROM TEXAS TO *NICOTIANA TABACUM* AND *N. REPANDA*. M. Reuveni, W. C. Nesmith and M. R. Siegel, Plant Pathology Department, University of Kentucky, Lexington, KY 40546.

Sporangia of *P. tabacina* obtained from infected, wild, *N. repanda*, plants near Uvalde, Texas were increased on *N. repanda*. Inoculation of *N. tabacum* (Ky 14) and *N. repanda* plants (7 wk



old, 4-5 leaves/plant) resulted in high disease ratings on both hosts. The results were consistent for inoculum collected through fifteen successive passages on both hosts. These findings differ from those obtained in similar previous experiments with Ky 79 isolate. To resolve this issue, the virulence of Texas and Ky isolates was compared directly on leaf disks of Ky 14 plants of different ages under controlled environmental conditions. Here the Texas isolate was more virulent to Ky 14 and this was not affected by changing the temperature during the infection period.

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**TARO ROOT AND CORM ROT CAUSED BY *PHYTHIUM MYRIOTYLIUM*.** J.J. Ooka and J.Y. Uehida. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Root and corm rots are serious recurring diseases of taro (*Colocasia esculenta* L.) in paddy culture in Hawaii. An association of *Pythium myriotyllum* Drechs. was seen with these diseases, and this fungus was readily isolated from root and corm rots. Pathogenicity of *P. myriotyllum* was established in greenhouse inoculation studies, fulfilling requirements of Koch's postulates for the first time with this disease. Following artificial inoculations, root and corm rots were equally severe on pathogen-free cultivars, Lehua Maoli and Ulaula Kumu. *In vitro* fungicide tests demonstrated that *P. myriotyllum* was inhibited by metalaxyl and ethazol, moderately sensitive to fosetyl-Al, and virtually unaffected by propanocarb. Metalaxyl and ethazol were effective at 8 mg a.i./100-cm<sup>2</sup> pot in greenhouse root-rot control studies.

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**USE OF EXCISED COTYLEDONS IN VITRO TO EVALUATE RESISTANCE TO COLLETOTRICHUM TRIFOLIUM IN ALFALFA.** J. Cucuzza and J. Kao, PLANT GENETICS, INC., 1930 Fifth Street, Davis, CA 95616

Resistance in alfalfa to *Colletotrichum trifolii* has been detected in excised cotyledons *in vitro*. Cotyledons, placed in prepared petri plates, were spray inoculated with conidia (5,000 spores/spray) and incubated at 24°C with a 16 hour light period for 14 days. In five experiments, 61.3 ± 8.5% of the cotyledons of our standard resistant cultivar (Saranac AR) exhibited either a hypersensitive response or remained healthy (resistant response). In our standard susceptible cultivar (Saranac), 98.7 ± 1.2% of the cotyledons were infected and the fungus sporulated (susceptible response). Excised cotyledons and the remaining seedlings were tested *in vitro* and in a greenhouse screen simultaneously. Ratings of Saranac AR and Saranac agreed in 86.5% and 96.2% of the observations, respectively, between the two screening techniques. Six cultivars were tested blindly and the percent resistance obtained using the *in vitro* screen agreed with published values. These results indicate the usefulness of this technique as an alternative resistance screening method.

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**USE OF RELATIVE REDUCTION IN SEED WEIGHT OF INFECTED OATS TO DEMONSTRATE RESIDUAL EFFECTS OF "DEFEATED" GENES FOR SEEDLING RESISTANCE TO *PUCCINIA CORONATA*.** M. D. Simons and K. J. Frey. ARS, U.S.D.A., Dept. of Plant Pathology; and Dept. of Agronomy, respectively, ISU, Ames, IA 50011.

Nine isolates of *P. coronata* that parasitized seedlings of one or more of 9 isolines of oats were used to inoculate field microplots of these lines. Seed weight of each isoline was expressed as a resistance index calculated by dividing values from rusted plots by corresponding values from control plots. In 1983, 9 line-isolate combinations had seed weight indexes from 0.53 to 0.68; 6 were higher ( $P = 0.05$ ) than the 0.55 of Lang, the recurrent parent of the isolines. In 1984, 24 combinations ranged in seed weight index from 0.60 to 0.86 and 17 were higher ( $P = 0.05$ ) than the 0.62 index for Lang. The data suggest that mutations from avirulence to virulence are commonly to incomplete virulence. The host resistance gene, then, retains residual effects that confer measurable protection.

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**USE OF PLANT SENESCENCE AS AN INTRA-CULTIVAR SELECTION CRITERION FOR SOYBEAN (GLYCINE MAX) RESISTANCE TO *MACROPHOMINA PHASEOLINA*.** C. H. Canaday, D. G. Helsel, and T. D. Wyllie, Depts. of Agronomy and Plant Pathology, Univ. of Missouri, Columbia, MO 65211.

Plant senescence was evaluated in field studies of four soybean

cultivars as a potential intra-cultivar selection criterion for general resistance or tolerance to *M. phaseolina*. Soybeans were rated by a team of observers for differences in senescence (SEN) during development stages R7 to R8 on a 0 to 4 scale, where 0=very premature SEN, 1=premature SEN, 2=average SEN, 3=delayed SEN, and 4=very delayed SEN. SEN was generally true-breeding, strongly related to the percentage of taproot discolored with microsclerotia of *M. phaseolina* at harvest maturity (%MS), and moderately related to yield (YLD). Correlation coefficients for SEN vs %MS were -0.99, -0.94, -0.65, and -0.99 for cv. Union, Williams 79, Cumberland, and Elf, respectively. Correlation coefficients for SEN vs YLD were 0.54, 0.77, 0.65, and 0.23, respectively. SEN appears to be a promising intra-cultivar selection criterion for soybean resistance to *M. phaseolina*.

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**DEVELOPMENT OF WHEAT LINES FOR RESISTANCE TO COMMON ROOT ROT.** R. D. TINLIE AND H. HARRING, AGRICULTURE CANADA, RESEARCH STATION, 107 SCIENCE CRESCENT, SASKATOON, SASKATCHEWAN, CANADA S7N 0X2.

From numerous wheat lines and cultivars screened for common root rot reaction on moderately resistant ones of diverse origin were selected. They were intercrossed in almost all paired combinations. Recurrent selection from the least-diseased single plant rows was practiced from the F3-F6 generations. The frequency of "resistant" lines increased from repeated selection; for example the percentage of lines from 6 crosses that appeared less diseased than the parents was 21, 30, 37 and 68 in the F3 to F6 generations. Advanced "resistant" lines are assessed for agronomic attributes to evaluate their potential usefulness as parents.

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**RELATIONSHIP BETWEEN PYRUS WOOD STARCH AND CANKER FORMATION BY *ERWINIA AMYLOVORA*.** A. L. Shigo, USDA Forest Service, Durham, NH 03824 and T. van der Zwet, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Twenty-five central leader stems (5x85cm) of pear (*Pyrus communis*) with cankers caused by *Erwinia amylovora* were collected in June and were cross sectioned to 30 cm below the lower limits of the cankers. Forty healthy stem sections (1x15cm) were collected from known resistant and susceptible trees in October. All transverse surfaces were stained with potassium iodide to detect starch. From the June collection, 18 stems had no starch 30 cm below the cankers, 3 stems had small amounts of starch 12-20 cm below the cankers, and 4 stems had moderate amounts of starch 3-5 cm below cankers that had well defined margins. There was no starch in the current growth ring. All October sections had moderate amounts of starch, indicating that resistant and susceptible trees store equal amounts of starch. The ability of trees to utilize starch reserves effectively after infection may be an important factor in fire blight resistance.

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**VIRULENCE OF *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* IN THE PHILIPPINES.** T.W. New & C.M. Vera Cruz. The International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Virulence of the bacterial blight pathogen was determined from 1972 to 1984 on rices carrying different resistance genes. A total of 490 isolates were tested. Inoculation was done on 40-day-old plants and scored 14 days later. A shift in virulence of the bacterial population was noted. Isolates virulent to Xa-10 gene and other popular native cultivars decreased in frequency since the mid-70's. On the contrary, isolates virulent to the Xa-4 gene, which conveys the resistance of widely planted rice cultivars such as IR36 and IR42, gradually increased in frequency and have dominated the bacterial population. Isolates with different or combined virulence were also detected but at a low frequency. The isolates from native cultivars appear to have a wide virulence in contrast to those from improved cultivars to which the virulence is relatively narrow.

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**IN VITRO SELECTION OF PHASEOLOTOXIN RESISTANT PLANTS USING MERISTEM CULTURE OF BEAN (*Phaseolus vulgaris*).** B.V. Gantotti, K.K. Kartha and S.S. Patil\*. Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan, S7N 0W9, Canada. \*Dept. of Plant Pathology, Univ. of Hawaii, Honolulu, HI 96822.

Phaseolotoxin is produced by the bacterium *Pseudomonas syringae* pv. *phaseolicola*, the cause of haloblight disease in beans. Since resistance to the pathogen does not confer resistance to the

toxin, field resistance is often affected by the environment. Selection for toxin resistance was carried out, *in vitro*, by culturing isolated bean apical meristems (ca. 0.5 mm) after treating with the toxin and inducing multiple shoot formation. The shoots produced by the surviving meristems further elongated on MS medium containing 1  $\mu$ M BA, 0.1  $\mu$ M NAA and 0.1  $\mu$ M GA<sub>3</sub>. After rooting on half-strength MS medium containing 1  $\mu$ M NAA the shoots gave rise to individual plants which were grown to maturity in pots. When tested by leaf bioassay for toxin resistance, about 20% of the regenerated plants exhibited varying degrees of resistance to the toxin. Further experiments to determine their resistance to the pathogen and the genetic stability of disease resistance are in progress.

### 310

**INHERITANCE OF RESISTANCE TO PUCCINIA RECONDITA IN PACIFIC NORTHWEST WHEATS.** M. E. Bjarko and R. F. Line, Dept. of Plant Pathology, Wash. State Univ. and ARS, USDA, Pullman, WA 99164.

Wampum and Borah, which have slow rusting resistance and a range of infection types, and Wared, which has high resistance (a low infection type), were crossed with each other and with a susceptible cultivar Twin. Parental, F<sub>1</sub>, F<sub>2</sub> and backcross plants were grown in the field. Leaf rust intensity was recorded when first observed and 7, 14, 21, 28 and 40 days after the initial recording, and infection type (IT) data were recorded 40 days after the initial intensity was recorded. The intensity data were transformed to area under the disease progress curve (AUDPC). Resistance was recessive in Borah and partially recessive in Wared for both IT and AUDPC. However, resistance in Wampum was recessive for IT and partially recessive for AUDPC. Based on crosses between the resistant parents, Borah, Wampum and Wared have different genes for resistance. In resistant x susceptible crosses, IT and AUDPC were positively correlated in F<sub>2</sub> and backcross generations.

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**RELATIONSHIP OF RUST INTENSITY, INFECTION TYPE, LATENT PERIOD, AND LESION LENGTH TO INHERITANCE OF A DURABLE TYPE OF STRIPE RUST RESISTANCE.** E. A. Milus and R. F. Line, Dept. of Plant Path., Wash. State Univ., and ARS, USDA, Pullman, WA 99164.

Gaines, Nugaines and Luke wheats, which have high-temperature, adult-plant resistance to *Puccinia striiformis*, were crossed to each other and to a susceptible line. In the field, rust intensity (RI) and infection type (IT) were positively correlated for both F<sub>2</sub> plants and F<sub>4</sub> rows. Gene action and number of genes controlling RI and IT were similar. At a 10-30 C gradually changing diurnal temperature cycle, IT on flag leaves of F<sub>2</sub> plants was negatively correlated with latent period (LP) and positively correlated with lesion length (LL); LP and LL were negatively correlated. Crosses involving Luke, the most resistant cultivar, had the highest correlations. The Nugaines x Gaines cross had the lowest correlations, because there was little segregation. Genes controlling this type of resistance have pleiotropic effects on RI, IT, LP and LL. Selection for one component should enhance resistance as measured by the others.

### 312

**SLOW RUSTING IN ASPARAGUS INFECTED WITH PUCCINIA ASPARAGI.** Dennis A. Johnson, Irrigated Agriculture Research and Extension Center, Box 30, Prosser, WA 99350

Rust development in the field was significantly slower on some asparagus cultivars than on others as measured by the area under the disease progress curve (AUDPC). When inoculated uniformly with urediospores of *Puccinia asparagi* in the greenhouse, the cultivars that rusted slowly in the field, collectively, had significantly longer latent periods and fewer uredia/cm<sup>2</sup> shoot than the more susceptible cultivars. However, latent period and uredia/cm<sup>2</sup> shoot of individual slow rusting cultivars did not differ significantly from those of some fast rusting cultivars. The length of latent period decreased and the number of uredia/cm<sup>2</sup> shoot increased in greenhouse tests as AUDPC in the field increased. The AUDPC was 29, 84, 184, 319, 429, 1112, 1480, and 1699 for 277E x 22-8, Jersey Centennial, Delmonte 361, 56 x 22-8, UC 157, Mary Washington, Wash T<sub>2</sub> and WSU 1, respectively. As asparagus shoots increased in age, both slow and fast rusting cultivars became more resistant to rust, as indicated by longer latent periods and fewer uredia/cm<sup>2</sup> shoot.

### 313

**NEW PATHOGENIC RECOMBINANTS THROUGH SELFING AND CROSSING OF COMMON STEM RUST RACES.** J. D. Miller and N. D. Williams,

USDA-ARS, North Dakota State Univ., Fargo, ND 58105.

Two new avirulence/virulence combinations were identified in progenies from random selfing and crossing of pycnia on barberry plants which had been inoculated with basidiospores from field teliospores produced on wheats in a nursery inoculated with stem rust races 15TLM, 15TMM, and 15IQSH. One recombinant produced a combination of high(H) and low(L) infection types (ITs) similar to those of TMN on single Sr gene lines, 'Len' and 'Coteau', but differed by showing virulence (HIT) on previously resistant 'Waldron', 'Olaf' and 'Alex'. The intermediate LIT on Len and LIT on Coteau showed that they have adequate genes conditioning resistance (R), and that Coteau may have an additional gene(s) for R. The second recombinant showed a different combination of genes for virulence and was coded LSK. It differed from the first recombinant by producing LITs on Sr7b, 9d, 9e and Tt-1, HITs on Sr6, 9b, and 13, and LITs on the above 5 cultivars. It differed from QSH by showing a LIT on Sr9d and HIT on Sr13.

### 314

**INHERITANCE OF ADULT PLANT RESISTANCE TO LEAF RUST IN ERA WHEAT.** B. Ezzahiri and A. P. Roelfs, Dept. de Phytopathologie, I.A.V. Hassan II, B.P. 6202, Rabat (Morocco) and Cereal Rust Lab., USDA, ARS, Univ. of Minn., St. Paul, MN 55108

A population of 600 lines representing 17 F<sub>2</sub> families of a cross between the susceptible cultivar Baart and the resistant cultivar Era was evaluated for adult plant resistance to leaf rust in both Morocco and the United States. Resistance was controlled by two complementary genes. In a cross between Era and one of its parents, Frontana (with adult plant resistance gene Lr13), no segregation was observed for adult plant resistance. Seedling tests showed that Era possesses the gene Lr10 plus an additional gene for seedling resistance different from the seedling resistance gene(s) in Frontana. The genes conferring adult plant resistance in Era were different from the seedling genes postulated to be present.

### 315

**ANTHRACNOSE DISEASE DEVELOPMENT, SEED INFECTION AND RESISTANCE IN SOYBEANS.** Manandhar, J. B., Hartman, G. L., and Sinclair, J. B. Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

Over 200 soybean cultivars in maturity groups 000 to X were evaluated for anthracnose (*Colletotrichum truncatum*) disease. Seedlings were inoculated using an atomized conidial suspension (3-5 x 10<sup>6</sup> conidia/ml) and incubated for 72 hr in a mist chamber programmed for 15-min misting/hr and 12-hr intermittent light. Symptoms developed in 52 to 60 hr. Disease severity increased with increased incubation. Plants were susceptible at all growth stages to the disease which resulted in death at V-1 stage. Defoliation occurred at all growth stages. Tarheel Black (VII), PI 95.860 (VI) and a few other cultivars were found to be resistant. Inoculated field soybeans, group 000 to IV at V-2, R-1 and R-6 stages using a hand-compression sprayer, resulted in high seed infection in the 000 cultivars Hidasta (49%), Pando (37%) and Sioux (30%), with few to no seed infection in other cultivars.

### 316

**VARIATION IN ANTHRACNOSE REACTION WITHIN NATIVE STYLOSANTHES CAPITATA POPULATIONS IN BRAZIL.** Jillian M. Lenné, Tropical Pastures Program, CIAT, A.A. 6713, Cali, Colombia.

*Stylosanthes capitata* is a promising perennial tropical pasture legume susceptible to anthracnose (*Colletotrichum gloeosporioides*) in several regions of tropical America. Two native *S. capitata* populations, approximately 50 km apart, were sampled in northern Minas Gerais, Brazil. Seed was collected from ten plants in each of two 1 m<sup>2</sup> quadrats at each site. Seed was pre-germinated and sown in trays in the glasshouse and resulting progenies were inoculated with one pathogenic isolate of *C. gloeosporioides*. Results clearly showed considerable variability in anthracnose reaction among progenies from single plants; among plants from the same quadrat and between quadrats at the same site, as well as between sites. Results suggest that diversity in anthracnose resistance within stable native *S. capitata* populations contributes to their persistence and imply the importance of maintaining diversity in improved *S. capitata* pastures.

### 317

**POTENTIAL OF MIXTURES OF STYLOSANTHES GUIANENSIS FOR CONTROLL-**

JNG ANTHRACNOSE. Jillian M. Lenné. Tropical Pastures Program, CIAT. A.A. 6713, Cali, Colombia.

Mixtures of six accessions of the perennial tropical pasture legume Stylosanthes guianensis CIAT 136, 1875, 1927, 1949, 2031 and 10136, ranging from susceptible to resistant to anthracnose (Colletotrichum gloeosporioides), were compared with their respective pure stands at the ICA-CIAT Research Station, Carimagua, Colombia during 1982 to 1983. Anthracnose severity was rated monthly; live plant yield and survival were evaluated bi-monthly. After six months, susceptible accessions CIAT 136, 1875 and 1927 in mixtures showed lower anthracnose levels, 88.5, 40.1 and 82.2% improvement in survival, respectively, and 66.5, 17.0 and 30.3% improvement in yield, respectively, over their pure stands. After one yr, CIAT 1927 continued with less anthracnose and showed 13.8% improvement in survival and 60.5% improvement in yield in mixtures vs. pure stand. Results indicate that mixtures of accessions of S. guianensis have some potential for controlling anthracnose.

### 318

DIFFERENCES IN FITNESS OF STRAINS OF COCHLIOBOLUS HETEROSTROPHUS NEAR-ISOGENIC FOR TOXIN PRODUCTION. C. J. R. Klittrich and C. R. Bronson, Dept. of Plant Pathology, Seed and Weed Sciences, Iowa State University, Ames, IA 50011.

Changes in the relative frequency of Cochliobolus heterostrophus (Helminthosporium maydis) strains were measured in the field to determine the effect of an "unnecessary" virulence gene on reproductive fitness. A Race T (toxin-producing) strain was backcrossed 5 times (1983) and 9 times (1984) to a closely-related Race O (non-toxin-producing) strain. Each year spores of siblings (one Race O and one Race T) from the last cross were mixed and used to inoculate 0.3 ha of N-cytoplasm corn. Over 900 (1983) and 1500 (1984) single-conidial isolates (one per lesion) were collected over the growing season. The percentage of the Race T strain decreased significantly both years, from 47% to 20% in 1983 (9 weeks) and from 24% to 15% in 1984 (6 weeks). These results support the hypothesis that the decline in field populations of Race T after 1970 may have been partly due to the presence of the gene for toxin production or a closely-linked gene.

### 319

EFFECT OF ACIDIC PRECIPITATION ON GERMINATION OF ALTERNARIA SOLANI AND ITS INFECTION EFFICIENCY ON POTATO. van Bruggen, A.H.C., Osmełowski, J., Heller, L., and Jacobson, J. Boyce Thompson Inst., Cornell University, Ithaca, NY 14853.

As part of a research project on the influence of acidic precipitation on plant disease management we studied the effect of acidity of conidial suspensions and simulated mist on germination of Alternaria solani and its infection efficiency on potato. Acidic solutions (pH 2.8-4.6) contained sulfuric and nitric acids (2:1 by weight) and background ions, mimicking ambient rain. In vitro germination of conidia increased curvilinearly with pH. During incubation the acidity of the conidial suspensions decreased, the decrease being linear with percent conidia germinated ( $r = -0.93$ ). Rooted potato cuttings, cv Norchip and Monona, were inoculated with conidial suspensions, and placed on turntables 3 m below pneumatic nozzles producing acidic mist (3.3 mm/hr) for 24 hr, and then returned to a greenhouse bench. The infection efficiency increased curvilinearly (from 0.04 to 1.58%) with pH of the mist. There were no significant cultivar differences. Foliar injury by acidity was only observed at pH 2.8.

### 320

TEMPERATURE RESPONSE MODELS OF SPORE GERMINATION AND SPORULATION FOR Ramularia tulasnei. V.J. Elliott. Dept of Plant Pathology, University of California, Berkeley, CA 94720

Spore germination of Ramularia tulasnei Sacc. on water agar was examined over a range of temperatures (5-38C). A time course analysis revealed that spore germination followed a logistic model. The rate of germination increased through a temperature range of 5 to 20C and then dropped to zero near 35C. Maximum percentages of germination dropped sharply above 30C. Sporulation was studied on detached leaves of the strawberry cultivar 'Chandler' and on Czapek Dox agar over a range of temperatures (5-35C). Spores were collected at periodic intervals and counted under a microscope. Several regression models were fitted to the data and Schrodter's sinus function most closely approximated the response

curves. Cardinal temperatures of 8, 18, and 30C are indicated for sporulation on both detached leaves and artificial media. The optimum temperature on artificial media showed a trend to shift from about 24C to 18C as the colony reached six days of age and older.

### 321

DISEASE PROGRESS OF PHYTOPHTHORA BLIGHT OF PEPPER. J.H. Bowers, D.J. Mitchell, and R.M. Sonoda. Plant Pathology Dept., Univ. of Florida, Gainesville, FL 32611, and ARC, Ft. Pierce, FL 33454.

Field plots were established in Delray Beach, FL in the spring and fall of 1984 to quantitate disease progress in the Phytophthora capsici-pepper pathosystem. Disease incidence (measured by incipient wilt and the presence of characteristic lesions) was assessed and the location of diseased plants mapped weekly. From point sources of inoculum (diseased plants), disease progressed along the row prior to bed-to-bed movement. The maximum levels of disease were 99.5% after 71 days in the spring and 93.8% after 126 days in the fall (average of eight subplots). Average mortality rates (determined by the use of the logit transformation which had higher  $R^2$  values than the Gompertz and monomolecular transformations) were 0.15 and 0.04/ unit/day for the spring and fall, respectively. The rates of disease progress and spatial spread of the pathogen were influenced by rainfall and the movement of water which accounted for the different mortality rates for the two tests.

### 322

THE RELATIONSHIP BETWEEN INOCULUM DENSITY OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE AND OBSERVED ROOT INFECTIONS OF NICOTIANA TABACUM. J.T. English and D.J. Mitchell, Plant Pathology Dept., Univ. Florida, Gainesville, FL 32611.

The average number of root infections per tobacco seedling (RIS) was determined by direct observation of sites of secondary inoculum production of P. parasitica var. nicotianae. Both the proportion of diseased tobacco seedlings and RIS increased in proportion to initial chlamydo-spore density after 2 weeks of seedling growth in autoclaved soil. Although the proportion of diseased seedlings approached an asymptote of about 0.96 at higher inoculum densities, the RIS continued to increase with increasing inoculum density. Both first-order linear and exponential functions described the relationship between RIS and inoculum density. Estimated numbers of infections per seedling, based on Gregory's multiple infection transformation [ $\ln(1/(1-Y))$ ], correlated well with numbers of RIS for all but the two highest inoculum densities examined. At the uppermost densities, this transformation underestimated RIS.

### 323

THE EFFECT OF PEANUT MOTTLE VIRUS INFECTION ON PHYTOMASS PRODUCTION IN SIX PEANUT GENOTYPES. H. A. Melouk and T. W. Popham. USDA-ARS, P.O. Box 1029, Plant Pathology Dept., Okla. State Univ., Stillwater, OK 74078

Six cultivated peanut entries (cv. Pronto, PI468202, PI468253, PI468315, BP2691 and a hybrid (F<sub>4</sub>) of Chico X PI475871) were planted at Perkins, OK in 3 m single row plots spaced at 0.9 m in a completely randomized design with four replications. Naturally occurring infections by peanut mottle virus (PMV) were observed on all entries about 45 days after planting (DAP). The presence of PMV was confirmed by a local lesion assay on Topcrop beans (Plant Disease 67:819-821, 1983). Ocular estimates (nearest 5%) of diseased area (DA) on plants were determined at 102 DAP. Plants were dug at 150 DAP. Pod number (PN), pod weight (PW) and top dry weight (TW) per plant were determined. Pooled over genotypes, negative and significant correlations were obtained between DA and PN, DA and PW, and DA and TW.

### 324

DISTRIBUTION AND HOSTS OF MAIZE DWARF MOSAIC VIRUS STRAIN A AND MAIZE CHLOROTIC DWARF VIRUS IN SOUTH CAROLINA. Graydon Kingsland and O. W. Barnett, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, S.C. 29631.

Maize dwarf mosaic virus strain A (MDMV) and maize chlorotic dwarf virus (MCDV) were identified by enzyme-linked immunosorbent assay (ELISA) from corn (Zea mays) and sorghum (Sorghum vulgare) in 4 counties with no previous records of these viruses. MDMV was identified from millet (Pennisetum glaucum), fall panicum (Panicum dichotomiflorum), and signal

grass (*Brachiaria platyphylla*) for the first time in SC. MCDV was identified from *P. dichotomiflorum*, *B. platyphylla*, crab grass (*Digitaria sanguinalis*) and *Leptochloa filiformis*. Acute anthocyanescence and chlorosis were associated with infection of *B. platyphylla* by MCDV; all plants (81) surveyed in nine 81-sq.m. plots in one field were characterized by this symptom.

**325**  
GEOGRAPHIC DISTRIBUTION AND AGGRESSIVENESS OF SEPTORIA TRITICI ON WHEAT IN THE UNITED STATES. D. Marshall, Texas A&M University Research and Extension Center, Texas Agricultural Experiment Station, 17360 Coit Road, Dallas, Texas 75252.

Cultivars of spring and winter wheat, respectively, were grown at three locations in California and 13 locations in nine other states in 1983 and 1984. Disease severity of septoria tritici blotch was assessed by area determination of disease progress curves (AUDPC). Greenhouse inoculation studies were made with *S. tritici* isolates collected from all locations during both years. Populations of *S. tritici* were deemed aggressive if they produced large AUDPC values in the field and extensive necrosis with abundant pycnidia in greenhouse studies. Populations of *S. tritici* determined to be most aggressive were found in central and north central California as well as northern regions of Indiana and Ohio. Those populations collected from northern Alabama, Mississippi, eastern Arkansas, southern Nebraska and central Kansas were least aggressive. Greater variability in aggressiveness was noted with the occurrence highly aggressive populations of *S. tritici*.

**326**  
TESTING A COMPUTERIZED FORECASTING SYSTEM FOR RICE BLAST DISEASE. Choong-Hoe Kim, D. R. MacKenzie and M. C. Rush, Dept. Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803.

Two watertight on-site microcomputer units, operating on alkaline batteries, collected temperature, leaf-wetness and relative humidity data from upland and flooded rice plots. Each microcomputer interpreted the microclimate information in relation to blast (*Pyricularia oryzae* Cav.) development and displayed daily blast unit of severity (BUS) values. BUS values were highly correlated with blast development on the two susceptible cultivars, Brazos and M-201, grown under upland conditions. The correlation was less with Brazos as field resistance became apparent about mid-season. M-201 was equally susceptible throughout the growing season. The BUS values obtained from flooded plots were not significantly different from those from the upland plot. A correlation was not observed between BUS values and blast development under flooded conditions as both varieties were resistant to leaf blast when flooded.

**327**  
SEASONAL FLUCTUATION OF FOLIAR BACTERIOSIS AND ANTHRACNOSE SYMPTOMS ON MEXICAN LIME IN COLIMA, MEXICO. J. J. STAPLETON, USDA, ARS, and T. Perez-Serrato, SARH, DGSV, Apartado 121, Tecoman, Colima, Mexico.

Bacteriosis, a presumed form of citrus canker (*Xanthomonas campestris* pv. *citri*), and anthracnose (*Colletotrichum linnetticolum*) are the principal foliar diseases of Mexican lime (*Citrus aurantifolia*) trees in Colima, Mexico. Incidence of bacteriosis pustules increased mainly during the dry, cool season (Nov.-May), remained high until the Oct. flush, and declined precipitously after the early Oct. (rainy season) flush. Occurrence of anthracnose symptoms peaked immediately after Oct. flush, and were at the lowest levels during the dry season. The % leaves infected, no. lesions/leaf, and no. lesions/infected leaf with bacteriosis range from 5.1-98.8, 0.1-3.8, and 1.0-4.5, respectively. The same parameters for anthracnose range from 2.6-75.7, 0.1-3.8, and 1.0-4.5, respectively. Foliar symptoms of the two diseases did not occur in high levels simultaneously.

**328**  
A PREDICTIVE SYSTEM FOR TIMING CHEMICAL APPLICATIONS FOR CONTROL OF BACTERIAL SPECK OF TOMATO CAUSED BY PSEUDOMONAS SYRINGAE PV. TOMATO. D.J. Jardine and C.T. Stephens, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312

Recommended sprays with fixed coppers at 7-day intervals have

provided inconsistent control of bacterial speck of tomatoes in Michigan. If chemical application could be timed to coincide with build-up of bacterial populations to the threshold necessary for symptom development, more consistent control might be achieved. A regression model relating temperature, rainfall, and previous population level to leaf bacterial populations was developed from 2 yr pooled data and validated. The model accounted for 85% of the observed variation in the populations. In 1984, plots sprayed using model predictions versus a calendar schedule received three less sprays with no significant difference in amount of infection. A more general, revised regression model was developed by pooling 3 yr of data. This equation accounted for 64% of the variation in the population.

**329**  
THE SOURCE AND SURVIVAL OF PRIMARY INOCULUM PRODUCED BY MARSSONINA PANATTONIANA, THE CAUSAL AGENT OF LETTUCE ANTHRACNOSE. C. L. Patterson and R. G. Grogan, Department of Plant Pathology, University of California, Davis, CA 95616

The primary inoculum of lettuce anthracnose caused by *Marssonina panattoniana* is soil-borne. Lettuce grown in soil collected from fields with a previous history of the disease developed anthracnose lesions after subjected to natural or simulated rainfall. Control plants grown in non-infested soil remained healthy. Lettuce planted in potted soil containing plants infected by *M. panattoniana* during the previous spring developed anthracnose symptoms after about 6.0-cm of rainfall. Microscopic examination of old infected lettuce lesions revealed that the fungus produces loosely packed multicellular structures, presumably microsclerotia. Non-infected lettuce tissue did not contain similar structures. Anthracnose lesions developed on lettuce plants in a mist chamber 7-10 days after inoculation with a suspension of the microsclerotia. Thus, the soil-borne microsclerotia, that persist over summer, are likely splashed onto lettuce leaves by rain or sprinkler irrigation and infect directly.

**330**  
COMPARISON OF DIAGONAL, W, AND STRATIFIED RANDOM SAMPLING PATTERNS USING SIMULATION. B. R. Delp, L. J. Stowell, and J. J. Marois, Department of Plant Pathology, University of California, Davis, CA 95616.

Fields with 0.01 to 10% disease incidence were simulated at five disease distributions, from random to highly aggregated. Fields were sampled with three sampling patterns (diagonal, W, and stratified random) at three sample sizes (20, 30, and 40 plants/sample site) and seven sample intensities from 0.05 to 4.4%. Percent error of the incidence estimates and standard deviation of percent error were lowest with the stratified random if intensity was  $\geq 0.2\%$  and highest with the diagonal if intensity was  $\geq 0.4\%$ . Error for all patterns decreased as intensity increased from 0.05 to 0.2%. Above this intensity, error for the diagonal and W patterns achieved a minimum plateau; however, error for the stratified random continued to decrease as intensity increased if disease was aggregated. Sample size had no apparent effect unless disease was aggregated at the lowest incidence. Error was directly related to incidence and indirectly related to aggregation.

**331**  
VIRULENCE, AGGRESSIVENESS, AND FITNESS OF PHYTOPHTHORA INFESTANS ISOLATES FROM SEXUAL AND ASEQUAL POPULATIONS. P. W. Tooley, J. A. Sweigard, and W. E. Fry, Dept. of Plant Pathology, Cornell University, Ithaca, NY, 14853.

Sixteen *Phytophthora infestans* isolates of A1 compatibility type collected from Mexico (sexual population) were compared with 16 isolates from the U.S. and Europe (asexual population). Virulence was assessed as the ability to produce sporulating lesions on detached leaflets of 9 differential (R-) potato genotypes. In growth chamber experiments with potato cultivar Norchip, aggressiveness was assessed as area (cm<sup>2</sup>) of blighted tissue per inoculated leaf at five days postinoculation; fitness was measured in terms of the exponential rate of increase of sporangial numbers from 0 to 5 days postinoculation. Isolates from the sexual population were more virulent than those from the asexual population. However, there were no consistent differences in aggressiveness or fitness between isolates from the two populations.

**332**  
FUNGAL COMMUNITY DEVELOPMENT ON APPLE LEAVES. Linda Kinkel and

Apple leaves were surface sterilized in the field in early August with hydrogen peroxide. The development of 'new' epiphytic communities was followed for eight weeks using leaf wash, imprint, and incubation techniques to enumerate species and individuals on each leaf. An average of 8,000 individuals and 7.3 species were present per leaf 12 hours after peroxide treatment. Numbers of filamentous fungal species per leaf fluctuated from six to 21 during the first two weeks of colonization and then stabilized around 12. Numbers of individuals increased throughout the sampling period. A significant proportion of species isolated were found only once, while six of the species were found on every leaf sampled. After eight weeks, treated leaves were indistinguishable from untreated leaves in number of individuals, number of species, or species composition.

### 336

ISOLATION AND CHARACTERIZATION OF PATHOGENICITY SPECIFIC GENES IN *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. D. K. Willis, P. B. Lindgren, R. C. Peet, S. E. Lindow, and N. J. Panopoulos, Dept. of Plant Pathology, Univ. of Calif., Berkeley, CA 94720.

Two Tn5 generated non-pathogenic mutants of *P. s. syringae* strain B728a have been isolated (Willis and Panopoulos 1984 *Phytopathology* 74:798). Both mutants fail to elicit any disease symptoms on either the pods or leaves of bean cultivars (*Phaseolus vulgaris*) which are susceptible to the wild-type parental strain B728a. This is in marked contrast to the ability of both mutants (designated as NPS3136 and NPS3139) to grow to the same extent as the parental strain both epiphytically on leaves and intercellularly within leaf tissue of bean. Utilizing a plasmid (pKW3) containing the Tn5 and flanking chromosomal sequences from mutant NPS3136 as a probe, we have isolated a clone from a *P. s. phaseolicola* library containing an intact EcoRI fragment corresponding to the fragment affected by Tn5 in NPS3136. The ability of this clone to restore pathogenicity to NPS3136 is under investigation.

### 337

CHARACTERISTICS OF A NONBACTERIAL ICE NUCLEUS CONSTITUTIVELY ASSOCIATED WITH WOODY TISSUES OF DECIDUOUS FRUIT TREES AND ITS ROLE IN FROST INJURY. D. C. Gross and E. L. Proebsting, Jr., Washington State University, Pullman, WA 99164; \*Irrigated Agricultural Research and Extension Center, Prosser, WA 99350.

Two types of ice nuclei, expressed at temperatures of -2 C and lower, are commonly associated with deciduous fruit trees in the Pacific Northwest. One type is ice nucleation-active (INA) *Pseudomonas syringae* and the other is a wood-associated ice nucleus (WIN). In comparisons of orchards harboring either high (>10<sup>6</sup> cfu/g) or low flower populations (<10 cfu/g) of INA *P. syringae*, similar percentages of flowers were killed following frosts of varying intensities (-2 to -5 C). Analysis of the parameters affecting activity for both types of ice nuclei show that the WIN, in contrast to the bacterial ice nucleus, is inactivated when stems are saturated with water. Peach stem segments (5-cm-long) soaked for 8 hr in water, supercooled about 1.5 C lower than segments not soaked. The activity of the WIN was fully restored upon air-drying of the stems.

### 338

USE OF PLASMID DNAs TO DIFFERENTIATE PATHOVARS OF *XANTHOMONAS CAMPESTRIS*. G. R. Lazo and D. W. Gabriel, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

Restriction endonuclease digests of plasmid DNAs extracted from different pathovars of *Xanthomonas campestris* were compared by agarose gel electrophoresis. Over 60 different strains from pathovars *glycines*, *malvacearum*, *phaseoli*, *vesicatoria*, and *vignicola* were examined. Between different pathovars the digestion patterns differed significantly and only rarely were fragments of similar size observed. Within the same pathovar the digestion patterns differed, but in most cases there were one or more DNA fragments of apparently identical size. To determine if particular plasmid DNA bands were unique to a pathovar, selected bands were isolated by electroelution, radioactively labelled, and hybridized to plasmid DNAs of other strains. Plasmid fragments were identified that are apparently highly conserved within individual pathovars. Such fragments may be useful in developing rapid diagnostic tests.

### 339

FOUR PLASMID DNA VARIANTS DISTINGUISHED IN 1984 FLORIDA CITRUS CANCER EPIDEMIOLOGIC. D. W. Gabriel. Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

A total of thirty confirmed isolates of *Xanthomonas campestris* pv. *citri* (Xct) were recovered in Florida last year by the Florida Division of Plant Industry. We examined all of them for plasmid content and found that fifteen isolates contained no detectable plasmids, thirteen contained a plasmid of ca. 40.6 kilobases (kb) in size, one isolate contained a 66.9 kb plasmid and one contained a ca. 85.8 kb plasmid. Southern hybridization analysis using the 40.6 kb plasmid as a probe

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GENETIC TRANSFER FROM *Erwinia carotovora* subsp. *carotovora* TO *Escherichia coli*. L.J. Ward and R.J. Copeman. Plant Science Dept., University of B.C., Vancouver, B.C. V6T 2A2.

Mobilization vector R68.45 was introduced into *E. carotovora* subsp. *carotovora* (Ecc) from *E. coli* by standard plate matings. Crystal violet pectate-positive (CVP<sup>+</sup>), kanamycin-resistant (50 ug/ml) colonies were mated back to virgin *E. coli* strain HB101 and transconjugates selected on Luria broth + kanamycin (50 ug/ml) + streptomycin (25 ug/ml). Virtually complete transfer of R68.45 to *E. coli* was observed. CVP<sup>+</sup> *E. coli* transconjugates were obtained at a frequency of 10<sup>-5</sup>. Erythromycin-resistant transconjugates were obtained at a frequency of 10<sup>-3</sup>. Most were CVP<sup>+</sup> and produced only diffuse plaques against three of four indicator strains. However, 1.7% were CVP<sup>+</sup> and produced clear plaques against all four bacteriocin indicator strains. Matings (4 h) between Ecc and *E. coli* without R68.45 mediation were performed in stiff culture. Transconjugates selected on Luria broth + streptomycin (25 ug/ml) + erythromycin (30 ug/ml) produced diffuse plaques against three of the four bacteriocin indicators and were CVP<sup>+</sup>.

### 334

BACTERIOGIN RECEPTOR IN *Erwinia carotovora* subsp. *atroseptica*. D.S. Smith and R.J. Copeman. Plant Science Dept., #248-2357 Main Mall, University of B.C., Vancouver, B.C. V6T 2A2.

The kinetics of killing of *E. carotovora* subsp. *atroseptica* (Eca) by bacteriocins produced by *E. carotovora* subsp. *carotovora* (Ecc) strain 379 was determined by measuring cell lysis and viable cell numbers. Supernatants of mitomycin C-induced (0.2 ug/ml) cultures of strain 379, containing up to 1000 lethal units/ml (LU), killed sensitive cells in a negative logarithmic fashion over time. Bacteriocin was similarly adsorbed irreversibly to cells of sensitive strains representing three different Eca serogroups. The activity of purified particulate bacteriocin preparations was neutralized by whole cell wall preparations from sensitive cells and by the Triton X-100 insoluble fractions obtained from them. Protein extracted from both the Triton-soluble and insoluble fractions did not reduce activity. Lipopolysaccharide (LPS) extracted from cells of sensitive strains neutralized at least 50 LU bacteriocin activity per ug 2-keto-3-deoxyoctonate (KDO). Together the data suggest that the receptor site is in the LPS.

### 335

MOLECULAR ANALYSIS OF THE *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* ICE GENE. D. R. Gies, D. K. Willis, N. J. Panopoulos, and S. E. Lindow. Department of Plant Pathology, University of California, Berkeley, CA 94720.

Two SstII fragments internal to the 4.2 Kb *ice* region of *Pseudomonas syringae* pv. *syringae* (Pss) Cit7 exhibited homology to unique EcoRI fragments from all *Ice*<sup>+</sup> strains of *P. fluorescens*, *Pseudomonas viridiflava*, *Erwinia herbicola*, and *P. syringae* examined by Southern blot analysis. Homology of *ice* DNA within different strains of Pss was higher than in interspecific comparisons by dot blot analyses although considerable variation in location of SalI restriction sites within Pss was observed. Two adjacent SstII fragments hybridized with each other as well as to other distal non-overlapping restriction fragments within the *ice* region of Pss strain Cit7. Forty-four percent of randomly selected Tn5 insertions in the Pss strain Cit7 *ice* region exhibit at least a partial *Ice*<sup>+</sup> phenotype. Many closely adjacent Tn5 insertions

revealed that only the 85.8 kb plasmid appeared to have any homology with the probe. All isolates with the 40.6 kb plasmid and the isolate with the 85.8 kb plasmid were traceable to the same nursery. All isolates without plasmids are traceable to a different nursery, and the isolate with the 66.9 kb plasmid was discovered at yet a third location. This data support the conclusion that Xct arose or was introduced via three or four independent events.

#### 340

CLONED DNA FRAGMENTS AS HYBRIDIZATION PROBES TO IDENTIFY *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI*. Don A. Roth and Jerry Johnson, Dept. Plant Science and Biochemistry, University of Wyoming, Laramie 82071.

Cloned DNA fragments of *Xanthomonas campestris* pv. *phaseoli* (Xcp) were used as hybridization probes to identify Xcp in pure and mixed cultures. Sau3A digestion fragments were cloned into plasmid pUN121 or bacteriophage M-13 and labeled with [ $\gamma$ - $^{32}$ P]ATP using polynucleotide kinase or [ $\alpha$ - $^{32}$ P] using DNA polymerase (Klenow fragment), respectively. Colony hybridization procedures were developed where bacterial colonies were lysed, the DNA denatured and fixed in situ on nitrocellulose prior to hybridization with radiolabeled probe. A 210bp fragment was identified which specifically hybridized to Xcp isolates but not to 25 other bacterial isolates including closely related xanthomonads, and other saprophytic and pathogenic bacteria commonly found in the Xcp ecosystem. This identification approach has inherent advantages which can be exploited to yield a highly specific, rapid and easily interpreted identification tool.

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CLONING OF A MACERATING FACTOR FROM *PSEUDOMONAS FLUORESCENS* W51. A. Schlemmer, M. Mayama, and N. T. Keen. Dept. of Plant Pathology, University of California, Riverside, CA 92521.

*Pseudomonas fluorescens* W51 produces a factor which macerates plant tissue, but is negative in the pectate lyase assay (Phytopath. 72:936;1982). It was not possible to obtain mutants completely lacking the macerating activity by transposon mutagenesis with Tn5, but in the same experiments five mutants were obtained which lacked a protease produced by the wild-type bacterium. Mutants lacking this protease still macerated cucumber tissue. This may mean either that Tn5 inserted at 'hot spots' or that the gene for the macerating factor is present in multiple copies. Since purification of this factor was difficult, cloning of the gene(s) was attempted. A pLAFR3 cosmid library yielded several clones which expressed macerating activity in *Escherichia coli*. Two of these clones showed a common restriction band of about 3 kb on agarose gel electrophoresis.

#### 342

CHARACTERIZATION OF A CONJUGATIVE PLASMID, pBPW1, AND ITS ABILITY TO CONFER SENSITIVITY TO PHAGE PRD1. Michael P. Quinnett and Paul D. Shaw, Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.

The conjugative plasmid, pBPW1, from *Pseudomonas syringae* pv. *labaci* strain BR2 encodes genes for phage PRD1 reception. A restriction map using nine restriction endonucleases has been prepared of the plasmid. The plasmid was found to be approximately 45 kilobases in length. Tn3 and Tn5 transposon mutagenesis was used to locate the genes involved in phage reception on a 25 kilobase HindIII restriction fragment. Four different phage-resistant mutants have been characterized, and all have lost transfer functions; however, they retain the ability to mobilize nonconjugative plasmids. One of these four mutants lacks a protein associated with the bacterial outer membrane.

#### 343 Withdrawn

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INVOLVEMENT OF LIPID PEROXIDATION IN THE DEVELOPMENT OF BACTERIALLY INDUCED HYPERSENSITIVE REACTION. L. Dale Keppler and Anton J. Novacky. University of Missouri, Columbia, Missouri 65211.

Concurrent increase in electrolyte leakage and membrane depolarization suggested alteration of cellular membranes of cucumber cotyledons infiltrated with *Pseudomonas syringae* pv. *psisi*, a hypersensitive reaction (HR) inducing bacterial pathogen. Separation of the two membrane potential (Em) components indicated

that the passive component was primarily affected. We tested the possibility of lipid peroxidation as a mechanism by which the passive component of Em was affected during HR. Lipid peroxidation and electrolyte leakage was followed and compared in cotyledons treated with the following live or heat-killed bacteria: 1) *Pseudomonas syringae* pv. *psisi* (HR inducing incompatible pathogen); 2) *Pseudomonas syringae* pv. *lachrymans* (compatible pathogen); 3) *Pseudomonas fluorescens* (a saprophyte). Lipid peroxidation and electrolyte leakage increased significantly in only the HR inducing combination.

#### 345

SOURCES AND CONTROL OF BACTERIAL EPIPHYTES IN DRY BEAN FIELDS. D. E. Legard and H. F. Schwartz, CSU, Dept. Pl. Path. & Weed Sci., Ft. Collins, 80523.

Field experiments were conducted during 1983-84 to evaluate the effect a copper spray schedule has upon foliar bacterial epiphytes in commercial dry bean plantings. The early season copper spray program effectively reduced *Pseudomonas syringae* pv. *phaseolicola* (Psp) and *Pseudomonas syringae* pv. *syringae* (Pss) epiphyte numbers, as well as overall bacterial populations. An early season (May-June 1985) survey of dry bean acreage was also conducted to identify potential sources of Psp and Pss inocula. A high incidence of volunteer beans was located within corn plantings throughout northeastern Colorado. Many of these volunteers were contaminated with pathogenic isolates of Psp and Pss. A limited survey of epiphytes in young commercial dry bean fields indicated that certified seed stocks were relatively free of Psp and Pss.

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LOCAL DIFFERENCES IN EPIPHYTIC BACTERIAL POPULATION SIZE AND SUPERCOOLING POINT OF CITRUS CORRELATED WITH TYPE OF SURROUNDING VEGETATION AND RATE OF BACTERIAL IMMIGRATION. G. L. Andersen and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Thirty-nine navel orange plantings containing trees untreated with copper fungicide were examined that were either free of weeds and surrounded by citrus plantings (Class 1 sites) or were weedy or adjacent to other crop or uncultivated plants shown to have higher epiphytic bacterial populations than navel orange (Class 2 sites). Total epiphytic bacteria as well as fluorescent and ice nucleation active (INA) bacterial population size on navel orange at Class 1 sites was significantly lower than in Class 2 sites during winter months of 1984 and 1985 ( $3.1 \times 10^4$  cfu/g fr. wt. and  $2.3 \times 10^5$  cfu/g fr. wt., total bacteria respectively). The average median supercooling temperature of navel orange leaves in Class 1 sites (-5.59 C) was significantly lower than in Class 2 sites (-5.04 C). The average rate of deposition of both total bacteria and INA bacteria onto petri dishes was higher within Class 2 sites than Class 1 sites.

#### 347

ARMILLARIA ROOT ROT OF ASPEN SPROUTS AFTER REPEATED SHORT ROTATIONS. Stanosz, G.R. and Patton, R.F. Department of Plant Pathology, University of Wisconsin-Madison, 53706.

Aspen (*Populus tremuloides* and *P. grandidentata*) root suckers and stump/root collar (S/RC) sprouts were sampled in short rotation plots established by the US For. Serv. in MN and by the Petawawa Nat'l For. Inst. in ONT (USDA For. Serv. Res. Paper NC-176, For. Chron. 54:265-267). Roots exhibited lesions and decay typical of those caused by *A. mellea*. S/RC sprouts were more frequently infected than suckers. Incidence was highest in a MN plot, sampled 5 years after the start of the third 8-year rotation, where 11 of 12 S/RC sprouts and 12 of 18 suckers were infected (77% overall). After three or more rotations of 4 or 5 years, in both locations, sprouting was severely reduced and sproutless stubs from previously cut sprouts were invariably colonized. After successive rotations, in all plots, decreases in stem numbers and sizes have occurred. Thus, Armillaria root rot may limit rotation length and number of times aspen stands can be successfully vegetatively regenerated.

#### 348

A DISC CAMERA SYSTEM FOR AUTOMATICALLY RECORDING SNOW COVER AND OTHER VISUAL DATA. M. Marosy<sup>1</sup>, A. H. Alberga<sup>2</sup>, C. B. Tanner<sup>2</sup> and C. D. Upper<sup>1,3</sup>. <sup>1</sup>/Dept. of Plant Pathology, <sup>2</sup>/Dept. of Soil Science, and <sup>3</sup>/ARS, USDA, Univ. of Wisconsin, Madison, WI 53706.

A method for recording the amount and duration of snow cover at

remote sites was required to study the relationship of snow cover to infection of red pine (*Pinus resinosa* Ait.) by the fungus *Gremmeniella abietina* (Lagerb.) Morelet. A Campbell Scientific CR-21 datalogger was coupled with a switch relay to automatically trigger a Minolta Disc-7 camera every second day. By focusing the camera on graduated stakes in the plot, the 15-exposure disc provided a 30-day record of snow depth. The equipment was checked, film changed, and CR-21 data collected once every 30 days. The system was sufficiently reliable to provide a continuous record of snow cover at two sites in Wisconsin throughout the winter of 1984-1985, when air temperatures as low as -36 C were recorded. This technique can be applied to many other experiments requiring visual records.

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SITE FACTORS ASSOCIATED WITH NECTRIA CANKER ON BLACK WALNUT TREES IN SOUTHWEST MICHIGAN. C. S. Thomas and J. H. Hart. Departments of Botany and Plant Pathology and Forestry, Michigan State University, East Lansing, MI 48824.

More than 75% of the black walnut trees (*Juglans nigra* L.) in some stands in southwest Michigan have multiple cankers caused by *Nectria galligena* Bres. A 5-county survey indicated that centers of severe *Nectria* canker incidence were associated with terminal moraines and till plains. Soil type, topography and surface geology were characterized at 30 of the survey sites. Soil texture, rooting depth and drainage features were not significantly associated with disease. Surface geology and topography were significantly correlated with disease levels as measured by several variables ( $P < 0.10$ ). Of the 189 survey observations, *Nectria* canker was more likely to be severe on black walnut trees growing on wetlands, kettles or depressions than on black walnut trees growing on uplands ( $P < 0.01$ ). Such lowland sites were more common on the rugged till plains and moraines.

### 350

THE RELATIONSHIP BETWEEN YEAR OF INFECTION, TREE AGE, TREE GROWTH AND NECTRIA CANKER OF BLACK WALNUT IN SOUTHWEST MICHIGAN. C. S. Thomas and J. H. Hart. Departments of Botany and Plant Pathology and Forestry, Michigan State University, East Lansing, MI 48824.

The incidence of *Nectria* canker (*Nectria galligena* Bres.) on black walnut trees (*Juglans nigra* L.) was mapped in a mixed hardwood plantation established in 1945-6 in southwest Michigan. Diameter breast height (DBH), number of cankers on the trunk 0.3-3.7 m above soil line, and canker shape (open or closed) were recorded for 2817 trees. The number of cankers per tree was not correlated with DBH or canker shape ( $R^2 = 0.05$ ). Seven infected and 4 uninfected trees were harvested to determine tree growth rates. Chronological year and age of trunk section at the time of infection were determined for 200 cankers dissected from the 7 infected trees. The number of infections per year (1961-83) increased from 1961 to 1979 with peak years 1978-80. Most trunk sections were 19-27 years old when infected, with peaks at 22 and 24 years. Tree growth rate was 30% less for black walnut trees infected with *Nectria* canker than for healthy trees ( $P < 0.01$ ).

### 351

SURVIVAL OF PHYTOPHTHORA LATERALIS IN SOIL AND ROOTS OF PORT-ORFORD-CEDAR. E.M. Hansen, P.B. Hamn, and J. Kalafarski. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

In pathogenicity tests all *Chamaecyparis lawsoniana* (POC) were killed by *P. lateralis*; *P. cinnamomi*, *P. cryptogea* and *P. megasperma* killed fewer trees. During the original epidemic on ornamental POC in the 1950s, both *P. lateralis* and *P. cinnamomi* killed trees in Oregon and Washington. Only *P. lateralis* was isolated from dying POC in a 1982 survey. Survival of *P. lateralis* was studied in forest soil, in pots, and in roots of large POC killed by the fungus. *P. lateralis* was recovered from all soil samples collected from beneath dying POC at time 0. After 30 months, recovery was 31%. Recovery from soil in large pots with intact root systems remained at 100% through 24 months, although propagule numbers declined by 30%. The fungus was recovered from 86% of larger, recently killed forest trees, 62% of trees dead for 1 or 2 years, and 0.0% of trees dead 3 or more years.

### 352

ANALYSIS OF THE DISTRIBUTION OF FOREST PATHOGENS IN THE UNITED STATES AND CHINA: TWO CASE STUDIES. M. M. Chen, Department of

Plant Pathology, University of California, Berkeley, CA 94720.

There are 740 major forest tree species in China and 1260 known forest pathogens, 93% of which are fungi. Mudanjiang, in NE China, and Wisconsin are at similar latitudes and share genera of forest trees and many pathogen species. Both regions are horizontal mountains. Many forest pathogens are also common to the Sierra Nevada and the eastern margin of the Tibetan plateau. They are similar in that both form large rain shadows, but the climate of the Sierra Nevada is influenced by the Pacific Ocean and Southeast China's climate is continental and strongly influenced by the high elevation of Qinghai-Xizhang (Tibetan) plateau. In spite of the great climate differences between the two regions, they share several coniferous genera and hardwood pathogens: dwarf mistletoes, rusts, decays and root diseases. The geographical distribution of fungi have been analyzed. A scientific analysis of the biogeography of pathogens is crucial to understand where and how pathogens may become a problem and for the application of quarantines.

### 353

A REEXAMINATION OF THE MORPHOLOGY OF ENDOTHIA AND CRYPHONECTRIA. J. A. Micales and R. J. Stipes, Dept. Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

The genus *Endothia* was recently divided into two genera, *Endothia* and *Cryphonectria*, based on differences in ascospore shape and septation, stromatic configuration, and the distribution of stromatic tissues. Isolates of *Endothia* reportedly produce nonseptate, allantoid ascospores, diatrypid stromata, and pseudoparenchymatous tissue; members of *Cryphonectria* form monoseptate, ellipsoid or ovoid ascospores, valsoid stromata, and prosenchymatous tissue. This separation is not accepted by all pathologists. These criteria were reexamined for three species of *Endothia* and eight species of *Cryphonectria*. The distribution of stromatic tissues was uniform and could not be used to separate the genera. The remaining criteria were correct despite some morphological variation associated with different hosts. The differentiation of *Endothia* from *Cryphonectria* is morphologically sound.

### 354

Sporulation of virulent *Endothia parasitica* cankers on American chestnut after exposure to virulent and hypovirulent inoculum for one year. W. L. MacDonald and M. L. Double, WVU Plant Path. and Ag. Micro., P.O. Box 6057, Morgantown, WV 26506-6057.

Artificially established 8-week-old virulent *Endothia parasitica* cankers were exposed to sources of virulent or hypovirulent inoculum to determine if these inocula effect subsequent sexual and asexual sporulation. After one year, sporulation was assessed by counting stromata/cm<sup>2</sup> and rating perithecial production on a scale from 0-4. Stromata production was greatest when cankers were exposed to vegetatively incompatible (sexually compatible) virulent inoculum (15.1/cm<sup>2</sup>) or to no inoculum (14.7/cm<sup>2</sup>-control). Perithecial production was greatest (rating of 3.1) after exposure to vegetatively incompatible virulent inoculum but almost eliminated (rating of 0.1) after exposure to vegetatively compatible (sexually incompatible) hypovirulent inoculum. Contamination of developing virulent cankers by hypovirulent inoculum appears to significantly reduce their potential to sporulate.

### 355

WOOD DECAY STUDIES OF THE WHITE-ROT FUNGI GANODERMA LUCIDUM AND G. TSUGAE. J.E. Adaskaveg and R.L. Gilbertson, Dept. of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721.

The in vitro effects of *G. lucidum* on wood from its deciduous hosts *Quercus hypoleucoides*, *Vitis vinifera*, and *Prosopis juliflora* and *G. tsugae* on its coniferous hosts *Abies concolor* and *Pseudotsuga menziesii* were determined over 20 wk at 26-27C using standard procedures. Each fungus also was tested on wood from the non-host species. The average percent decay of each of the woods caused by three isolates of each fungus (five replicated/isolate) was: *Vitis* - *G. lucidum* 64.9% / *G. tsugae* 43.0% / Control 4.0%; *Quercus* - 37.6%/18.4%/0.9%; *Abies* - 10.6%/5.7%/0.5%; *Pseudotsuga* - 7.4%/4.1%/0.3%; *Prosopis* - 2.2%/3.3%/1.4%. Based on Klason lignin and chlorite holocellulose analyses of ground extracted wood, isolates of *G. lucidum* selectively delignified *Vitis*, *Quercus*, and *Abies* wood, but not *Pseudotsuga*, while *G. tsugae* isolates selectively delignified wood of the non-hosts *Vitis* and *Quercus*, but not the host woods of *Abies* or *Pseudotsuga*. *Prosopis* was not tested.

CULTURAL AND TEMPERATURE RELATIONSHIPS OF SEVERAL NORTH AMERICAN AND EUROPEAN GANODERMA SPECIES. J. E. Adaskaveg and R.L. Gilbertson. Dept. of Plant Pathology, Univ. of Arizona, Tucson, Arizona 85721.

Cultural studies were made of putative species of the wood-rotting fungus *Ganoderma* collected in Europe (*G. valesiacum*, *G. resinaceum*) and North America (*G. oregonense*, *G. tsugae*, *G. lucidum*). The species were divided into two groups based on cultural morphology and temperature studies. *G. lucidum* and *G. resinaceum* produced chlamydospores indistinguishable from each other in size and appearance as determined by brightfield and scanning electron microscopy. *G. tsugae*, *G. oregonense*, and *G. valesiacum* did not produce chlamydospores. The optimal temperatures for radial growth for isolates of *G. lucidum* and *G. resinaceum* on malt extract agar were 30-34C, with growth rates ranging from 5.5 to 7.8 mm/day. These temperatures for *G. tsugae*, *G. oregonense*, and *G. valesiacum* ranged between 20-25C, with growth rates ranging from 2.1 to 2.8 mm/day. The latter three species did not grow above 34 C during the 2-wk study.

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WOOD STRENGTH REDUCTION BY BASIDIOMYCETES ISOLATED FROM AIR SEASONING DOUGLAS-FIR UTILITY POLES. C. M. Sexton and M. E. Corden. Department of Forest Products, Oregon State University, Corvallis, Oregon 97331.

Basidiomycetes isolated from Douglas-fir utility poles air seasoning in the Pacific Northwest were tested for their effect on wood strength (toughness) as an early predictor of ability to decay wood. Douglas-fir heartwood specimens (6 x 1 x 50 mm) incubated with the test fungi for 4 weeks were tested for strength by measuring impact bending and breaking radius. Among 234 isolates of 26 species, the brown rot fungi (*Poria xantha*, *P. placenta*, *P. carbonica*, *Fomitopsis cajanderi*, *P. pinicola*, *Antrodia serialis* and *Crustoderma dryinum*), and the white rotter *Coriolus versicolor* caused extensive strength losses. White rot fungi, although isolated in greater numbers than the brown rotters generally caused only minor strength losses. *P. carbonica* and *P. placenta*, the major decay fungi of Douglas-fir poles, produced significant strength losses before weight loss could be detected.

## 358

PATTERNS OF CELL WALL DEGRADATION IN ADVANCED STAGES OF WOOD DECOMPOSITION BY WHITE ROT BASIDIOMYCETES. Robert A. Blanchette. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Advanced stages of white-rotted deciduous wood, decayed by *Ganoderma*, *Ischnoderma*, and *Phellinus* species as well as other Basidiomycetes, had different micromorphological patterns of cell wall degradation. 1) A simultaneous removal of all cell wall components resulted in the localized erosion of cell wall layers. Lignin, cellulose and hemicellulose were degraded in approximately equal proportions. 2) A complete degradation of fiber and parenchyma cell walls but not vessel elements; the ratio of lignin to cellulose remained the same as sound wood. 3) Extensive loss of middle lamellae caused by selective degradation of lignin and hemicellulose. The resulting wood was composed primarily (>95%) of cellulose.

## 359

ASCOSPORE PRODUCTIVITY AND INFECTION OF HYBRID POPLARS BY *MYCOSPHAERELLA POPULORUM*. C. J. Luley and H. S. McNabb, Jr. Iowa State University, Ames, Iowa 50011.

Ascospore productivity and infection of hybrid poplars by *Mycosphaerella populorum* was monitored weekly in a central Iowa plantation. Productivity was measured with an ascospore liberation tunnel and petroleum coated microscope slides. Foliar and stem infections of susceptible (NE-5272) and moderately resistant (NE-5271) clones were determined by exposing potted cuttings within the plantation before their removal to a greenhouse. Ascospores were collected the first week of sampling (April 20) and ascospore productivity measured by the two collection methods was significantly correlated (R=0.77). Spore productivity peaked in late May and declined to extinction in early August. Foliar infection occurred over a range of environmental and inoculum density conditions. Stem infection of both clones, attributed solely to ascospores, occurred in a single week during maximum discharge. Ascospores appear to be important in the epidemiology of both foliar and stem infection.

## 360

ISOLATION OF VIRUS-LIKE PARTICLES FROM LEAFROLL-INFECTED GRAPEVINES. F. Zee, D. Gonsalves, A. Goheen, R. Lee, and R. Pool; New York State Agricultural Experiment Station, Geneva, NY 14456; USDA, University of California, Davis, CA 95616; University of Florida, Lake Alfred, FL 33850.

Virus-like particles, which were similar in morphology to Closteroviruses, were recovered in relatively high concentrations from leafroll-infected grapevines (*Vitis vinifera*) cv. 'Melon' and 'Pinot Noir' using procedures described by Gugerli, Brugger, and Bovey (Rev. Suisse Vitic. Hortic. 16:299-304, 1984). The procedure included pulverizing leaf tissue in liquid nitrogen and stirring the powdered tissues in pH 8.2, 0.5 M Tris-HCl buffer containing 4% water-insoluble polyvinylpyrrolidone, 0.5% bentonite, 1X triton X-100 and 0.2% mercaptoethanol. The extract was then subjected to low and high speed centrifugations, clarified with an equal volume of ether:carbon tetrachloride mixture, and concentrated by high speed centrifugation. Virus-like particles were not recovered from similarly treated leaves of healthy plants. The virus-like particles are being characterized serologically and biochemically.

## 361

DOUBLE-STRANDED RNA IN GRAPEVINES AFFECTED WITH LEAFROLL DISEASE. H. R. Cameron and Michael H. Walter, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331

Double-stranded RNA (dsRNA), extracted from dormant cane phloem of grapevines showing leafroll-disease symptoms, formed a set of high molecular weight bands on non-denaturing polyacrylamide gels. Only the highest molecular-weight dsRNA bands differed in pattern from that of symptomless canes. Controls were certified to be virus-free. Double-stranded RNA from citrus infected with citrus tristeza virus served as a marker at high molecular weight. The possibility exists of using dsRNA gel-electrophoretic patterns as a diagnostic technique for identifying virus content of imported grapevine stock.

## 362

INDEXING PEACHES FOR RNA VIRUS INFECTION BY ISOLATION OF REPLICATIVE VIRAL RNA. C. K. Hanson and R. S. Halliwell, Dept. of Plant Pathology & Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

Double-stranded RNA (dsRNA), a replicative form of RNA viruses, is peculiar to plants infected with these viruses. An electrophoretic system was adapted to index peach material containing known viruses. Only leaves were assayed for this research. Both virus-free and virus-infected leaves were extracted with phenol. The dsRNA was isolated by Whatman CF-11 cellulose chromatography and analyzed on 5% polyacrylamide gels. Resulting bands were visualized by ethidium bromide under ultra-violet light. Cucumber mosaic and tobacco mosaic viruses were used as controls and molecular weight markers. Peach material containing Prunus necrotic ringspot virus tested positive for dsRNA. The structure of the dsRNA was confirmed by resistance to RNase digestion in a 0.3 M NaCl solution.

## 363

A technique for the differentiation of strains of individual maize dwarf mosaic virus particles in a mixed preparation. J. D. Alexander and R. W. Toler. Texas Agricultural Experiment Station, College Station, TX 77843.

Immuno-electron microscopy was coupled with immuno-gold decoration to differentially label virus particles of two strains of maize dwarf mosaic virus (MDMV). The purpose of pursuing this technique is for the study of mixed infections of MDMV and/or sugarcane mosaic virus strains. Carbon coated parlodian grids, treated with a mixture of anti-MDMV-A and anti-MDMV-B IgG, were used to trap approximately equal densities of MDMV-A and MDMV-B particles from infected sudangrass sap. Colloidal gold (14.5 nm), stabilized with protein A and conjugated to IgG specific to one strain of the virus, was used to specifically label virus particles of that strain in the mixture attached to the grids. The grids were then stained with PTA and examined under the electron microscope. Optimum labelling resulted in an average of 10 to 15 gold labels per homologous virus particle.

## 364

THE POSSIBLE ROLE OF POLLEN IN THE SPREAD OF ASPARAGUS VIRUS 2



IN ASPARAGUS. T.A. Evans and C.T. Stephens, Dept. of Botany and Plant Pathology, Michigan State Univ., East Lansing, MI 48824

The exine of pollen from virus-infected anthers may carry plant viruses, which has implicated pollen as a virus "vector". In the present study, asparagus virus 2 (AV-2) antigen was localized in the exine of pollen from AV-2-infected asparagus by enzyme-linked immunosorbent assay and virus specific IgG-latex conjugate. AV-2 antigen was easily washed from the exine and infective virus was associated with these pollen washes. Pollen washes of some but not all virus-infected pollen were determined to contain large quantities of AV-2 antigen, which was not detected within virus-infected or virus-free pollen. More IgG-latex spheres adhered to the surface of virus-infected pollen than to either virus-infected pollen "blocked" with IgG specific for AV-2 antigen or virus-free pollen. Up to 70% of virus-free asparagus plants rub-inoculated with sap from AV-2 infected asparagus were infected with AV-2 after 2 months.

### 365

TRANSMISSION OF HUANGLUNGBIN AGENT FROM CITRUS TO PERIWINKLE BY DODDER. Chung Ke and James H. Tsai. Fujian Academy of Agric. Sciences, Fuzhou, Fujian, People's Republic of China and University of Florida, FLREC, Fort Lauderdale, FL 33314.

For the first time in China, citrus huanglungbin agent has been successfully transmitted from *Citrus sinensis* and *C. reticulata* to periwinkles (*Catharanthus roseus*) by dodder (*Cuscuta campestris*). Three of 15 periwinkles tested by dodder developed yellowing symptoms within 3-4 mo. Initial symptom includes chlorosis along the veins followed by general chlorosis and thickening of leaves. General chlorosis often started from one branch and moved to other branches. Electron microscopy revealed the presence of bacterial-like organisms (BLO) in the sieve tubes of leaf midribs of symptomatic plants. These BLO had elongated and round forms and a triple-layered cell wall indistinguishable from those of the agent in the infected citrus. The titre of BLO in periwinkle was much higher than in citrus. Several attempts to transmit BLO in same manner by *C. chinensis* and *C. japonica* were unsuccessful.

### 366

LACK OF AN ASSOCIATION OF BLUEBERRY LEAF MOTTLING VIRUS WITH A NEMATODE VECTOR. A.M. Childress and D.C. Ramsdell, Dept. of Plant Pathol., Physiol. & Weed Sci., VPI & SU, Blacksburg, VA 24061 and Dept. of Botany and Plant Pathol., Michigan State Univ., East Lansing, MI 48824.

Blueberry leaf mottle virus (BBLMV) is a putative member of the nepovirus group. Although BBLMV has been associated with pollen from infected blueberry (*Vaccinium corymbosum*), it has not been associated with a nematode vector. Surveys of 5 blueberry 'Jersey' fields demonstrated a poor association of *Xiphinema americanum* and *Longidorus* spp. with virus-infected bushes. *Trichodorus* spp. were ubiquitous in these fields and represented the highest population in all fields sampled. Virus was not detected by radioimmunoassay in the 3 genera of nematodes tested. *Nicotiana glauca* bait plants were not infected following a 14-day inoculation access period with *X. americanum* or *Longidorus* spp. obtained near infected bushes. No weed species in 17 genera harbored BBLMV whether or not potential nematode vectors were present. Thus BBLMV does not appear to be associated with weed species or nematode vectors in the field.

### 367

INTERACTIONS BETWEEN MAIZE MOSAIC AND MAIZE STRIPE VIRUSES IN THEIR VECTOR, PEREGRINUS MAIDIS, AND IN MAIZE PLANTS. E. D. Ammar<sup>1</sup>, R. E. Gingery<sup>2</sup>, and L. R. Nault<sup>3</sup>. <sup>1</sup>Faculty of Agriculture, Cairo University, Giza, Egypt, <sup>2</sup>USDA-ARS, Dept. of Plant Pathology and <sup>3</sup>Dept. of Entomology, The Ohio State University, OARDC, Wooster, OH 44691.

Maize mosaic virus (MMV) and maize stripe virus (MStpV), members of distinct virus groups, have the same insect vector and similar plant host ranges. The effect of each of these viruses on the insect transmission of the other and the cross-protection between the two viruses in maize were determined. Two biotypes of *Peregrinus maidis* and two cultivars of maize were tested. There was mild cross-protection between the two viruses in both maize cultivars and pronounced interference between the viruses in both biotypes of *P. maidis*, with MMV usually dominant over MStpV, suggesting a competitive interaction between the two viruses. Interference of MMV with MStpV in the vector appeared to be independent of the time of acquisition of MMV relative to that of MStpV.

### 368

NUMBER OF POTYVIRUS PARTICLES REQUIRED FOR TRANSMISSION BY APHIDS. T. P. Pirone and D. W. Thornbury, University of Kentucky, Lexington, KY 40546.

Aphids (*Myzus persicae*) were allowed a 10 min acquisition access to a solution which contained helper component, 1  $\mu$ g/ml of tobacco vein mottling virus (TMV), and 1.25  $\mu$ c Na<sup>125</sup>I. Individual aphids were then counted in a gamma counter and placed on a tobacco seedling to test for virus transmission. Aliquots of the test solution were counted to determine specific radioactivity (cpm/ $\mu$ l). Volumes acquired by aphids typically ranged from ~300  $\mu$ l to ~1  $\mu$ l (the lower limit of detection). The number of virus particles contained in these volumes was calculated to be from ~4000 to ~10. In 3 experiments, 5-17% of the aphids transmitted TMV and there was no correlation between the numbers of particles acquired and the ability to transmit. Aphids which acquired fewer than 100 particles accounted for almost half of the transmissions.

### 369

ULTRASTRUCTURE OF DIODIA VIRGINIANA INFECTED WITH A WHITEFLY-TRANSMITTED VIRUS-LIKE DISEASE AGENT. Richard C. Larsen and K. S. Kim, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

*Diodia virginiana* exhibiting chlorosis and vein-clearing symptoms was found to be widely distributed in Arkansas. The disease was transmitted by the abutilonea whitefly, *Trialeurodes abutilonea*. Ultrastructural studies of diseased leaves revealed the consistent presence of flexuous rod-shaped virus-like particles in the cytoplasm of phloem parenchyma cells and sieve elements. The particles, ca. 12 nm in diameter with undetermined length were associated with membranous vesicles containing fibrils similar to those induced by known closteroviruses, including the recently identified lettuce infectious yellows virus transmitted by the whitefly, *Bemisia tabaci*. Many phloem cells contained a greatly proliferated tubular endoplasmic reticulum which appeared to give rise to the vesicles. It is suggested, therefore, that the infectious agent is a possible member of the closterovirus group. The abutilonea whitefly has not been shown to transmit any plant virus.

### 370

RIBONUCLEASE IN BEETLE REGURGITANT IS A DETERMINANT IN PLANT VIRUS TRANSMISSION. R. C. Gergerich, H. A. Scott and J. P. Fulton, University of Arkansas, Fayetteville, AR 72701.

Regurgitant from leaf-feeding beetles (*Cerotoma trifurcata*, *Epilachna varivestis*, and *Diabrotica undecimpunctata*) contains ribonuclease (RNase) activity equivalent to 0.1 mg to 1.0 mg per ml of pancreatic RNase, the amount of enzyme activity varying with the species of beetle. When plants were inoculated with mixtures of regurgitant and plant viruses using a technique which mimics beetle feeding, infection by non-beetle-transmissible viruses was prevented while infection by beetle-transmissible viruses was unaffected. When pancreatic RNase, at a level equivalent to the RNase activity in beetle regurgitant, was mixed with various viruses, the same pattern of selective inhibition was apparent, i.e. only infection by beetle-transmissible viruses occurred. Pancreatic RNase at activities five times that found in beetle regurgitant was not effective in preventing transmission of two beetle-transmissible viruses, while RNase at activities one-half that found in beetle regurgitant prevented infection of non-beetle-transmissible viruses.

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RNA POLYMERASE ACTIVITY ASSOCIATED WITH NAKED dsRNA CONTAINED IN VESICLES OF A HYPOVIRULENT STRAIN OF ENDOTHIA PARASITICA. N.K. Van Alfen, H. Aloni, D.R. Hansen, and W.A. Powell, Department of Biology, Utah State University, Logan, UT 84322.

Hypovirulence in certain strains of *Endothia parasitica* is believed to be associated with the presence of dsRNA. This dsRNA is similar to a dsRNA fungal virus genome but no viral particles have been found. We have shown that the dsRNA is packaged in vesicles of fungal origin. RNA polymerase activity has been detected in vesicles isolated from a hypovirulent strain (EP773) of *E. parasitica*. These vesicles demonstrated RNA polymerase activity because with dsRNA as a template, in the presence of actinomycin D, radiolabeled UMP was incorporated into TCA precipitable products. Similar vesicles isolated from a virulent, non-dsRNA containing strain (EP155) were also assayed using dsRNA as a template with no polymerase activity detected. A polymerase associated with the dsRNA containing vesicles would seem to reiterate the virus-like nature of the dsRNA.

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HOMOLOGY RELATIONSHIPS WITH MICHIGAN HYPOVIRULENT STRAINS OF *ENDOTHIA PARASITICA*. C. P. Plant and D. W. Fulbright. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824-1312.

A correlation exists between cytoplasmic hypovirulence and the presence of double-stranded RNA (dsRNA) in *Endothia parasitica*. Various strains of *E. parasitica* exhibit different banding patterns of dsRNA on agarose gels. To determine whether the dsRNAs from these various strains show any sequence homology, dsRNA from selected strains was transferred from the gel to nitrocellulose and hybridized to <sup>32</sup>P-labeled dsRNA from the Michigan hypovirulent strains GH2 and R1. Neither GH2 or R1 dsRNA showed homology to dsRNA from strains from Europe or other states. GH2 and R1 show no homology to each other. GH2 exhibited homology to dsRNA from all other Michigan strains tested, while R1 did not show homology to any other Michigan strains tested.

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SEQUENCE RELATIONSHIPS AMONG DOUBLE-STRANDED RNA SPECIES OF A HYPOVIRULENT STRAIN OF THE CHESTNUT BLIGHT FUNGUS *ENDOTHIA PARASITICA*. S. Hiremath, B. L. Hostis, S. A. Chabrial, and R. E. Rhoads, Departments of Biochemistry and Plant Pathology, University of Kentucky, Lexington, Ky 40546.

A recombinant DNA probe complementary to dsRNA from the French-derived hypovirulent strain EP-711 of *Endothia parasitica* was constructed. Nucleotide sequence analysis revealed that the insert was 194 bp and included an enzymatically added poly(A) tract, suggesting that the probe represented the 3'-terminus of one strand of a dsRNA species. All of the four major dsRNA species from EP-711 hybridized equally well to the probe, indicating sequence homology. Even more hybridization occurred, however, to RNAs of faster electrophoretic mobility which were either minor components or undetectable by staining with ethidium bromide. Digestion with a single-strand specific nuclease caused either the disappearance or electrophoretic shift of the new bands, suggesting that some of them may represent messenger RNAs transcribed from the dsRNA.

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CHARACTERIZATION OF SYRINGOMYCIN PRODUCTION MUTANTS OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* CAUSED BY TN5 INSERTIONS. G. W. Xu and D. C. Gross, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164.

Genetic analysis of syringomycin production in *Pseudomonas syringae* pv. *syringae* is being used to evaluate the role of the phytoalexin in virulence. The suicide plasmid vehicle pGS9 was used to deliver Tn5 into pv. *syringae* strain B301D. Subsequent screening of kanamycin resistant transconjugants for in vitro syringomycin production identified three classes of mutants: (Class I) hyperproducers, 1.2%; (Class II) hypoproducers, 2.8%; and (Class III) nonproducers, 0.4%. In pear seedling pathogenicity assays, 92% (11/12) of the nonproducers were avirulent. Southern blot analysis indicated that these mutants were the result of independent insertions into distinct EcoRI fragments. The Tn5 and flanking chromosomal DNA from two avirulent, nontoxic strains has been cloned. The cloned fragments are being used to isolate the corresponding wild-type syringomycin genes from strain B301D.

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ANALYSIS OF A *PSEUDOMONAS FLUORESCENS* ICE NUCLEATION GENE BY COMPLEMENTATION, AND IDENTIFICATION OF ITS PRODUCT. Gareth Warren, Loren Corotto, and Paul Wolber; Advanced Genetic Sciences, 6701 San Pablo Avenue, Oakland CA 94608.

A cloned 5.5 kb segment of DNA from *P. fluorescens* MS1650 encodes ice nucleation activity (INA). Gene replacement experiments showed that this segment is essential for INA in MS1650. We tested a number of INA<sup>-</sup> mutations for complementation. All mutations fell into a single complementation group, suggesting that this region encodes a very large single gene. The product of this gene has been identified as a band on SDS-polyacrylamide gels: this band is removed by mutations throughout the region, and its mobility indicates an appropriate molecular weight.

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REGULATION OF ANTIBIOTIC BIOSYNTHESIS IN *PSEUDOMONAS*

*FLUORESCENS* STRAIN HV37a. Neal I. Gutterson and Gareth J. Warren, Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., Oakland, CA 94608.

*Pseudomonas fluorescens* strain HV37a can control the *Pythium ultimum*-induced damping-off disease of cotton. This pseudomonad also produces an antibiotic that is responsible for a significant proportion of disease control. Mutants deficient in antibiotic biosynthesis have been isolated, and wide-host-range cosmids containing these genes have been identified. Fusions were constructed between these cosmids' biosynthetic operons and the lac-operon, and then returned to the pseudomonad. With the production of beta-galactosidase as an assay, the regulation of antibiotic biosynthetic genes has been studied. In particular, glucose induces the expression of some genes. Genes involved in regulation have been identified by the absence of glucose induction of genes involved in antibiotic biosynthesis.

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AN Fe<sup>+++</sup> ANTAGONIZED FUNGISTATIC AGENT FROM *PSEUDOMONAS FLUORESCENS* THAT IS DISTINCT FROM THE FLUORESCENT SIDEROPHORE. Paul R. Gil and Gareth J. Warren, Advanced Genetic Sciences, Inc., Oakland, CA 94608

We have identified a plant growth promoting rhizobacterium, *P. fluorescens* NZ130, that exhibits antibiosis against the phytopathogen, *Pythium ultimum*. The antibiosis is induced if the Fe<sup>+++</sup> concentration is below 10<sup>-6</sup>M. Gel filtration of material secreted by this strain shows that the fluorescent siderophore, (S<sub>α</sub>), can be separated from the antifungal activity, (S<sub>β</sub>), demonstrating that S<sub>α</sub> is distinct from S<sub>β</sub>. We generated mutants with the phenotypes: S<sub>α</sub><sup>-</sup>S<sub>β</sub><sup>+</sup>, S<sub>α</sub><sup>+</sup>S<sub>β</sub><sup>-</sup> and S<sub>α</sub><sup>-</sup>S<sub>β</sub><sup>-</sup>, showing that S<sub>α</sub> and S<sub>β</sub> are the products of different but related biosynthetic pathways. DNA sequences required for the biosynthesis of S<sub>α</sub> and S<sub>β</sub> were identified by complementation of mutants using a cosmid library, and the sequences required for S<sub>α</sub>S<sub>β</sub><sup>+</sup> complementation are distinct from those required for S<sub>α</sub>S<sub>β</sub><sup>-</sup> complementation. Using β-galactosidase gene fusions, we have shown that transcription of regions required for S<sub>α</sub> and for S<sub>β</sub> production is regulated by the level of available Fe<sup>+++</sup>.

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CLONING AND CHARACTERIZATION OF THE IAA-LYSINE GENE AND ITS POSSIBLE ROLE IN VIRULENCE OF *PSEUDOMONAS SAVASTANOI*. N. Louise Glass and Tsune Kosuge. Department of Plant Pathology, University of California, Davis, CA 95616.

*Pseudomonas syringae* pv. *savastanoi* induces gall formation on olive and oleander plants by the bacterial synthesis of the phytohormone indoleacetic acid (IAA). The genes for IAA biosynthesis reside on a plasmid (pIAA) in oleander isolates of *P. savastanoi* and are organized in an operon. Oleander isolates are capable of further metabolizing IAA to 3-indole-acetyl-L-lysine. The gene for IAA-lysine synthetase was cloned from an oleander isolate into *E. coli* and characterized by restriction mapping. The *iaal* locus was mapped to a location 2kb upstream from the IAA operon by Tn5 insertion mutagenesis. The specific activity of the IAA-lysine synthetase is 47 times higher in the *E. coli* transformant than in *P. savastanoi*. Confirmation of the presence of IAA-lysine in the *E. coli* clone was confirmed by HPLC. The conversion of IAA to IAA-lysine may help regulate IAA pool size in the bacterium and therefore modulate virulence as assayed by gall size.

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PLASMID-MEDIATED COPPER RESISTANCE IN *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. C. L. Bender and B. A. Cooksey, Department of Plant Pathology, University of California, Riverside, CA 92521.

Twenty strains of *P. syringae* pv. *tomato* (Pat) were tested for resistance to copper sulfate (CuSO<sub>4</sub>). The Pst strains were grouped into three sensitivity classes based on the minimum inhibitory concentration (MIC) of CuSO<sub>4</sub>. MIC values were 0.4 - 0.6 mM CuSO<sub>4</sub> for Group I (8 strains), 1.2 mM for Group II (2 strains), and 1.6 - 2.0 mM for Group III (10 strains). Pst strain PT23 (Group III) was mated with two Cu<sup>s</sup> strains of *P. syringae* pv. *syringae* (Pss) having MIC values of 0.1 mM CuSO<sub>4</sub>. Pss transconjugants recovered on media with 0.4 mM CuSO<sub>4</sub> contained a 101 kb plasmid from PT23. The Pss transconjugants served as donors of Cu<sup>r</sup> in subsequent matings; this was always associated with transfer of the 101 kb plasmid.

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TRANSPOSON INDUCED MUTANTS OF *PSEUDOMONAS GORRUGATA*. Wesley Chun

and J. V. Leary, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

*Pseudomonas corrugata*, the causal agent of tomato pith necrosis, produces a phytotoxin *in vitro* and *in planta*. To determine the role of the toxin in pathogenicity, transposon mutagenesis was performed using the suicide vector pSUP2021::Tn5. *P. corrugata* (0782-6) was mated with *E. coli* S17(pSUP2021::Tn5) on 0.22 µm Millipore filters and grown for 2 days on King's Medium B. The bacteria were resuspended and dilutions plated on a minimal medium containing kanamycin (50 µg/ml) and trimethoprim (100 µg/ml), allowing growth only of *P. corrugata* cells that had undergone the transpositional event and received the kanamycin resistance (Km<sup>R</sup>) from Tn5. This resulted in the recovery of 480 *P. corrugata* Km<sup>R</sup> transconjugants. Of the 480 Km<sup>R</sup> transconjugants recovered, three Tox<sup>-</sup> isolates did not cause any symptoms on inoculated plants. When tested after recovery from tomato plants, these three isolates were still Km<sup>R</sup> and Tox<sup>-</sup>.

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B-GALACTOSIDASE AS A METABOLIC MARKER IN XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS. M. Kawalek and N. W. Schaad, Dept. Pl., Soil & Ent. Sci., Univ. of Idaho, Moscow, ID 83843.

For studies on the leaf colonization ability of *Xanthomonas campestris* pv. *campestris* (Xcc) the gene of B-Galactosidase (B-Gal) was used as a metabolic marker. It was investigated after finding antibiotic markers unstable. In view of earlier success (Hemming, B.C., 1984, J. Cellular Biochem. Suppl. 8B:252) in transferring the B-Gal gene to pseudomonads, the triparental mating system was attempted, with *Escherichia coli* helper plasmid 2013 and plasmids 5002 & 5003. Recombinants were isolated by plating on selective lactose and starch media. Four strains were successfully transformed with efficiencies of 0.01-0.03%. Plasmids 5002 and 5003 have been stable in these strains for at least 25 generations. Since wildtype Xcc does not metabolize lactose, transformed strains used in leaf colonization studies could be readily differentiated from natural strains on lactose medium.

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DETECTION OF A CYTOKININ GENE HOMOLOGUE IN CORYNEBACTERIUM FASCIANS. M. A. Mellano and D. A. Cooksey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Cytokinin production has been implicated in the induction of fasciation symptoms by *Corynebacterium fascians*. To study the genetics of cytokinin production by *C. fascians*, we used a cytokinin gene from the T-DNA of *Agrobacterium tumefaciens* as a probe for homologous sequences in *C. fascians*. A 1.9 kb Bam HI/Hind III fragment containing the cytokinin gene for isopentenyl transferase was cloned from the *tmr* locus of the T-DNA of *A. tumefaciens* C58. The fragment was nick translated and used as a heterologous probe to Southern transfers of restricted *C. fascians* plasmid and chromosomal DNA. A sequence with high homology was detected in chromosomal digests of three different *C. fascians* isolates.

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INTERACTION OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA GENE PRODUCTS IN THE DEGRADATION OF POTATO TUBER TISSUE. D. P. ROBERTS and G. H. LACY. Dept. Plant Pathol., Physiol. & Weed Sci. VPI&SU, Blacksburg, VA 24061.

*Erwinia carotovora* subsp. *carotovora* strain EC14 chromosomal DNA was cloned into the PstI or BamHI sites of plasmid pBR322. Plasmid pDR1, a PstI clone, confers tetracycline resistance and mediates the production of a number of pectate lyases in *Escherichia coli* strain HB101. HB101 harboring pDR1 did not macerate potato tuber slices. Cloning into the BamHI site resulted in a number of ampicillin-resistant hybrid plasmids which mediate the production of pectolytic enzymes. *Escherichia coli* cotransformants containing pDR1 and each of the ampicillin resistant hybrid plasmids were constructed and analysed by gel electrophoresis. These *E. coli* cotransformants were screened for the ability to macerate potato tuber slices.

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THE ISOLATION AND CHARACTERIZATION OF PECTOLYTIC ENZYME GENES FROM ERWINIA CAROTOVORA SUBSP. ATROSEPTICA. C. Allen and G. H. Lacy, Dept. of Plant Pathology, Physiology, and Weed Science, VPI & SU, Blacksburg, VA.

*Erwinia carotovora* subsp. *atroseptica* (Eca), incitant of potato blackleg disease, is the least genetically characterized of the soft rot erwiniae. We constructed a clone library of Eca strain SR-8 DNA in *Escherichia coli* using the plasmid cloning vector pBR322. Two different types of pectolytic recombinant clones were recovered. These Eca clones were compared with various pectolytic clones carrying genes from *E. carotovora* subsp. *carotovora* and from *E. chrysanthemi* using restriction mapping, Southern hybridization, and enzyme profiles. Preliminary results indicate that some degree of homology exists among pectolytic enzyme genes from all three taxa.

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SEQUENCING AND HIGH LEVEL EXPRESSION OF A pI 9.8 PECTATE LYASE GENE FROM ERWINIA CHRYSANTHEMI EC16. S. Tamaki and N. T. Keen, Advanced Genetic Sciences, Oakland, CA and Dept. of Plant Pathology, University of California, Riverside, CA 92521.

The previously cloned pectate lyase (PL) gene in pPL742 was further subcloned as a 1.2 kb Eco RI-Sal I fragment. This fragment led to high levels of pectate lyase production in *E. coli* (10-20% of cell protein) when inserted in the proper orientation into pUC18 or the expression vector pIN III<sup>143</sup> A-1. Greater PL production occurred in the presence of IPTG and the enzyme was secreted efficiently into the periplasm and medium. The 1.2 kb fragment was found to contain a single open reading frame coding for 385 amino acids, including a signal peptidase recognition sequence. An efficient Shine-Dalgarno sequence was well positioned 5' to the translational start ATG codon and a near-consensus binding site for the *E. coli* catapalt activator protein was located further upstream from the coding region. In addition, an 11 base pair palindromic sequence was identified which is a possible operator region.

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GENETIC AND PHYSICAL CHARACTERIZATION OF VIRULENCE GENES CONTAINED IN RECOMBINANT PLASMID pES1044 FROM ERWINIA STEWARTII. D. L. Coplin, R. D. Frederick, and D. Majerczak. Department of Plant Pathology, OARDC, Wooster OH, 44691.

Clones restoring virulence to avirulent mutants of *Erwinia stewartii* were obtained from a cosmid library constructed in pVK100. Clone pES1044 (containing a 23 kb insert) restored water-soaking ability to mutants of complementation group II which is comprised of the capsular Mu pF7701-induced mutants MU43, MU51, MU136, and MU141 and a spontaneous mutant RDP6011. pES1044 also suppressed the avirulence mutation of acapsular strain MU14110 from group I. A restriction map of pES1044 was prepared. The virulence of all of the mutants except RDP6011 was restored by a subclone pRF203 that contained a 9.3 kb Hind III-BamHI fragment. Southern blot hybridizations with pRF203 probe DNA have associated the Mu pF7701 insertions in MU43, MU51, MU136, and MU141 with this fragment. Additional virulence genes have been located on an adjacent 4.6 kb Hind III fragment by Tn5 lac mutagenesis.

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PYTHIUM NUNN- A POTENTIAL BIOLOGICAL CONTROL AGENT. T. Paulitz, M. Windham, and R. Baker. Dept. of Plant Pathology

*Pythium nunn* is a recently described mycoparasite of *Pythium* spp. The effects of *P. nunn* on population densities of *Pythium ultimum* and pre-emergence damping-off were tested in a greenhouse experiment. Zero or 300 CFU/g of *P. nunn* were added to aerated steamed soil amended with 0, 100, 300, or 1000 CFU/g of *P. ultimum*. Population densities of both *Pythium* spp. were monitored weekly with a selective medium. Treatments were planted with pea and cucumber to assay soil suppressiveness. After 7 days, *P. nunn* significantly reduced population densities of *P. ultimum* in all treatments. Reductions in *P. ultimum* population densities continued throughout the 40 day experiment. Percent emergence of cucumbers planted in *P. nunn* treatments was significantly greater than in treatments without *P. nunn*. However, damping-off of peas was not controlled by the mycoparasite.

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KENTUCKY BLUEGRASS LITTER HARBORS MICROFLORA SUPPRESSIVE TO *COCHLIOBOLUS SATIVUS*. D. S. Egel and R. M. Endo, Department of Plant Pathology, University of California, Riverside, CA 92521

A selective enrichment procedure was used to identify possible antagonists from *Poa pratensis* litter. The litter was shaken in sterile distilled water (SDW), and the wash was filter-sterilized, heated at 50, 60 or 70 C for 30 min, or left untreated. These treatments were added to sterile, filter paper inoculated with conidia of *C. sativus*. Fungistasis was assessed by counting conidiophores. Abundant conidiophores were produced on SDW, however, complete fungistasis was observed with untreated wash and the 50 C treatment. Filtering the wash, or heating it at 60 or 70 C negated fungistasis. Elimination of fungistasis was also observed at 10 and 100 X dilutions of the 50 C and untreated wash, respectively. Dilution plating on media revealed numerous microbes present in the 50 C and untreated wash that were not present in other treatments. These results indicate the fungistatic effect of litter may be due to microbial antagonists.

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SELECTION OF FUNGICIDE TOLERANT ISOLATES OF *PYTHIUM OLIGANDRUM*. F. N. Martin and J. G. Hancock, USDA-ARS, Salinas, CA 93915 and University of California, Berkeley, CA 94720, respectively.

*Pythium oligandrum* is an effective biological control agent for *Pythium* damping-off of sugar beet. Preliminary investigations indicate that it also has promise for controlling damping-off of ornamental crops. A major short coming is its sensitivity to several fungicides. Its growth in culture is completely inhibited by 0.5 ppm metalaxyl (met). By the use of UV mutagenesis, variants have been selected which are tolerant of met concentrations higher than 200 ppm. Four successive vegetative transfers on non-met amended media did not influence the level of tolerance. One hundred percent of the oospore progeny had greater met tolerance than the wild type isolate. However, not all progeny had the same levels of tolerance as the parental variant strain. Progeny with aberrant morphological characteristics had lower levels of tolerance while those with wild type morphology had greater levels of tolerance. Enhancement of growth rates and fungicide tolerance were obtained by repeated selection of progeny demonstrating these characteristics.

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INHIBITION OF SPORANGIENESIS OF *PHYTOPHTHORA MEGASPERMA* F. SP. *GLYCINEA* BY 18 ISOLATES OF *TRICHOHECTIUM ROSEUM*. Al-Heeti, M. B., Sinclair, J. B., Dept. of Plant Path., Univ. of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801

Eighteen isolates of *Trichothecium roseum* grown separately on modified Czapek Dox broth, and their culture filtrates diluted to 5% in water, inhibited sporangienesis by *Phytophthora megasperma* f. sp. *glycinea* (Pmg) with the control producing  $2 \times 10^8$  zoospores/ml. Different concentrations of the culture filtrates were added to lima bean agar and inoculated with 5-mm plugs of Pmg, incubated for 7 days at 25°C. Colony diameter at 20% concentration ranged from 3.2 mm for isolate 7 to 22.8 mm for isolate 6, and 63.2 mm for the control. Mycelial dry weight of *T. roseum* on modified Czapek Dox broth after 21 days at 25°C ranged from 0.672g for isolate 3 to 0.966 g for isolate 8.

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COMPARISON OF THE HERBICIDAL EFFICACIES OF TWO FUNGI PATHOGENIC

An anthracnose-causing *Colletotrichum* sp. and a wilt-inducing *Fusarium* sp. were isolated from a mature, wilting *Crotalaria spectabilis* (showy crotalaria) plant and single-spore cultured. Both were virulent, caused 100% mortality of showy crotalaria seedlings, and were host specific to the target weed based on tests of 50 to 70 plant species in 14 families. When seedlings of comparable age were inoculated by foliar spray ( $10^6$  conidia/ml, *Colletotrichum*) or soil drench ( $10^6$ /g soil, *Fusarium*), the mean lag time between inoculation and 100% seedling mortality was 10 d for *Colletotrichum* and >30 d for *Fusarium*. The need for soil infestation with *Fusarium* vs the facility of foliar application with *Colletotrichum* further distinguished the efficacies. These results and current approaches to herbicide development suggest that the *Colletotrichum* sp. is a better mycoherbicide candidate for this weed than the *Fusarium* sp.

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COLONIZATION OF SCLEROTIA OF *SCLEROTINIA MINOR* BY *PENICILLIUM CITRINUM*. C. N. Akem and R. A. Melouk, Department of Plant Pathology, USDA-ARS, Oklahoma State University, Stillwater, OK 74078

Sclerotia of *Sclerotinia minor* were soaked in a conidial suspension ( $1.3 \times 10^7$  conidia/ml) of *Penicillium citrinum* at 25 ± 2 C for 1 hr. This resulted in coating each sclerotium with about  $3.7 \times 10^5$  conidia. Treated sclerotia were either incubated in diffused light on damp Whatman No. 1 filter paper or in pasteurized soil at 25 ± 2 C, for up to six weeks. Seventy four percent of sclerotia incubated in pasteurized soil were colonized with *P. citrinum*, whereas seventy percent colonization occurred in sclerotia incubated on damp filter paper. Field observations at Stillwater, Oklahoma indicate that about 50% of sclerotia of *S. minor* recovered from soil were colonized by *P. citrinum*. This suggests a potential use of *P. citrinum* as a biocontrol agent for *S. minor*.

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COLONIZATION OF POTATO TUBERS AND REDUCTION OF BACTERIAL SOFT ROT. John Bahme, M. N. Schroth and A. R. Weinhold, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Certain strains of *Pseudomonas fluorescens* when inoculated onto potato seedpieces subsequently colonized the periderm of field-grown daughter tubers in peat-loam and loamy-sand soils. This was associated with a reduction in the postharvest incidence of bacterial soft rot. In the heavy peat-loam soil, only tubers attached < 6 cm from the seedpiece were colonized ( $\bar{x} = 3 \times 10^4$  cfu/tuber), resulting in an overall soft rot reduction of 30%. Tubers grown in the lighter loamy-sand soil were colonized in all areas of placement averaging  $8 \times 10^3$  and  $1 \times 10^5$  cfu per tuber distal and near to the seedpiece, respectively. Incidence of soft rot was reduced 45%. Reduction of bacterial soft rot of progeny tubers was positively related to colonization, and was influenced by soil type. To improve colonization, a slow-release bacterial granule formulation ( $10^9$  cfu/g) was incorporated into the top 15 cm of the potato bed at planting. After the first irrigation, populations of the bacterium within the treated zone averaged  $1.5 \times 10^6$  cfu/g soil.

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PREPARATION OF PROTOPLASTS FROM CONIDIA OF *TRICHODERMA*. T. E. Stasz and G. E. Harman, Dept. of Horticultural Sciences, Cornell University, NY 14856

*Trichoderma* spp. are promising biocontrol fungi, and strains with various desirable traits have been isolated. Protoplast fusion may provide a means to cross selected parents for strain improvement by parasexuality. As a first step, we have prepared protoplasts from conidia, which are uninucleate. Walls of very young (< 24 hr), but not older, conidia fluoresced when stained with 0.2 mg/ml Calcofluor white M2R. Conidial suspension was freed of hyphal fragments and mixed 1:1 (v/v) with 0.45 M filtered enzyme/osmoticum solution containing 20%  $\beta$ -glucuronidase type H-2, 20 mg/ml Driselase, 50  $\mu$ g/ml chitinase (O. C. Yoder, personal communication) and 1.2 M sucrose. Spheroplasts formed within two hr at 37 C as indicated by loss of wall staining; protoplasts formed within four hr as indicated by cell enlargement, vacuole formation and development of blebs. Bleb-protoplast interfaces stained, indicating cell wall synthesis.

BINDING OF ENTEROBACTER CLOACAE TO CONSTITUENTS OF FUNGAL AND HIGHER PLANT CELL WALLS. T. E. Stagg and G. E. Harman, Dept. of Horticultural Sciences, Cornell Univ., NYSAES, Geneva, NY 14456

The biocontrol agent E. cloacae binds to Pythium hyphae and colonizes higher plant root surfaces. The ability of E. cloacae to bind to specific polysaccharides was tested. Non-substituted (crystalline or  $\alpha$ ) cellulose did not agglutinate a bacterial suspension but ionic or esterified (carboxymethyl or DEAE) cellulose did. Pectin and polygalacturonic acid were positive. Hemicellulosic materials varied in their reaction; gum arabic and arabinogalactan were negative, but xylans, carrageenan, barley gum, locust bean gum, and gum tragacanth agglutinated the bacterial cells. Some  $\beta$  1,3- or  $\beta$  1,6- linked glucans, similar to those in fungal cell walls, were positive. None of a variety of synthetic dextrans (Ficoll, Sephadex or DEAE Sephadex) or other polysaccharides (agar or gum xanthan) caused agglutination. These data indicate that E. cloacae cells contain agglutinins capable of binding to various components of fungal and higher plant cell walls.

POTENTIAL OF DICHTOMOPHTHORA PORTULACAE FOR BIOLOGICAL CONTROL OF COMMON PURSLANE. J. M. Klisiewicz, USDA-ARS, Department of Plant Pathology, University of California, Davis, CA 95616.

Dichotomophthora portulacae causes blackening and constriction of the basal stem of purslane in nature. Growth and reproduction of the fungus, and its pathogenicity on leaves and stems of purslane were studied in a controlled environment. D. portulacae produces sclerotia and conidia on diseased tissue. It readily sporulates and produces large quantities of conidia on artificial media. Optimum temperatures for production of conidia and germination were 24 and 33 C, respectively. Conidia germinated within 1 hr at 15 to 42 C and infection occurred within 2 to 8 hr at 15 to 33 C. Moisture is required for germination and infection. Necrotic sunken lesions formed on infected leaves within 48 hr after inoculation. Leaves turned yellow and dropped within 4 to 7 days. Branches developed black lesions and terminal dieback. Progression of disease in lateral branches and stems led to collapse and death of the plant.

LARGE SCALE PRODUCTION OF PRUNIPHAGE FOR BIOCONTROL OF PRUNUS BACTERIAL SPOT DISEASE IN FIELD. Randhawa, P.S. and Civerolo, E.L. USDA Fruit Laboratory, Beltsville Agricultural Research Center -W, Beltsville, MD 20705.

High titre phage lysates ( $10^{11}$  -  $10^{12}$  pfu/ml) were prepared by incubating Xanthomonas campestris pv. pruni (Xcp) and pruniphage in nutrient glucose sodium chloride broth for 24 h. Pruniphage was purified by precipitation from lysates adjusted to 7.5 % polyethylene glycol (PEG) and 0.5 M sodium chloride. Crude phage lysates diluted in tap water and containing  $10^7$  to  $10^9$  pfu/ml reduced bacterial spot disease on apricot fruits but lysates in which infectivity was neutralized with anti-phage serum were ineffective. Partially purified phage in tap water reduced bacterial spot disease on foliage of commercial peach trees at two of four selected locations. On young symptomless leaves on phage-treated peach trees 42-50 % of the Xcp population was pruniphage resistant and different in plasmid DNA content from the phage propagating strain.

TALAROMYCES FLAVUS, AN EFFECTIVE MYCOPARASITE OF SCLEROTINIA SCLEROTIIFORMIS. D.L. McLaren<sup>1</sup>, H.C. Huang<sup>2</sup>, and S.R. Rimmer<sup>1</sup>. Department of Plant Science, University of Manitoba, Winnipeg, Manitoba<sup>1</sup> and Research Station, Agriculture Canada, Lethbridge, Alberta, Canada<sup>2</sup>.

Laboratory and field investigations indicate that T. flavus is a promising agent for biological control of sclerotinia wilt of sunflower. T. flavus is destructive to hyphae and sclerotia of Sclerotinia sclerotiorum, the causal agent of sclerotinia wilt of sunflower. In dual culture, hyphae of T. flavus grew toward the host and coiled around the host hyphal cells. The coiling effect intensified as the hyphae of T. flavus branched repeatedly on the host surface. Tips of hyphal branches often invaded the host by direct penetration of the cell wall. Infection of host cells resulted in plasmolysis, granulation of the cytoplasm and finally collapse of cell walls. Results of a 3-year field study showed that T. flavus significantly reduced the incidence of sclerotinia wilt in sunflower. Wilt incidence for T. flavus-treated plots was 4, 0, and 2% compared with 47, 54, and 50% in control plots.

A CYTOPLASMIC HYPOVIRULENT STRAIN OF ENDOTHIA PARASITICA WITHOUT DOUBLE-STRANDED RNA (dsRNA). Dennis W. Fulbright, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Hypovirulence in E. parasitica has been found to occur as transmissible and nontransmissible forms. Transmissible hypovirulence has been associated with cytoplasmic-borne dsRNA while nontransmissible hypovirulence has been associated with nuclear genes. Another form of hypovirulence has been found in a strain of E. parasitica (CL25) recovered from a blighted but surviving American chestnut tree found within an infected, declining American chestnut grove in Michigan. This hypovirulent strain has no detectable dsRNA and induced a host defense reaction in cankers established by vegetatively compatible, virulent strains. Unlike dsRNA-associated hypovirulence, the hypovirulent phenotype in CL25 was maternally inherited in a limited ascospore study.

EFFECT OF PUCCTINIA CHONDRILLINA ON THE POPULATION DENSITY OF CHONDRILLA JUNCEA (RUSH SKELETONWORM) IN CALIFORNIA. D. M. Supkoff, D. B. Joley, and J. J. Marois\*, California Department of Food and Agriculture, Biological Control Services Program, 3288 Meadowview Road, Sacramento, CA 95832 and \*Department of Plant Pathology, University of California, Davis, CA 95616.

Three biological control agents, P. chondrillina (rust), Cystiphora schmidtii (gall midge) and Aceria chondrillae (gall mite) were established on C. juncea in California. Plant population densities and control agent densities were monitored yearly for 6 yr at five sites. The best correlation of plant density and control agent population was between the incidence of P. chondrillina on the rosette and the next season's plant density. At five separate sites the linear correlation coefficient for this relationship ranged from -0.54 to -0.78 ( $p < 0.01$ ). During the six years of the study, the decrease in plant populations ranged from 57% to 82%. The percent incidence of rust ranged from 4% to 83%, depending upon the year and site.

IMPORTANCE OF MYCOPARASITISM IN THE BIOLOGICAL CONTROL OF RHIZOCTONIA SOLANI WITH GLIOCLADIUM VIRENS. C. R. Howell, USDA, ARS National Cotton Pathology Research Laboratory, P.O. Drawer JF, College Station, TX 77841

Gliocladium virens is a mycoparasite of Rhizoctonia solani. It encircles and penetrates the hyphae of the plant pathogen, resulting in rapid death of the host mycelium. When G. virens is grown in a peat moss- czapek's broth mixture (PMC2B) and applied to cottonseed at planting in R. solani infested soil, the emerging cotton seedlings are protected from damping-off. When a strongly parasitic strain of G. virens was irradiated with ultraviolet light, several mutant strains were isolated that showed no mycoparasitic activity. When PMC2B cultures of these strains were compared with the parent isolate as seed treatments in pathogen infested soil, all were equally capable of controlling damping-off of cotton seedlings. These results indicate that mycoparasitism is not an important mechanism in the biological control of cotton seedling disease by this method.

INOCULUM PRODUCTION FROM WINTER WHEAT STEMS TREATED WITH BENZIMIDAZOLE FUNGICIDES FOR THE CONTROL OF PSEUDOCOSPORELLA HERPOTRICHOIDES. T. D. Murray, Dept. of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

Stems of winter wheat treated with Topsin-M, Mertect 340-F, and Benlate PNW for the control of strawbreaker foot rot, caused by Pseudocosporella herpotrichoides, were collected from plots after harvest and assessed for disease severity. Stems from individual plots were incubated outside during autumn to stimulate production of conidia. Conidia were then washed from the stems and counted. The number of conidia per stem increased with increasing disease severity. Stems treated with Topsin had the lowest disease index (2.09) and the fewest conidia per stem ( $1.48 \times 10^5$ ). Mertect-treated stems had the highest disease index (3.02) and the most conidia per stem ( $11.39 \times 10^5$ ), and Benlate-treated stems had an intermediate disease index (2.65) and number of conidia per stem ( $6.75 \times 10^5$ ). There may be a long-term benefit to applying a fungicide that controls disease and reduces inoculum production for subsequent crops.

PERSISTENCE OF PENCYCURON FOR THE CONTROL OF RHIZOCTONIA BROWN PATCH OF ST. AUGUSTINEGRASS. M.P. GRISHAM, US Sugarcane Field Laboratory, P.O. Box 470, Houma, LA 70361.

Brown patch of St. Augustinegrass (*Stenotaphrum secundatum*) caused by *Rhizoctonia solani* was effectively controlled in College Station, TX, by four fungicides when applied at the time of first disease symptoms in the fall and again two weeks later. Effective concentrations of wettable powders in grams of active ingredient per 100 m<sup>2</sup> were for penycuron, 28.3, 56.7, or 113.3; PCNB 366.2; furmecyclox, 91.5 or 183.1; or iprodione, 30.5, respectively. A single application of these fungicides applied at the time of first disease symptoms provided effective control for 3 weeks. Ten weeks after the single fungicide application only penycuron-treated plots (with <12% increase in disease area) differed from the untreated control (with >50% increase in disease area). Following winter dormancy, the percentage of dead stolons in the turf was <10% in the penycuron-treated plots, 14% in the higher rate furmecyclox-treated plots, and >50% in the plots of all other treatments.

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Control of *Alternaria* leafspot on *Cossypium barbadense*. M.W. Olsen and P.J. Cotty. Dept. of Plant Pathology, University of Arizona, Tucson 85721

Growers in central Arizona became concerned about yield losses in Pima cotton (*Gossypium barbadense*) during recent years when high incidences of *Alternaria* leafspot occurred. The activities of three systemic fungicides against *Alternaria macrospora* were studied. Tilt, Baycor and Bayleton were evaluated in vitro at 1 ppb, 100 ppb and 10 ppm on 10% V-8 agar at 27 C under 12 hr diurnal light. All three fungicides reduced radial growth at 10 ppm, only Tilt reduced growth at 100 ppb and none was effective at 1 ppb. Plants were sprayed to runoff with either 135 ppm Tilt, 150 ppm Bayleton or 150 ppm Baycor 48 hr prior to inoculation with 4,500 spores/ml and subsequent incubation in a humidity chamber for 48 hr. Tilt, the most efficacious treatment, reduced lesion development 98% and thus may be useful in evaluating yield losses in Pima cotton.

## 406

ATTENUATION OF METALAXYL ON/IN POTATO LEAVES OVER TIME AND BY ACIDIC RAIN. van Bruggen, A.H.C., Milgroom, M., Osmeloski, J., Fry, W.E., and Jacobson, J., Boyce Thompson Inst. and Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

As part of a research project on the influence of acidic precipitation on plant disease management we studied the effect of acidic rain on metalaxyl concentration on/in potato leaves. Rooted potato cuttings, cv Norchip and Monona, were sprayed with 200 ppm metalaxyl (Apron 25 WP) 78, 30, 6 and 0 hr before being exposed to simulated acidic rain (pH 2.8, 3.4, 4.0 and 4.6) for 0, 10, 20, or 30 min at a rate of 8 mm/hr. Metalaxyl concentrations were assessed on leaf discs using a bioassay with *Phytophthora boehmeriae*. Treated and untreated plants were then inoculated with *P. infestans* and incubated in a mist chamber at 18C. The metalaxyl concentration declined exponentially with time and rainfall duration (despite its systemic nature). The rate of fungicide wash-off was not affected by pH or cultivar, but there were several statistically significant interactions. Despite a fast decrease in metalaxyl concentration, residual concentrations were still sufficient to control late blight.

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CONTROLLING SEED-ROT AND SEEDLING DISEASES IN RICE. M. C. Rush, Dept. of Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803.

In Louisiana seed-rot and water-mold diseases of water-seeded rice are caused primarily by *Achlya klebsiana*, *A. conspicua*, *Pythium dissotocum*, and *P. spinosum*. Seedling blight on dry-seeded rice is caused primarily by seedborne fungi, such as, *Bipolaris oryzae*, *Curvularia lunata*, *Curvularia* spp., *Fusarium solanum*, and *Fusarium* spp. Seed-protectant fungicide tests were conducted using three water-management-planting systems. These were water-seeded, drained and flushed (flooded and drained) as needed; water-seeded, continuous flood; and drill-seeded, flushed as needed. The planting-management systems and the seed-protectant fungicides both affected stand establishment. The percent stands obtained with untreated seeds over 9 years of tests were 41.3, 27.3, and 48.5, respectively, for the three manage-

ment systems. Using the most effective fungicidal treatment in each test, the stands were increased to 55.3, 42.3, and 66 percent, respectively.

## 408

PARASITIC FITNESS OF BENOMYL-RESISTANT AND BENOMYL-SENSITIVE *MONILINIA LAXA*. Victor M. Cañez, Jr. and Joseph M. Ogawa. Department of Plant Pathology, University of California, Davis, CA 95616

Isolates of *Monilinia laxa* resistant and sensitive to 1.0 mg/L benomyl were obtained from almond orchards and examined for parasitic fitness. Results obtained from similar experiments using a single spore isolate or a combination of 4 single spore isolates did not differ. Almond blossoms inoculated two days prior to bloom with various spore concentrations of benomyl-sensitive isolates resulted in a greater number of blighted blossoms than inoculations with benomyl-resistant isolates. Almond blossoms inoculated with sensitive isolates resulted in a shorter latent period and greater spore production than with resistant isolates. Almond twigs inoculated with mycelial plugs from a sensitive isolate resulted in larger cankers than with a resistant isolate. Canker lengths resulting from blighted blossoms did not differ between isolates. Overwintering ability and spore production was greater with the sensitive isolate than with the resistant isolate.

## 409

EFFECT OF SURFACTANTS ON EPICUTICULAR WAX AND INFECTION OF GRAPE BERRIES BY BOTRYTIS CINEREA. J. J. Marois, A. M. Sledsoe and W. D. Gubler. Department of Plant Pathology, University of California, Davis, CA 95616.

Mature berries of grape, *Vitis vinifera* cv. Thompson Seedless or Emperor, were sprayed until runoff with several commonly used agricultural surfactants. Grape berries dipped in chloroform for 10 sec to remove most of the epicuticular wax and berries sprayed with distilled water only were used as controls. After the berry surface was dry, 20 µl of water containing 4 x 10<sup>4</sup> conidia of *B. cinerea* was placed on each berry. The berries were incubated for 100 hr at 22 C and 95% RH. The development of fungal growth was monitored daily to determine disease incidence. Ten berries were used for each of five replicates and the entire experiment was repeated three times. Disease was lowest in the nontreated control (12.74% after 48 hr) and highest in the chloroform treatment (98.18%). Disease in the surfactant treatments was significantly (p <= 0.05) less than the chloroform control and significantly greater than the nontreated control.

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SUPPRESSION OF BOTRYTIS CINEREA LESIONS IN FRESH MARKET TOMATO FRUIT WITH FUNGICIDES. R. T. Manji and J. M. Ogawa. Department of Plant Pathology, University of California, Davis, CA 95616

Development of *B. cinerea* lesions was prevented with a post-harvest treatment of 3% imazalil in wax 0, 4, and 8 hr after inoculation of mature green tomato fruit. Lesions on tomatoes developed when treated 16 and 24 hr after inoculation but 3% imazalil in wax was significantly better than the combination of 1.5% dicloran (DCNA) plus 2.5% orthophenolphenate (OPP) in wax. Tomato fruit inoculated postharvest with a *B. cinerea* spore suspension and treated with imazalil in wax in a commercial treater had significantly fewer rots than fruit treated with DCNA plus OPP in wax. However, both postharvest treatments were significantly better than no treatment. Imazalil residues of 1-2 µg/g are necessary for effective control of *B. cinerea* in fresh market tomatoes.

## 411

INCIDENCE OF FUSARIUM YELLOWS OF CELERY GROWN ON ORGANIC SOILS AFTER CROP ROTATION AND FLOODING. R. T. Awuah and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Two organic soils with histories of *Fusarium* yellows of celery caused by *Fusarium oxysporum* f. sp. *apii* race 2 and with relatively high *Fusarium* Yellows Indices (FYI) of 3.8 and 3.6 were not reduced in their conduciveness to the disease by two croppings of celery (highly resistant) or onion (non susceptible) in greenhouse pot experiments. The FYI ranged from 0 (healthy

plants) to 5 (dead plants). An organic soil with no history of the disease, but with FYI = 1.0 when tested in pot culture (pathogen already present), became conducive after two successive plantings with healthy celery seedlings. When one soil (FYI = 3.8) was flooded prior to planting with celery in pots, the FYI decreased to 1.4 after 2 wk flooding. The average height of celery plants increased from 22.5 cm in the unflooded control to 27.7 in flooded soil. The population of the pathogen was reduced from 1,033 propagules/g soil to 600 after 2 wk flooding and to 175 after 6 wk flooding.

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FUNGICIDAL EFFECT OF CHEMICALS ON NONGERMINATED AND GERMINATED STIGMINA SPORES. A. Tigchelaar, Graduate Student from the Department of Phytopathology, Agricultural University, Wageningen, The Netherlands and J. M. Ogawa, Department of Plant Pathology, University of California, Davis, CA 95616.

"Protectant" fungicides applied in stone fruit orchards for control of the shot hole disease were tested under laboratory conditions on nongerminated and germinated *Stigmina carpophila* spores. The cellophane-transfer bioassay method was used to evaluate effect of test chemicals on fungal germination or growth. Spores on cellophane placed onto Difco-PDA medium controls had an average germination rate of 96%. When the medium was amended with 1.8 g/L ziram or 1.8 g/L CCA 448, both nongerminated and germinated spores were inactivated within 24 and 47 hr exposure, respectively, whereas 0.64 g/L chlorothalonil inactivated nongerminated spores within 47 hr but did not affect germinated spores even after 98 hr exposure. Cooper hydroxide at 1.8 g/L showed no effects on either nongerminated or germinated spores after 98 hr exposure period. Further tests are being done to evaluate fungicidal and fungistatic values of these chemicals on *Stigmina* spores when applied in the orchard.

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COMPETITIVE FITNESS AND SURVIVABILITY OF METALAXYL-TOLERANT AND PHOSPHOROUS ACID-TOLERANT ISOLATES OF PHYTOPHTHORA CAPSICI ON GREEN PEPPERS AND IN SOIL. Leslie A. Bower and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Sandy loam soil was infested with metalaxyl-tolerant (MT), phosphorous acid-tolerant (PAT) and parental sensitive (SEN) isolates of *Phytophthora capsici*. Soil was mixed in ratios of 1:3, 1:1 and 3:1 for MT:SEN and PAT:SEN. When soil populations were assayed after 2, 4 and 8 wk greenhouse incubation, MT and PAT were recovered in higher proportions than SEN, and accounted for > 90% recovered colonies at all ratios by 4 wk. Zoospores of MT, PAT and SEN were mixed in ratios of 1:20, 1:10, 1:3, 1:1, and 3:1 for MT:SEN and PAT:SEN. After 24 hr incubation on green pepper seedlings, recovery of MT and PAT was 100% even at ratios of 1:20 in competition with SEN. Spore ratios of 1:3, 1:1 or 3:1 MT:PAT yielded equal recovery of MT and PAT. MT and PAT isolates generated in the laboratory with MNNG mutagenesis, survive well in soil and compete favorably with SEN in soil and on plants.

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FOSETYL-AL AND PHOSPHOROUS ACID, DIRECT OR INDIRECT MODE OF ACTION? M. E. Fenn and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

It has been reported that aminoxyacetic acid (AOA) and glyphosate, inhibitors of phenylpropanoid metabolism, reverse the antifungal activity of fosetyl-Al in tomato leaflets inoculated with *Phytophthora capsici* (Phytlatr.-Phytopharm. 30:257-272). In our experiments glyphosate did not reverse the activity of fosetyl-Al or phosphorous acid ( $H_3PO_3$ ). At suboptimal concentrations for inhibition the efficacy of fosetyl-Al (130  $\mu$ g/ml) and  $H_3PO_3$  (90  $\mu$ g/ml) was reduced by 0.08 mM AOA. However, fosetyl-Al at 260  $\mu$ g/ml and  $H_3PO_3$  at 180  $\mu$ g/ml were not affected by AOA. With *P. capsici* in liquid culture 0.08 mM AOA caused a 62% reduction in uptake of  $H_3PO_3$  over 3 hr. A laboratory developed mutant of *P. capsici*, which was  $H_3PO_3$ -tolerant in vitro, was also insensitive to both  $H_3PO_3$  and fosetyl-Al in vivo.

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IN VIVO EFFICACY OF FIVE PHOSPHITE COMPOUNDS AGAINST PHYTOPHTHORA CAPSICI ON PEPPER PLANTS. D. Oulmette and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Pepper seedlings (cv. Yolo Wonder) were placed in 0.005 M solutions of diethyl phosphite (DEP), dimethyl phosphite (DMP), sodium monoethylphosphite (FOS-NA), hypophosphite (HP), or phosphite (PA) and simultaneously inoculated with zoospores of either *P. capsici* (P1319), or a PA-tolerant mutant (H36) derived from P1319 by nitrosoguanidine mutagenesis. After one week at 26°C, plants were assayed for the % infection (as determined by recovery of *Phytophthora* from plant tissue), and disease severity, as determined by a Disease Severity Index (DSI), in which 0 = no disease and 3 = plants dead. Plants inoculated with P1319 and treated with PA, DMP and DEP all had DSI values of 0, with a % infection of 0, 20 and 80, respectively. Plants inoculated with P1319 and treated with FOS-NA and HP were 100% infected and had DSI values of 1.8 and 2.6, respectively. Plants inoculated with H36 and treated with PA and DMP had DSI values of 1.4 and 2.2, respectively. Plants inoculated with H36 and treated with DEP, FOS-NA and HP had DSI values of 3.0, and were 100% infected.

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IN VITRO AND IN VIVO ASSESSMENT OF PHYTOPHTHORA PALMIVORA STRAINS RESISTANT TO PHOSPHOROUS ACID. T. E. Dolan and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Isolates of *Phytophthora palmivora* resistant to  $H_3PO_3$  (PA) were obtained by treating zoospores with the chemical mutagen MNNG. Isolates exhibiting low, moderate, and high resistance according to radial growth characteristics on PA amended media were selected for in vivo resistance assessment. Tomato seedlings were exposed to a concentration series of PA and inoculated with zoospores of the parental or resistant strains. PA at 100  $\mu$ g/ml completely controlled the parental and low-resistance strains, but failed to control strains exhibiting moderate or high in vitro resistance to PA. PA also affected in vitro zoosporangium production. The  $EC_{50}$  value for PA in the parental strain was < 2  $\mu$ g/ml, while  $EC_{50}$  values for PA for resistant strains were 5-20 times higher. Similar experiments with fosetyl-Na paralleled results obtained with PA. The in vitro and in vivo activity of phosphate against the parent and PA resistant strains is also being examined.

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EVALUATION OF FIELD INOCULATION TECHNIQUES FOR SCREENING CORN GENOTYPES AGAINST KERNEL INFECTION BY ASPERGILLUS FLAVUS IN MISSISSIPPI. Natale Zummo and G. E. Scott, USDA-ARS and Mississippi State Agricultural and Forestry Experiment Station, Mississippi State, Mississippi 39762

The pin bar inoculation technique for screening corn genotypes against *Aspergillus flavus* infection in the field was compared with (a) needle application of inoculum through the silk channel directly onto the ear, (b) infested toothpick in the ear, (c) infested toothpick in the silk channel, and (d) infested string around the silks. Pin bar and toothpick inoculation in the ear caused some mechanical damage to ears, whereas no injury occurred using the other three techniques. Kernel infection percentages were 10.4 for the needle, 10.1 for pin bar, 5.0 for toothpick in the ear, 3.3 for string around the silks, and 2.5 for toothpick in the silk channel. Although the needle method did not produce more kernel infection than the pin bar technique, it was easier to apply and did not require hand shelling of inoculated ears.

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LOCALIZATION OF INFECTION BY FUSARIUM MONILIFORME, CEPHALOSPORIUM ACREMONIUM, AND ASPERGILLUS FLAVUS IN CORN KERNELS IN MISSISSIPPI. Natale Zummo, USDA-ARS and Mississippi State Agricultural and Forestry Experiment Station, Mississippi State, Mississippi 39762.

Surface sterilized kernels of eight uninoculated corn genotypes were cut in half transversely and plated on Czapek solution agar (CSA) with and without 7.5% sodium chloride. On amended CSA, *Fusarium moniliforme* was isolated from the pedicle (cob) end of 20% of the kernels and from the apical end of 9% of the kernels. Isolations from the same crosses on unamended CSA showed that 20% of the kernels were infected with *Cephalosporium acremonium* at the pedicle end while 2% were infected at the apical end. Among five corn genotypes in which ears were pin bar inoculated with *Aspergillus flavus*, 4% of the *A. flavus* infection occurred at the pedicle end and 5% at the apical end. When kernels from ears inoculated with *A. flavus* were dissected aseptically and the scutellum and embryo removed from the endosperm and plated on unamended CSA, 0.4%, 0.0%, and 0.2% *A. flavus* infection was recorded from the endosperm, scutellum, and embryo, respectively.

EVALUATION OF SEED TREATMENTS TO PREVENT GERMINATION OF *Tilletia indica* TELIOSPORES. J.L. Smilanick, M. Dupler, K. Wiese, and J.A. Hoffmann. USDA-ARS, Logan, Utah 84322

Wheat seed lots containing 5% seeds infected with the Karnal bunt fungus, *Tilletia indica*, were treated with disinfectant solutions or fumigants. To assess germinability after treatment, teliospores were removed from seeds and incubated 4 wk at 15C on water agar. Seeds were incubated 4 days at 25C on moist paper. Immersion in formaldehyde solution (5-10 mg/l, w/v, 10-20 min) or 40% ethanol (10 min) prevented teliospore germination without affecting seed viability. Sodium hypochlorite (5%, v/v, pH 6, 10 min), chlorine dioxide (50 mg/l, w/v, 45 min), or hot water (54C, 30 min) reduced teliospore germination 90% without affecting seed viability. Cupric acetate (1%, v/v, pH 5) or other ethanol concentrations were phytotoxic at dosages insufficient to prevent teliospore germination. Chloropicrin, sulfur dioxide, or methyl bromide fumigations at 32-322, 7-235, or 30-1300 mg/l for 24 hrs, respectively, were phytotoxic at rates lower than would inhibit teliospore germination. None of the fumigants reduced teliospore germination more than 75%.

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COTYLEDON LESIONS AND BLEMISHING OF JAPANESE DAIKON SPROUTING RADISH CAUSED BY MECHANICAL INJURY OF SEED. P. M. Beckman. Nickerson-Zwaan Veg. Res. Center, P.O. Box 1787, Gilroy, CA 95021

Pacific Northwest seed production of sprouting radish has rapidly increased since 1981. Blemished cotyledons, which seriously reduce seed marketability, are characterized by blackened or chlorotic lesions typically on cotyledonary undersides, margins or other surfaces contiguous to the seed coat. Reduced vigor and size and malformation of the sprout can also occur. A microbial etiology was not indicated because surface sterilization or fungicidal treatment did not affect blemishing, lesions did not enlarge under various incubation conditions, and isolations yielded no organism or non-pathogenicity, even after wounding. Comparisons of seed samples taken both at combining and after subsequent handling and milling showed extensive differences in damage in 3 seriously affected lots (P<.01). Symptoms could be duplicated by vigorously shaking seed in a glass jar or by mechanical scarification, with damage increasing with severity of treatment (P<.01). Handling procedures that minimize dropping or shearing actions on seed will reduce cotyledon damage due to mechanical injury.

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Development of soybean seedcoat structures: relevance to field seed pathogen infection and breeding for resistance. Duncan A. Vaughan, R.L. Bernard, USDA and Department of Agronomy, University of Illinois, Urbana, IL 61801, and J.B. Sinclair, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Microscopic and ultra-microscopic natural openings or areas of weakness such as micropyle, pores (holes) and pits (depressions) on the surface of the soybean seedcoat afford potential entry routes for the infection of soybean seeds by pathogens. The development of the soybean seedcoat surface, as seen by scanning electron microscopy, is traced in PI 181550 (reported to be resistant to *Diaporthe phaseolorum* var *sojae*) and cultivar Union. Observations of field fungi entering the developing seed suggest the importance of the micropyle as an ever present natural opening in the seedcoat. Variation in the soybean germplasm exists with respect to these various seedcoat structures. The relevance of this variation to breeding for resistance is discussed.

## 422

DETECTING HYPHAE OF ENDOPHYTIC FUNGI IN SEEDLING MERISTEMS OF PERENNIAL RYEGRASS AND TALL FESCUE. R.E. Welty, M.D. Azevedo, K.L. Cook. USDA ARS, Dept. Botany and Plant Pathology, Oregon State University, Corvallis 97331.

A test was developed to detect endophyte (*Acremonium loliae* and *A. coenophialum*) hyphae in meristems and leaf sheaths of perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*). Seeds were soaked 1 hr in captafol (4F) at 14,780 µg/ml or 7,390 µg/ml in deionized water, air-dried overnight, and germinated (7 days at 5 C in dark, followed by 14 days at 8 hr light 25 C and 16 hr dark at 15 C). Seedlings were digested 16-18 hr in 5% NaOH with 0.1% trypan blue, boiled 20 min in lactophenol with 0.1% trypan blue, squash-mounted, and examined at x 200. Captafol at 7,390 µg/ml reduced seedborne species of *Alternaria*, *Cladosporium*, and *Epicoccum*, allowed

growth of *A. coenophialum* and *A. loliae* in meristems or leaf sheaths, and caused no phytotoxicity. Captafol at 7,390 µg/ml stunted roots. The technique provides a rapid method for detecting viable endophyte in these grass species.

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SURVIVAL OF ENDOPHYTE HYPHAE IN SEEDS OF TALL FESCUE STORED ONE YEAR. R. E. Welty and M. D. Azevedo. USDA ARS, Dept. Botany and Plant Pathology, Oregon State University, Corvallis 97331.

Seeds of tall fescue (*Festuca arundinacea*) were stored 1 year at 10, 20, or 30 C above saturated aqueous solutions providing 5 relative humidities (RH) from 12-95%. At monthly intervals, seeds were germinated and presence of live *Acremonium coenophialum* was determined by a grow-out test. Only data for 85% RH are presented. Germination of seeds stored at 10 C for 1, 6, and 12 months was 98, 96, and 94%, and endophyte survival was 98, 74, and 24%, respectively. Germination of seeds stored at 20 C for 1, 6, and 12 months was 100, 84, and 10%, and endophyte survival was 100, 20, and 10%, respectively. Germination of seeds stored at 30 C for 1, 6, and 12 months was 96, 4, and 0%, and endophyte survival was 82, 2, and 0%, respectively. Seed moisture content (12-month mean) at 10, 20, and 30 C was 19.4, 17.8, and 16.6%, respectively. No storage condition tested maintained seed viability at 50% or greater with a concomitant reduction in viable endophyte of 5% or less.

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PREVALANCE OF NON-CHOKE-INDUCING ENDOPHYTES IN TURF AND FORAGE GRASSES. P. M. Halisky, D. C. Saha, and C. R. Funk. N.J.A.E.S., Cook College, Rutgers University, New Brunswick, NJ 08903.

Non-choke-inducing (NCI) endophytes (*Acremonium* spp.) systemically infect ryegrasses and tall fescue without inducing external symptoms. A survey was conducted to determine the prevalence of NCI endophytes in turf and forage grasses by seed analysis. A total of 534 cultivars and selections were processed using an alkali-soak-stain method and examined microscopically for endophyte mycelium. Endophyte was observed in perennial ryegrass, tall fescue, hard fescue, strong creeping red fescue, Chewings fescue, but not in either Kentucky bluegrass or blue sheeps fescue. The presence of endophyte in fine fescues enhanced insect resistance and turf performance. New Jersey Agricultural Experiment Station, Publication Nos. K-11130-2-85 and K-15267-7-85.

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DEVELOPMENT OF TECHNIQUES FOR INTERNATIONAL TRANSFER OF MICROORGANISM-FREE RICE GERMLASM. K. Kaymanesh and N. C. Rush, Dept. of Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803.

The major pathogens of rice, except for viruses, are seedborne. These include the majority of fungal pathogens, the major bacterial pathogen, and the white-tip nematode. Some of the microflora associated with rice seed are suspected pathogens with their etiological relationships still undefined. A method is needed for shipment of microorganism-free rice germplasm among cooperating international and government institutions. Detached embryos were successfully sterilized with hydrogen peroxide, mercuric chloride, and mercuric iodine in 50% ETOH without significant phytotoxicity. Embryos maintained in the dark on culture media survived for 4 weeks, a period considered the maximum time necessary for shipment. Methods for controlling germination and growth while in shipment or storage are being investigated. Shipment of germplasm in sterile culture should significantly reduce the time required for quarantine procedures.

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A SEED-WASHING AND AGAR PLATING ASSAY FOR *XANTHOMONAS CAMPESTRIS* PV. *CAROTAE*. R. E. Williford and N. W. Schaad, Dept. Pl., Soil and Ent. Sci., Univ. of Idaho, Moscow, ID 83843.

Bacterial blight of carrots, caused by *Xanthomonas campestris* pv. *carotae* (XCC), is seed transmitted and a problem in seed production fields. We have developed a seed assay utilizing XCS agar (Williford, R. E. and Schaad, N. W., Phytopath. 74:1142), a semiselective medium, for detecting XCC contaminated seed lots. To extract the pathogen, 10,000 seeds are shaken in 80 ml sterile 0.85% NaCl plus 0.01% Tween 20 at 5°C for 16-18 hr. Samples of 0.1 ml are plated, in triplicate, onto XCS agar. Sensitivity was determined by assaying 10,000



healthy seeds to which was added: 1) known numbers of colony forming units (cfu) of XCC, and 2) one and two naturally contaminated seeds with  $10^3$  to  $10^6$  cfu of XCC per seed. As few as  $1.5 \times 10^3$  cfu of XCC or a single naturally contaminated seed was detected. In addition, XCC was detected in 44 of 51 commercial seed lots with counts ranging from  $10^3$  to  $10^7$  cfu/10,000 seed.

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A SEMISELECTIVE MEDIUM FOR ISOLATION OF CORYNEBACTERIUM MICHIGANENSE SUBSP MICHIGANENSE. M. Fatmi and N. W. Schaad, Dept. Pl., Soil & Ent. Sci., Univ. of Idaho, Moscow, ID 83843

Corynebacterium michiganense subsp michiganense (C.m.) is a destructive seedborne pathogen of tomato. To determine the presence of C.m. in seeds, we developed a semiselective basal agar medium. The selective C. michiganense (SCM) agar contains the following: sucrose, nicotinic acid, L-tryptophane,  $KH_2PO_4$ ,  $K_2HPO_4$ ,  $MgSO_4 \cdot 7H_2O$ , LiCl, nalidixic acid, cycloheximide and potassium tellurite. Colonies become visible after 4-5 days at 26°C. After 8 to 10 days, they are slightly mucoid, convex, round, shiny, dark grey in color and 1.5 mm in diameter. Over 99% of the saprophytic bacteria of tomato seed which grew on nutrient broth yeast (NBY) agar were inhibited on SCM agar. Recoveries of five strains of C.m. ranged from 35 to 106% on SCM and 8 to 63% on C. nebraskense selective (CNS) medium of Gross and Vidaver, as compared to NBY agar. A further advantage of SCM agar over CNS medium is that colonies of C.m. are more easily differentiated from tomato seed flora.

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SCAB OF WHEAT AND BARLEY IN SOUTHERN IDAHO. L. J. Mubata and R. L. Forster; University of Idaho Research & Extension Center; Route 1, Kimberly, ID 83341.

Scab epidemics in wheat and barley occurred in southern Idaho in 1982 and 1984 and resulted in estimated yield losses of up to 50%. Laboratory analyses of 12 wheat and one barley seed samples obtained from scab infested fields showed 0.1 and 1.0 ppm vomitoxin contamination in two wheat samples and in the barley sample, respectively. Twenty five seeds/sample were plated on acidified corn meal agar after which suspected Fusarium colonies were transferred to carnation leaf agar for species identification. Fusarium spp. infested from 0 - 84% (mean = 26%) of the seeds in the 13 samples. Of the 81 Fusarium isolates obtained, 81% were E. culmorum. Other species included: E. avenaceum, E. graminearum, E. acuminatum, and E. soliseti. Germination of scab infested seed on blotter paper was between 78 and 91% (mean = 86%). Additional germination, species identification, and seed treatment studies are in progress.

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A COMPARISON BETWEEN ISOLATES OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA FROM SOYBEAN SEEDS IN IOWA AND STEM CANKERED SOYBEANS IN SOUTHERN STATES. D. C. McGee and J. Biddle, Department of Plant Pathology, Seed and Weed Sciences, Iowa State University, Ames, IA 50011.

Twenty three seed-borne isolates of Diaporthe phaseolorum var. caulivora Athow and Caldwell (DPC) from soybeans grown in different locations in Iowa, and 16 isolates from stem cankered plants from Mississippi, Georgia and Florida were tested for pathogenicity against seedlings of varieties Bragg, Tracy, Harsoy, Hawkeye, Williams and BSR 201 under laboratory conditions. All Iowa isolates were moderately virulent on all six varieties. Ten southern isolates were highly virulent on Bragg and avirulent on the other varieties and 6 were moderately virulent on Bragg and BSR 201, and avirulent on the others. Cultural characteristics, determined by growing isolates for four weeks on PDA plates at 25 C under constant light, showed that the Iowa isolates were easily distinguishable from southern isolates. The data confirmed that the northern population of DPC is different from the southern, which seemingly is not present in Iowa.

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PROTECTION OF SEEDLINGS FROM DAMPING OFF CAUSED BY RHIZOCTONIA SOLANI WITH HYPOVIRULENT R. SOLANI. B. Sneh, M. Ichtelevich-Auster, I. Barash & Y. Koltin, Dept. of Botany and Dept. of Microbiology, Tel Aviv University, Israel 69978.

Seeds of radish, carrot, cotton or wheat were inoculated with a

hypovirulent strain (AG4) either in TWA plates or in a soil mix (1 g mycelium/kg soil). They were challenged after three days with virulent strains of different AG, either by transfer to a soil mix infested with the virulent strain, or by adding the virulent strain on soil surface as a suspension. Seventy-six to 94% of the plants inoculated with the avirulent strain were protected from damping off symptoms. When soil was infested with both virulent and hypovirulent strains prior to sowing, 69% were protected when the ratio of virulent to hypovirulent was 1:1 and 78% were protected at the ratio of 1:2. Addition of either medium A broth or hypocotyl extract 3 times during 3 days after challenge inoculation did not reduce protection, indicating that protection was not due to competition for nutrients.

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INCREASED GROWTH RESPONSE IN PLANTS INDUCED BY NON-PATHOGENIC RHIZOCTONIA SOLANI. B. Sneh, M. Zeidan, I. Barash & Y. Koltin, Dept. of Botany & Dept. of Microbiology, Tel Aviv University, Israel 69978.

More than 100 isolates of R. solani were obtained from plant debris particles in soil samples collected from various locations in Israel. Pathogenicity was determined in TWA plates on 11 different host plants. Some of the non-pathogenic isolates to all hosts tested have demonstrated induction of enhanced germination and growth of the seedlings. One isolate (AG4) was chosen for further studies. Inoculum was prepared on autoclaved wheat seeds. Infested grains were placed either below surface of soil mix (soil, peat, vericulite 1:1:1, 10 grain/kg in pots), or in the furrow (50 grains/m row in mini plots of  $1 \times 1 \text{ m}^2$ ) of a sandy loam soil (5 replicates). Autoclaved infested grains served as control. Radish, carrot, lettuce or wheat were planted immediately after inoculation. Pots were placed in a growth chamber at 25C. After a growing period, plants growing in the inoculated soil weighed 30-90% more than the ones growing in the control soil.

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ECOLOGY OF RHIZOCTONIA SOLANI AND BINUCLEATE RHIZOCTONIA-LIKE FUNGI IN PEANUT IN INTEGRATED PEST MANAGEMENT. D. K. Bell, D. R. Sumner, J. R. Stansell, and E. D. Threadgill, Coastal Plain Experiment Station, Tifton, GA 31793

Florunner peanut was grown on Bonifay sand in a 2-yr rotation with a corn-lima bean double-crop from 1981-1983. Whole plots were tillage treatments (disking, in-row subsoiling, or mold-board plowing); subplots were day or night sprinkler irrigation; and sub-subplots were PCNB-fensulfothion (PCNB-F) over the row vs no fungicide-nematicide (NF-N). Each year 100 visibly sound seed from each sub-subplot were collected from pods attached to plants at digging. Seed were incubated on tannic acid-benomyl agar and cultures of Rhizoctonia solani and binucleate Rhizoctonia-like fungi (RBN) identified. There were no differences among tillage and irrigation treatments in numbers of cultures isolated. Less R. solani AG-4 (P=0.05) was isolated from PCNB-F than from NF-N treatments in 1981 (1.3 vs 3.3%) and 1983 (1.1 vs 4.1%), but not in 1982 (2.2 vs 0.6%). There were no differences between PCNB-F and NF-N in percentages of R. solani AG-2 (type 1 or 2) or RBN isolated (0.03-0.40% each year).

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ECOLOGY OF RHIZOCTONIA SOLANI AND RHIZOCTONIA-LIKE FUNGI IN A CORN-PEANUT ROTATION IN MICROPLOTS. Donald R. Sumner and Durham K. Bell, University of Georgia Coastal Plain Station, Tifton, GA 31793.

Microplots of Dothan loamy sand were fumigated with metam sodium in 1982 and separately infested with Rhizoctonia solani AG-4, AG-2 type (T) 1, or AG-2T2; Rhizoctonia-like (binucleate) CAG-2, CAG-3, CAG-4, or CAG-5; or noninfested. Corn or peanut was planted alternately in the spring for three years. Microplots were fallow in the winter. Crown and brace root rot (CBRR) was present on 41, 60, and 66% of the roots in microplots infested with AG-2T2, and 10, 6, and 57% of the roots on 4-6-wk-old corn in microplots infested with AG-2T1, respectively, 1982-1984. CBRR was not observed, or was negligible in other treatments. In soil infested with AG2T1 or AG2T2, respectively, AG2T1 or AG2T2 was isolated from 0.8-7.0% of visibly sound peanut seed each year at digging. The other basidiomycetes (except CAG-2) were isolated from 0.8-1.8% of the peanut seed in respective infested soil in 1984.

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ANASTOMOSIS GROUPING OF SIX MULTINUCLEATE ISOLATES OF

#### RHIZOCTONIA SOLANI FROM PACIFIC NORTHWEST CEREALS.

E. N. Bassett, D. M. Weller, and R. J. Cook, WSU, Pullman, WA

One isolate of *Rhizoctonia solani* from roots of barley from Clyde, WA, 2 from culms and one from roots of common wheat from Hermiston, OR, and 2 from roots of durum wheat from Tetonia, ID, were paired with each other and with 10 isolates representing anastomosis groups (AG) 1, 2, 2-1, 2-2, 3, 4, and 5. None of the 6 anastomosed with any of the tester isolates available. Three AG's were distinguished within the 6 isolates; 1 consisted of the 2 isolates from durum (D-1 and D-23), the second of the 2 isolates (H-18 and H-24) from common wheat culms and the third of the isolates from barley (C-1) and common wheat (H-1) roots. All 6 caused root-rot of wheat in greenhouse tests. On PDA, D-1 and D-23 grew rapidly with profuse aerial hyphae and produced small brown sclerotia randomly within 10 days at 20 C. H-18 and H-24 grew slower, but produced numerous small dark-brown sclerotia in concentric rings within a week. C-1 and H-1 grew slowest and produced light-brown sclerotia infrequently.

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*Rhizoctonia solani* and *R. solani*-like binucleates associated with potato plants and soils with varied cropping histories. D.E. Carling and R.H. Leiner. Univ. of Alaska, 533 E. Fireweed, Palmer, AK 99645.

*Rhizoctonia* disease of potato is a common problem for growers in Alaska. Surveys of soils and potato plants were conducted in 1984, and isolates of *R. solani* were categorized by anastomosis group (AG). Of 224 isolates recovered from plants (71 lesion, 80 hymenial, 73 sclerotial), 73.7% were AG-3, 20.1% AG-2-1 and 3.6% were multinucleates that did not anastomose with tester isolates from any of the described groups. These nonanastomosing multinucleates may constitute an undescribed anastomosis group. The remaining 2.7% were binucleate. Binucleates and nonanastomosing multinucleates were recovered from lesions and hymenia. AG-3 and AG-2-1 were recovered from lesions, hymenia and sclerotia. The beet seed baiting technique was used to isolate *R. solani* from soil. AG-3 was recovered from soils where *Rhizoctonia* diseased potatoes had been grown one or two years previously, but not from plots free of potatoes for five or more years. AG-2-1 was recovered from all soils tested, suggesting it may be indigenous. Binucleates and nonanastomosing multinucleates were also recovered from soil.

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#### SCLEROTINIA SCLEROTIIFORM CAUSING CROWN AND STEM ROT OF ALFALFA.

R. G. Gilbert, USDA-ARS, Irrig. Agric. Res. & Ext. Center, P.O. Box 30, Prosser, WA 99350.

Sclerotinia crown and stem rot has caused considerable losses in dense stands of seed alfalfa in the Pacific Northwest. Initially, *Sclerotinia trifoliorum* was assumed to be the causal agent. However, apothecial production occurred from March through May and ascospore development was not dimorphic. The average spore size was 11.3 x 6.4 u. These results indicated that *Sclerotinia sclerotiorum* was the disease causing species. The production of sclerotia was determined in plant and soil samples collected monthly. Alfalfa stems and surface plant residue contained from 70 to 80% of the sclerotial inoculum, while the soil contained 20 to 30%. Results have shown that fall burning (Oct-Nov) reduced the number of sclerotia per M<sup>2</sup> of plant canopy by 95% and decreased the viability of the remaining 5% of the sclerotia in the surface soil to 14%. The viability of sclerotia in controls was >95%.

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A SEEDLING TEST TO EVALUATE VIRULENCE OF *SCLEROTINIA SCLEROTIIFORM* ISOLATES ON SUNFLOWER. Berlin Nelson, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

A seedling technique was developed to evaluate virulence of *Sclerotinia sclerotiorum* isolates on sunflower. Surface sterilized seeds of hybrid 894 were placed in sterile, wet, seed germination paper rolls (Anchor Paper) and incubated 7 days at 20C. The seedlings were then inoculated by placing mycelium + agar plugs from PDA cultures of *S. sclerotiorum* onto the bases of the stems. Inoculated seedlings were incubated for 72 hours at 20C. Virulence was measured as the length of decayed stem tissue. Statistically significant differences in virulence among isolates were detected. Maintaining sterility throughout the procedure was essential for repeatability. The technique is proposed as a tool for identifying virulent isolates used for research on *Sclerotinia* wilt of sunflower.

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INOCULUM PREPARATION OF *SCLEROTINIA SCLEROTIIFORM* FOR INFECTION OF SUNFLOWER AND OTHER HOSTS. Berlin Nelson, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

A method of preparing inoculum of *Sclerotinia sclerotiorum* was developed for infection of sunflowers and other susceptible crops. Three 11 x 12 cm sheets of bathroom tissue (Charmin, plain, white, unscented) were placed in 100 x 15 mm petri plates and 15 ml of a solution of 1% yeast extract, 1% casamino acids and 2% dextrose was added to each plate. The tissue medium was sterilized, then small plugs of agar with mycelium (from 7-10 day old PDA cultures) were placed around the periphery of the tissue. A dense mycelium covered and permeated the tissue after 7-10 days incubation at 20C. The tissue + mycelium was cut into 35 x 35 mm pieces and inoculated onto sunflower, bean and soybean. All inoculations were successful. The advantages of the tissue + mycelium inoculum are 1) ease of preparation, 2) rapid and uniform mycelial growth, 3) adjustable inoculum size, 4) retention of moisture, 5) ease of placement on plant parts and 6) consistent disease development.

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HABITAT CHARACTERIZATION OF *TRICHODERMA HARZIANUM* IN NATURAL SOIL BY MICROSITE ANALYSIS. D. M. Eastburn, E. E. Butler, J. J. Marois, and J. M. Dunaway, Dept. of Plant Pathology, University of California, Davis, CA 95616.

In an attempt to identify factors associated with the natural soil habitat of *Trichoderma harzianum*, microsites composed of 50 ms samples were removed from a natural field soil and assayed for 57 different factors. Samples were taken at depths of 0 to 15.2 cm in increments of 1.3 cm from 7.6 cm diam soil cores. Individual samples were assayed for nine abiotic factors, total populations of fungi, bacteria, and actinomycetes, and individual populations of forty five different fungi. Regression analysis of data from four initial sample periods (94 samples) resulted in a multiple regression model composed of ten variables. Variation in the population of *T. harzianum* was positively correlated with the presence of plant roots, two types of *Fusarium oxysporum*, two *Penicillium* spp., and negatively correlated with the electrical conductivity of the soil solution, total fungal population, a *Verticillium* sp., and two unidentified fungi.

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THE EFFECTS OF *TRICHODERMA HARZIANUM* ON ROOTING OF CHRYSANTHEMUM CUTTINGS. T. Paulitz, M. Windham, and R. Baker. Dept. of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO. 80526.

*Trichoderma harzianum* has recently been shown to cause increased plant growth independent of any pathogen involvement. Cuttings of chrysanthemums, 'White Marble' and 'Charisma' were rooted in steamed peat-perlite medium containing peat-wheat bran inoculum of *T. harzianum* isolates T-12 or T-95 (1%, V/V). Both *T. harzianum* isolates caused significant increases in shoot height, shoot dry weight, and number of nodes.

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USE OF SEED PRIMING FOR CONTROL OF *PYTHIUM ULTIMUM* SEED DECAY AND DAMPING-OFF OF SUGAR BEETS. R. M. Osburn and M. N. Schroth, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Seed priming reduces seed germination time, and is particularly beneficial under suboptimal temperature and moisture conditions. The effect of priming on sugar beet seed and seedling infection by *Pythium ultimum* was studied in growth chamber experiments (21 C day/16 C night, 0.15 bar  $\mu$ m). Primed seed was prepared by initially washing with water, then priming in 0.34 M NaCl for 7 days. Primed seed advanced seedling emergence by ~3 days, and significantly reduced seed coat infection by *P. ultimum* from 83.3% to 6.7%, and seed decay and seedling damping-off from 44.8% to 10%, compared to an untreated control. Application of *Pseudomonas putida* strain R20 to primed seed eliminated seed and seedling disease. In experiments using 0.1 M MgSO<sub>4</sub> for priming, washing of seed prior to priming significantly improved control of seed coat infection compared to priming alone (55.9% reduction of infection compared to 26.7%). Primed seed releases a reduced amount of exudate upon imbibition, which may result in a reduced stimulation of *P. ultimum* and consequently a reduced level of infection and disease.

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Detrimental effect of surface sterilization on the isolation of *Pythium*

spp. from feeder roots. M.E. Stanghellini and W.C. Kronland, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Feeder roots are commonly surface sterilized in sodium hypochlorite or ethyl alcohol prior to isolation attempts. Our objective was to determine the effect of surface sterilization on the isolation of *Pythium dissotocum* from infected lettuce roots. Infected roots, collected from lettuce plants grown hydroponically, were cut into 35-52 mm length segments. A portion of the segments (11) was washed in running tap water for 3 min., blotted dry, and placed on water agar. A second portion (24 segments) was placed in 70% ethyl alcohol for 1 min. and a third portion (20 segments) was placed in 0.5% sodium hypochlorite for 1 min. After treatment, roots were rinsed in running tap water for 3 min., blotted dry, and placed on water agar. The fungus was consistently isolated from all non-surface sterilized roots but not from any of the surface sterilized roots after 48-96 hr incubation at 25C. Surface sterilization had the same detrimental effect on the isolation of *P. aphanidermatum* from infected spinach roots.

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THE NUCLEOTIDE SEQUENCE OF THE CODING REGION OF BARLEY STRIPE MOSAIC VIRUS RNA. G. D. Gustafson and S. L. Armour, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285

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FINE STRUCTURE OF THE TURNIP CRINKLE VIRUS CAPSID PROTEIN GENE. J. C. Carrington and T. J. Morris, Department of Plant Pathology, University of California, Berkeley, CA 94720.

TCV contains a single-component, single-stranded RNA genome of 4.0 kb. Neither genomic, nor two major subgenomic RNAs contain significant stretches of polyadenylation. We constructed cDNA clones representing 3'-proximal sequences of the TCV genome, which is the region proposed to harbor the coat protein gene. The nucleotide sequence of a 1451 bp insert was determined by the chemical cleavage method. This revealed the presence of a 3'-noncoding region of 257 nucleotides. This is preceded by an open-reading-frame of 1053 nucleotides which probably codes for coat protein. Expression of this gene has been shown by others to require subgenomic RNA. The sequence upstream from the initiation codon of this putative gene is particularly adenosine and cytidine rich, and contains a 12-nucleotide direct repeat at positions -31 to -20 and -17 to -6 (relative to the AUG). Homology between TCV and carnation mottle virus RNA non-coding regions will also be discussed.

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COMPARISON OF THREE SATELLITE-LIKE RNAs ASSOCIATED WITH TOMBUSVIRUSES. B.I. Hillman and T.J. Morris, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Several electrophoretically distinct, low molecular weight, satellite-like RNAs have been observed in association with tomato bushy stunt virus (TBSV). Two such species have been isolated and shown to be individually infectious, provided any of several tomosvirus genomic RNAs was present. These were compared to one another and to a satellite RNA associated with cybidium ringspot virus, another member of the tomosvirus group, in terms of infectivity, efficiency of encapsidation, symptom attenuation in *Nicotiana clevelandii* and nucleotide sequence relationships as determined by blot hybridization. While infectivities for the three were similar, the CyRSV satellite was much more efficiently encapsidated than the TBSV-associated RNAs. The larger of the two TBSV satellites had the greatest symptom attenuating effect, while the CyRSV satellite had very little. Both RNAs associated with TBSV hybridized to cDNA clones derived from the viral genome, while that associated with CyRSV did not.

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SYNTHESIS OF FULL-LENGTH cDNA CLONES OF TMV. D. L. Beck, D. Knorr, G. Grantham, and W. O. Dawson, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

The genome of tobacco mosaic virus (TMV), a 6.4 kb (+) sense RNA virus, was copied into cDNA and cloned in pBR322 in *E. coli*. Overlapping cDNA clones representing different parts of the TMV genome were generated by poly A tailing virion RNA and oligo-(dT) primed reverse transcription, followed by second

strand synthesis. cDNA corresponding to the 3' and 5' ends of the viral RNA was synthesized by oligonucleotide priming. The 3' and 5' specific primers, 12 and 14 deoxyribonucleotides long respectively, contained terminal restriction sites to allow exact excision of the TMV sequences. Internal and end-specific clones were ligated into full-length TMV sequences and cloned in the PstI site of pBR322. Several combinations of genomic length TMV cDNAs have been assembled, each conforming exactly to the predicted restriction enzyme patterns from the published sequence for TMV (PNAS 79:5818, 1982) for all (>15) restriction enzymes tested. In addition, the 5' end "variable region" has been sequenced and conforms exactly to the published sequence.

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THE STRUCTURE OF TOBACCO RINGSPOT VIRUS SATELLITE RNA. Jamal M. Buzayan, Wayne L. Gerlach and George Bruening, Department of Plant Pathology, University of California, Davis, CA 95616.

The ca. 360 nucleotide residue satellite RNA of tobacco ringspot virus (STobRV RNA) replicates extensively only in tissue that also is infected with its supporting virus, tobacco ringspot virus (TobRV). STobRV RNA reduces TobRV accumulation and the severity of symptoms induced by TobRV alone but lacks any obvious structural similarity to TobRV genomic RNAs. Covalent dimeric and trimeric forms of STobRV RNA self-process in an autolytic reaction that requires no enzyme. We have analyzed several isolates of STobRV RNA with the intent of understanding the structural basis for the three functions that have been identified: template for transcription, autolysis and encapsidation in TobRV coat protein. Both nucleotide sequences and secondary structures were conserved for the terminal regions of the isolates but two variations of an internal sequence and structure were found. In addition, the folding of the two monomeric units of the dimeric RNA resembles very closely the folding of monomeric RNA.

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RNA PRODUCTS OF IN VITRO TOBACCO MOSAIC VIRUS REPLICATION. Nevin Young, Peter Palukaitis, and Milton Zaitlin. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

The structure of plant viral replicative intermediates is poorly understood. We have analyzed the labeled products of "run-off" replication in cell-free extracts prepared from infected tobacco tissue. Replicative form (RF, double-stranded genomic TMV), replicative intermediate (RI, rapidly-labeled, partially RNase A-sensitive structures), and three lower molecular weight components similar in size to the double-stranded forms of TMV subgenomic RNAs were observed upon gel electrophoresis. Separation of replicate products on denaturing gels led to several bands of discrete sizes. Denaturing gel electrophoresis of isolated RI produced bands similar in size to some of those obtained from the complete replicate reaction. Experiments with RNase will be described which assess the single and double-stranded nature of the RI.

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PURIFICATION, SEROLOGY, AND CYTOLOGY OF PEANUT MOTTLE VIRUS. Z. Xiong, D. E. Purcifull, and E. Hiebert. Dept. Plant Pathology, University of Florida, Gainesville FL 32611.

Peanut mottle virus (PMoV) was purified from infected *Pisum sativum* tissue by extraction in 0.5 M potassium phosphate, pH 8.0, containing 20 mM Na<sub>2</sub>EDTA and 20 mM Na<sub>2</sub>SO<sub>3</sub>; clarification with 25% of a mixture of CHCl<sub>3</sub> and CCl<sub>4</sub>(1:1,v/v); concentration by PEG precipitation; and by density gradient centrifugation in Cs<sub>2</sub>SO<sub>4</sub>. The cylindrical inclusions (CI) of PMoV were partially purified by treatment of the low speed centrifugation pellet from the virus purification procedure with 2% Triton X-100. Antisera were produced to purified virions, and to capsid and CI proteins purified by preparative SDS-PAGE. Structures with striations similar to those of the nuclear inclusions (NI) induced by tobacco etch virus (TEV) were observed. CI and NI were detected in PMoV-infected pea tissue by immunofluorescent staining with antisera to PMoV CI and TEV NI, respectively. SDS-immunodiffusion tests confirmed that PMoV NI and TEV NI were antigenically related, although they were not identical.

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MAPPING OF THE PEANUT MOTTLE VIRUS GENOME BY IN VITRO TRANSLATION. Z. Xiong, D. E. Purcifull, and E. Hiebert. Dept. of Plant Pathology, University of Florida, Gainesville FL 32611.

Purified peanut mottle virus (PMoV) RNA was translated in the rabbit reticulocyte lysate and wheat germ systems. The translational products were analyzed by immunoprecipitation with antisera to several potyviral proteins. Proteolytic processing of the *in vitro* translational products was apparent when products formed in the presence of dithiothreitol (DTT) were compared with those formed in its absence. In the absence of DTT, PMoV-RNA was translated into 84k and 210-250k polypeptides, which were subsequently cleaved into smaller proteins by incubation with added DTT. A proposed map of the PMoV genome from the 5'-terminus to the 3'-terminus is as follows: the genome-linked protein, 34k unknown protein, 50k helper protein, 42k unknown protein, 68k cylindrical inclusion protein, 50k nuclear inclusion protein, 53k nuclear inclusion protein, 34k coat protein and the poly A tail.

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**PHOTOAFFINITY LABELING OF VIRAL INDUCED NUCLEOTIDE BINDING PROTEINS.** D. A. Roth, B. E. Haley and R. K. Evans. Dept. Plant Science and Biochemistry, University of Wyoming, Laramie, WY 82071.

The photoaffinity analogs 8-azidoadenosine 5'-triphosphate [ $8N_3ATP$ ] and 8-azidoadenosine 5'-triphosphate [ $8N_3GTP$ ] can be utilized to identify and study nucleotide binding proteins induced by viral pathogenesis. A 120,000 Mr protein and a 72,000 Mr protein were specifically labeled with  $8N_3ATP$  and  $8N_3GTP$ . The following criteria for specific incorporation have been satisfied: a) photodependent insertion into a very few proteins in a system containing numerous proteins, b) utilization of the probe as a substrate or reversible inhibitor, c) saturation effects, and d) decreased photoincorporation of probe in the presence of appropriate concentrations of the natural nucleotides. The 120,000 Mr and the 72,000 Mr proteins have nucleotide binding characteristics expected of a RNA dependent RNA polymerase and a protein kinase, respectively.

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**SEQUENCE ANALYSIS OF THE  $\gamma$  RNAs OF THE TYPE AND ND18 STRAINS OF BARLEY STRIPE MOSAIC VIRUS.** R. Hanau, G. D. Gustafson\*, R. G. Hunter, S. L. Armour\*, and A. O. Jackson, Dept. of Botany & Plant Pathology, Purdue Univ., W. Lafayette, IN 47907 and \*Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46205

There are strain specific differences in the length of BSMV RNA $\gamma$ . For example, RNA $\gamma$  of the Type strain is about 3600nt long, while RNA $\gamma$  of the ND18 strain is about 3250nt. The increased size of the Type RNA $\gamma$  probably results from a 366nt direct repeat which we have located near the 5'-end of this RNA. The repeat is within an open reading frame (ORF) and thus could also explain the differences observed in the M $_r$  of the polypeptides translated *in vitro* from (M $_r$ =82,000) and ND18 (M $_r$ =71,000)  $\gamma$  RNAs. Both  $\gamma$  RNAs contain a second ORF followed by a poly(A) tract and a tRNA-like structure at the 3'-end. The 3'-ORF sequence is also present in a subgenomic RNA of approximately 500nt which is produced by both strains and which directs the synthesis *in vitro* of an M $_r$ -20,000 protein.

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**EVIDENCE OF PROTEOLYTIC PROCESSING OF POTYVIRAL POLYPEPTIDES IN INFECTED PROTOPLASTS.** Z. Xu, R. E. Rhoads and J. G. Shaw, Depts. Plant Pathology and Biochemistry, University of Kentucky, Lexington, KY 40546.

Small amounts of high molecular weight, viral-specific polypeptides were detected in extracts of tobacco protoplasts infected with tobacco vein mottling virus (TMV). These products appeared at approximately the same time after inoculation that the TMV helper component (HCP) and cylindrical inclusion (CIP) proteins were first detected. Among these products, two major polypeptides of 90 kD and 150 kD were shown to be immunologically related to HCP and CIP, respectively. Incubation of protoplasts at high temperature (37°C) and with the protease inhibitor TLCK failed to increase the production of the 90 kD and 150 kD polypeptides. However, a new polypeptide of 125 kD, which was related to the 49 kD nuclear inclusion protein, was enhanced in the protoplasts treated with TLCK.

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**SEQUENCE ANALYSIS AT THE 3' TERMINUS OF THE GENOMIC RNA OF SONCHUS YELLOW NET VIRUS.** Louis A. Heaton, Douwe Zuidema, and A. O. Jackson. Dept. of Botany and Plant Pathology, Purdue

University, West Lafayette, IN, USA 47907.

Sonchus yellow net virus (SYNV) is a plant rhabdovirus with a negative-stranded RNA genome. The viral genome is about 13,000 nucleotides long and presumably codes for five nonstructural mRNAs. The nucleotide sequence at the 3' end of the genomic RNA was determined by a combination of sequencing methods. Hybridization of 3' end-labeled genomic RNA with RNA from SYNV infected tobacco plants revealed a "leader" RNA that differed in both its size and sequence from "leader" RNAs found in cells infected with animal rhabdoviruses. The sequence adjacent to the "leader" RNA contained an antisense open reading frame that probably encodes the mRNA for the N-protein. One class of cDNA clones constructed from poly(A)<sup>+</sup> RNAs of SYNV infected tobacco cross-hybridized with cDNA clones generated from the 3' end of the viral genome. These clones were used to establish the sequence of the putative N-protein coding gene.

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**cDNA PROBES FOR DETECTION AND CHARACTERIZATION OF THE STRAWBERRY NECROTIC SHOCK STRAIN (NS) OF TOBACCO STREAK VIRUS (TSV).** D. C. Stenger, T. J. Morris, and R. H. Mullin, Department of Plant Pathology, University of California, Berkeley, CA 94720.

cDNA probes which detect TSV-NS RNAs in *Nicotiana glauca* and *Fragaria* spp. have been developed. Randomly primed cDNA probes were synthesized by reverse transcription of template RNAs extracted from purified virions. Cloned cDNA probes specific for TSV-NS RNA 3 were constructed using *E. coli* plasmid UCB and labeled by nick translation. Both random and cloned cDNA probes complementary to TSV-NS RNA were capable of detecting TSV-NS RNA in dot and northern blots of virion RNA or total ssRNA extracts from plants. TSV-NS random or cloned probes did not hybridize to virion RNAs of TSV strains WC and M, tobacco mosaic virus, or total ssRNA extracts from healthy tissue. TSV-WC random cDNA probe hybridized with TSV-WC and M RNAs, but did not hybridize with TSV-NS RNAs. Similar strain relationships were observed using ELISA and superinfection assays, indicating strain NS is distinct from the more closely related strains WC and M.

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**RATE REDUCING DISEASE RESISTANCE IN PHASEOLUS VULGARIS TO ISARIOPSIS GRISEOLA.** D. J. Hagedorn and R. E. Rand, Department of Plant Pathology, University of Wisconsin-Madison 53706.

Cultural or chemical controls for angular leaf spot of red kidney beans in NW Wisconsin are only partially effective so we searched for resistance among red-seeded bean PIs. They were grown in 12.5 cm pots, the 3/4 expanded 1st trifoliolate leaves spray-inoculated with  $10^5$  spore/ml, placed in a mist chamber for 48 h, and then in a growth chamber at 24 C. Resistance affecting rate of lesion development was found in PI 209488 from Costa Rica. Susceptible Montcalm developed many conspicuous 2+ mm dia. lesions 10 days after inoculation; 4 days later lesions were at least 5 mm dia. and leaves abscised. PI 209488 showed no symptoms after 10 or 14 days, and after 20 days only very few pinpoint-0.5 mm lesions which enlarged very slowly, rarely reaching the maximum lesion size on Montcalm, and leaf abscission was greatly delayed.

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**EVALUATION OF TOBACCO HAPLOIDS FOR RESISTANCE TO TMV, PVY, AND MELOIDOGYNE INCOGNITA USING DETACHED LEAVES.** R. C. Rufty, E. A. Wernsman, and G. V. Gooding, Jr., Departments of Crop Science & Plant Pathology, North Carolina State Univ., Raleigh 27695-7620.

Development of disease-resistant cultivars in self-pollinated crops such as tobacco can be greatly accelerated by evaluating populations of haploid plants derived from F1 progeny resistant to various diseases. A serious disadvantage of haploid breeding is the need to assess reactions to multiple pathogens on a single plant of a unique genotype. In order to avoid confounding systemic or lethal effects of diseases, we inoculated detached leaves separately with tobacco mosaic virus and potato virus Y. Resistance to *Meloidogyne incognita* was identified by the associated reaction of entries to the MN strain of PVY. Detached leaves were maintained by immersing their petioles in water until symptom expression. Symptoms of viruses in detached leaves were similar to those in intact plants. The original intact plants with identified disease resistance could then be evaluated for other traits and the susceptible genotypes discarded.

resistant lines. Total spore production was least on leaves from 16-wk old plants, but rankings of lines by total spore production were independent of plant age. Spore production on NC 3033 was 3X that on Robut 33-1, 6X that on NC 5, and 7X that on PI 259747 or NC Ac 17133 (RF).

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DETECTION OF *CROMARTIUM QUERCUM* F. SP. *FUSIFORME* IN *PINUS TAEDA* EMBRYOS USING THE ENZYME-LINKED IMMUNOSORBENT ASSAY. F. C. Spaine, H. V. Amerson and J. W. Moyer. Depts. of Botany, Forestry and Plant Pathology respectively, North Carolina State University, Raleigh, North Carolina 27695-7612.

Indirect (ID) and indirect preabsorption (IPA) ELISAs were compared for the detection of *Cromartium quercuum* f. sp. *fusiforme* (C.q.f.). The ID ELISA detected C.q.f. homogenate as low as 20 ug/ml of fungal fresh weight, however when healthy pine homogenates (HP) were mixed with C.q.f., fungal binding to the ELISA plate was reduced. The IPA ELISA eliminates HP interference; thus C.q.f. diluted in HP and C.q.f. homogenates alone are detected with equal efficiency. Initial IPA ELISAs detect C.q.f. to 10 ug/ml fungal fresh weight with current progress expected to increase this level of detection. Different families of *Pinus taeda* embryos were inoculated *in vitro* with C.q.f. basidiospores and infection quantitatively measured over time. This assay is being evaluated as a possible *in vitro* screening assay for fusiform rust resistance in *P. taeda*.

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CORRELATIONS BETWEEN REACTIONS OF SWEET CORN HYBRIDS TO GOSS' WILT, STEWART'S BACTERIAL WILT AND NORTHERN CORN LEAF BLIGHT. J. K. Pataky, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Reactions to Goss' wilt, Stewart's wilt and northern corn leaf blight were highly correlated for 75 mid-to late-season sweet corn hybrids evaluated in the 1984 Illinois sweet corn disease nursery and in greenhouse trials. In the disease nursery, correlations between severity of Goss' and Stewart's wilt ranged from 0.64 to 0.77. Hybrid rank correlations were from 0.66 to 0.74. Similar results were obtained in greenhouse trials. Correlations of hybrid reactions to the two bacterial diseases may have resulted from plant morphological characteristics or from genetic resistance. Correlations between the two bacterial diseases and northern corn leaf blight (race 1 and race 2) ranged from 0.34 to 0.63. Correlations between race 1 and race 2 of northern corn leaf blight ranged from 0.61 to 0.83; and, probably were due to polygenic resistance which displayed little or no race specificity. Hybrid reaction to rust was not correlated with reactions to the other diseases.

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SESQUITERPENOID PHYTOALEXIN PRODUCTION IN WILD, TUBER-BEARING *SOLANUM* SPECIES, K. L. Deahl, Vegetable Laboratory USDA, ARS, BARC-West, Beltsville, MD 20705

In interspecific hybrids between *S. tuberosum* and *S. demissum*, hypersensitive resistance (HR) is controlled by resistance genes (R) derived from *S. demissum*. Incompatible races of *Phytophthora infestans* elicit HR in tuber tissues characterized in part by rapid accumulation of the fungitoxic sesquiterpenoids rishitin, phytuberin and lubimin. Several races of *P. infestans* induced HR and triggered terpenoid accumulation in 3 of 15 wild, tuber-bearing *Solanum* species lacking R genes. There was considerable variation in response to *P. infestans* in 8 of the species, from extreme HR without terpenoid induction (e.g., *S. bulbocastanum*) to cases where tuber tissues displayed only susceptible reactions with copious terpenoid production (e.g., *S. phureja*). This report presents data on several previously unanalyzed species of potential use for host resistance, but questions the contribution of the terpenoids as primary factors in their resistance.

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INDUCTION OF PHYTOALEXINS IN SUNFLOWER BY FUNGAL INOCULATION. Beni Tal and David J. Robeson, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568-2685.

Sunflower (*Helianthus annuus* L., Compositae) is an agronomic crop of major economic importance in many areas of the world. The crop has been severely attacked in recent history by

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POTENTIAL SOURCES OF HEAT STABLE MELOIDOGYNE *INCOGNITA* RESISTANCE IN LYCOPERSICON SPP. I. J. Thomason and M. Ammati, Dept. of Nematology, University of California, Riverside, CA 92521 and Royaume du Maroc, Institut Agronomique et Veterinaire, Complexe D'Agadir, BP. 438, Agadir, Morocco.

The  $M_1$  gene, obtained from *Lycopersicon peruvianum*, is the source of resistance to *Meloidogyne* spp. in tomato. Resistance mediated by the  $M_1$  gene breaks down at soil temperatures above 28 C above and does not provide resistance to *Meloidogyne hapla*. Lines of *L. glandulosum* and *L. peruvianum* with resistance to several *Meloidogyne* spp., including *M. hapla*, have been isolated. Seedlings of *L. esculentum* cvs. Rutgers and VFN 8 ( $M_1$  gene) and clonal plants of *L. peruvianum* 128,657 (source of  $M_1$  gene), *L. peruvianum* 129,152 and 270,435 and *L. glandulosum* 126,443 were exposed to *M. incognita* inoculum (5000 eggs/600 cc soil) at either 25 or 32 C. Exposure time was 750 °D base 10 C. Significant root galling and egg production occurred only on the susceptible cultivar Rutgers at 25 C. At 32 C numerous eggs occurred on Rutgers, VFN 8 and line 128,657. Resistance in *L. peruvianum* 129,152 and 270,435 and *L. glandulosum* 126,443 was stable at 32 C.

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RESISTANCE OF SELECTED SOYBEAN LINES TO CYST NEMATODE, RACES 3 AND 5. A. S. Al-Jalili, V. T. Sopra, and R. P. Pacumbaba, Dept. of Natural Resource and Environmental Studies, Alabama A&M University, Normal 35762.

Eighteen soybean entries of maturity groups V to VIII were screened for two years both in the greenhouse and the field for resistance to soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, races 3 and 5. Evaluation for resistance was made at  $V_2$  (4th-trifoliolate) growth stage. Bedford, J74-51, and D77-8 were resistant to SCN, races 3 and 5. Resistant plants had from 0.5 to 2.3 cysts in the roots. Cultivars Essex, Ransom, Davis, and Lee had an average of 36 cysts in the roots and exhibited severe inhibition of rhizobium nodule formation. Yield reduction of 42-70% was observed on susceptible plants. Except for plant height, parameters such as number of cysts, nodulation/plant, and yield were significantly correlated.

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PLANT AGE EFFECTS ON EXPRESSION OF RESISTANCE TO LATE LEAFSPOT IN PEANUT. B. B. Shew<sup>1</sup>, J. C. Wynne<sup>1</sup>, and M. K. Beute<sup>2</sup>, Departments of Crop Science (1) and Plant Pathology (2), North Carolina State University, Raleigh 27695.

Leaves detached from 8, 12, or 16-wk old plants of peanut lines having high [NC Ac 17133 (RF), PI 259747], moderate (NC 5), or very low (NC 3033, Robut 33-1) resistance to late leafspot were inoculated with *Cercosporidium personatum* and incubated under intermittent mist for 41 da. Infection rates were highest on leaves from 8-wk old plants of all lines except NC Ac 17133 (RF). Rankings of lines by % lesions sporulating (% LS) varied with age until day 29; thereafter, % LS was greatest on Robut 33-1 and least on PI 259747 for all ages tested. At least 10% of lesions sporulated by day 25 for NC 3033, Robut 33-1 and NC 5, compared to day 31 for the more

various pathogens. Nevertheless, *H. annuus* has apparently received scant attention in terms of disease resistance. We recently initiated an investigation of this species for the production of antimicrobial compounds which are induced by fungal inoculation. This study resulted in the identification of two antifungal coumarin derivatives, ayapin (6,7-methylenedioxy-coumarin) and scopoletin (6-hydroxy-7-methoxy-coumarin), which may be regarded as sunflower phytoalexins. Quantitative data are presented for the kinetics of accumulation *in planta* and degradation *in vitro* following inoculation with fungi pathogenic and non-pathogenic to sunflower.

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GROWTH INHIBITION OF CLADOSPORIUM CARYIGENUM BY CONDENSED TANNIN AND ISOQUERCITRIN FROM PECAN. D. W. Laird, C. H. Graves, & P. A. Hedin, Dept. of Plant Pathology & Weed Science, Miss. State Univ., Miss. State, MS 39762, and Boll Weevil Research Lab., ARS USDA, Miss. State, MS 39762.\*

Condensed tannin and isoquercitrin extracted from mature pecan trees (*Carya illinoensis*) were tested for growth inhibition of a selected laboratory isolate and two wild type isolates of *Cladosporium caryigenum*, the pecan scab incitant. The two wild types were isolated from Van Deman (highly susceptible) and Stuart (somewhat resistant) cultivars of pecan. The quantitative assay was conducted using measured amounts of compounds in potato dextrose broth. Isoquercitrin was CA. three times more active in growth inhibition over all isolates than condensed tannin, though both were active. The laboratory isolate exhibited the least response for both compounds followed by the Van Deman isolate and then the Stuart isolate. These results indicate that these compounds may be involved in resistance to scab via a variability of isolate tolerance to these compounds between cultivars.

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Lasis of Resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybeans. K. D. Simcox, J. D. Paxton, and I. S. Bhandal. Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

Involvement of the phytoalexin glyceollin in the resistance of 1 to 4-week-old soybean plants to *Pmg* was studied using heat shock to inhibit the accumulation of glyceollin. Seven-, 14-, 21-, and 28-day-old Harosoy-63 plants were heat shocked with running 44C water at a 1.0 cm hypocotyl section for 60 min. Control plants were treated at 23C. Plants were then inoculated in the treated hypocotyl section with mycelium of *Pmg* race 1, avirulent on H-63. After 3 days, susceptibility and amount of glyceollin at the inoculation site was determined. Heat shock inhibited the accumulation of glyceollin at the site of inoculation in all plants and 7- and 14-day-old plants became susceptible. In 21- and 28-day-old plants, reduced accumulation of glyceollin did not result in increased susceptibility. Examination of stem cross-sections indicated that resistance of older plants may be due to increased lignification at the vascular bundles, rather than to the accumulation of glyceollin.

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EFFECT OF RESISTANCE AND FUNGICIDE SPRAY INTERVALS ON SEVERITY OF LATE LEAFSPOT ON THREE PEANUT CULTIGENS. G. R. Watson, T. A. Kucharek, F. M. Shakes, and D. W. Gorbet, Plant Pathology Dept., Gainesville, Quincy AREC, and Mariana ARC, University of Florida.

Severity of late peanut leafspot, caused by *Cercosporidium personatum*, was significantly lower at 113 days after planting without fungicide sprays on the cultigens Southern Runner and UF 81206 when compared to Florunner in a split-plot experimental design. Chlorothalonil used at 2.48 L/ha significantly decreased leafspot severity when used on Florunner but not on Southern Runner or UF 81206 with 10 or 20-day spray intervals. This significant cultivar x fungicide interaction was present in each of three canopy regions where leafspot was assessed.

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RATE LIMITING RESISTANCE TO PYRENOPHORA LEAF BLOTCH IN SPRING OATS. J. A. Frank and H. G. Marshall, USDA-ARS, The Pennsylvania State University, University Park, PA 16802.

Several breeding lines of spring oats from the USDA breeding program in Pennsylvania were identified as having a degree of

resistance to *Pyrenophora* leaf blotch in 1979 and 1980 field trials. These lines were evaluated for components of rate limiting resistance in greenhouse and field tests from 1981-1984. In comparison to susceptible cultivars, the relative infection efficiencies were similar. Resistant lines had smaller lesions and their lesion expansion was minimal. Latent periods were generally twice as long in resistant lines, and sporulation rates per unit of lesion area were comparable. However, if sporulation rates were calculated on a leaf area basis, susceptible leaves had a 3-5 fold increase in lesion area as compared to resistant leaves and sporulation rates increased correspondingly. Results in field and greenhouse tests were comparable except that latent periods were longer in the field.

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GREENHOUSE AND FIELD SCREENING OF SUDANGRASS ACCESSIONS FOR RESISTANCE TO STRAIN A AND B OF MAIZE DWARF MOSAIC VIRUS. Stephen R. Vann, Robert W. Toler, Dept. of Plant Pathology and Microbiology, and Frederick R. Miller, Dept. of Soil and Crop Sciences, Texas Agricultural Experiment Station, College Station, TX 77843

Seventy-three sudangrass (*Sorghum sudanense* Hitch.) accessions were screened for resistance to maize dwarf mosaic virus strains A and B. Seedlings were inoculated at the 3-6 leaf stage with a DeVilbiss spray gun at 6.33 kg/cm<sup>2</sup>; greenhouse inoculations were made with MDMV strain B and field inoculations with strain A. Symptom expression was rated after 3 weeks. Symptoms varied from mild mosaic to redleaf development. Of the accessions tested, 2 were resistant, 50 intermediate, and 21 susceptible. Resistant candidates were Krasnaplenejaja and Sweet Sudan. Examples of intermediate candidates included TE-1004 and Greenleaf; and susceptible candidates included Piper and Tift.

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HISTOPATHOLOGY OF VERTICILLADIELLA PROCERA IN SCOTS AND EASTERN WHITE PINE. W. Elliott Horner and S. A. Alexander, Dept. of Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

A histological study of diseased stem tissue was made to elucidate the colonization pattern of *Verticilladiella procera* in *Pinus strobus* and *P. sylvestris*. Naturally infected, symptomatic trees of both species were collected from commercial plantings; artificially inoculated, asymptomatic field-grown *P. strobus* were also collected. Stained sections showed that *V. procera* preferentially colonized parenchymatous cells: predominantly ray parenchyma, less frequently the epithelial cells of resin ducts, and least frequently, the axial tracheids. Penetration of cell walls was generally through pit pairs and occasionally via narrow diameter bore holes. Resin-soaking was usually present between black-stained tissue containing hyphae of *V. procera* and clear sapwood.

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GERMINABILITY OVER TIME OF VERTICILLADIELLA PROCERA PROPAGULES IN ARTIFICIALLY INFESTED SOIL AND THEIR INABILITY TO CAUSE DISEASE. Kathy J. Lewis, S. A. Alexander, Dept. Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

Germinability of *Verticilladiella procera* propagules (conidia and mycelial fragments) in artificially infested soils was studied by dilution plating on a selective medium. Under controlled temperature conditions an average of 15% of the propagules germinated after 10 months in moist soil at low temperatures (-5 and 10C). Germinability fell quickly in air dry soil at 20 and 30C. Fiberglass mesh tubes containing artificially infested soil samples were reburied in the A horizons at five different sites. Numbers of germinable propagules decreased gradually over 8 mo. Seedlings planted in an artificially infested sand-soil mix at propagule densities from 10 sp/gm to 10<sup>6</sup> sp/gm did not develop disease symptoms after up to 8 mo. in a greenhouse. These results suggest that soil-borne propagules are relatively short-lived and may not be of primary importance in disease development.

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SUSCEPTIBILITY OF SHORTLEAF AND LOBLOLLY PINE SEEDLINGS TO PHYTOPHTHORA CINNAMOMI. S. W. Fraedrich, Department of Forestry, Clemson University, Clemson, SC 29631.

Shortleaf pines and, to a lesser extent, loblolly pines, are

affected in the Piedmont by a disease termed littleleaf. High hazard sites are low in soil fertility and have inadequate soil aeration. Controversy exists as to the exact role of *Phytophthora cinnamomi* in this disease complex. Shortleaf and loblolly pines were grown from seed in sand culture for six weeks and subsequently root-dip inoculated for two-hr periods in a series of concentrations of *P. cinnamomi* zoospores. Seedlings were incubated hydroponically for 48 hrs prior to culturing lateral root tips for *P. cinnamomi*. Preliminary results indicate that 128 spores/ml are needed to infect 50% of the shortleaf pine root tips as compared to 180 spores/ml for loblolly pine. At concentrations > 2,400 spores/ml, all root tips of both species became discolored within 48 hrs. Frequency of sporangial production after 48 hrs was greater on infected roots of shortleaf pine (44%) than loblolly pine (28%).

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SOURCES OF *SPHAEROPSIS SAPINEA* INOCULUM IN FOREST TREE NURSERIES. M. A. Palmer, R. E. McRoberts, and T. H. Nicholls, North Central Forest Expt. Station, St. Paul, MN 55108

Inoculum potential of *Sphaeropsis sapinea* infected pine tissues, commonly found in forest tree nurseries experiencing outbreaks of shoot blight, was determined by monitoring seedling infection and spore dispersal from *Pinus resinosa* cones, seedlings, and litter. Infected nursery seedlings and cones collected from nursery windbreaks and from beneath windbreaks had the greatest inoculum potential. The number of spores produced by individual cones varied among and within windbreaks as did the period of peak spore production of individual cones. Disease gradients in seedbeds of 1-year-old *P. resinosa* were characteristic of spore dispersal gradients from an elevated inoculum source. The inoculum potential of cones and the pattern of disease distribution in the seedbeds suggest that cones on windbreak trees are the primary inoculum source in seedbeds of 1-year-old *P. resinosa*. The resulting infected seedlings are a source of secondary inoculum.

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FUNGI ASSOCIATED WITH THREE PINE BARK BEETLES IN SOUTH AFRICA. M. J. Wingfield, I. A. Strauss and G. D. Tribe, Plant Protection Res. Inst., Private Bag X5017, Stellenbosch, 7600 South Africa.

Three pine bark beetles, *Orthotomicus erosus*, *Hylurgus ligniperda* and *Hylastes angustatus*, are known to occur in South Africa. In order to determine their possible role as vectors of pathogenic fungi, weekly isolations were made from insects collected from *Pinus radiata* trap logs over a six month period. Isolations from approximately 1 000 insects of each species were made on a medium selective for *Leptographium* spp. and on malt extract agar. *Ceratocystis* spp. and *Leptographium* spp. were the most commonly isolated genera. *Orthotomicus erosus*, *H. ligniperda* and *H. angustatus* carried six, three and four *Ceratocystis* spp. and two, three and four *Leptographium* spp. respectively. Of these, *C. ips*, *L. serpens* and an unidentified *Leptographium* sp. were most commonly isolated and occurred on all three insect species.

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PATHOGENICITY OF *Bursaphelenchus xylophilus* TO SCOTS PINE. Peter

J. Bedker and Robert A. Blanchette, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Eighteen 10- to 15-year-old Scots pine trees (*Pinus sylvestris*) were inoculated with the pine wood nematode (*Bursaphelenchus xylophilus*) on August 2, 1984, with an equal number of trees treated as controls. Wounds, 1 cm x 3 cm that exposed the xylem, were made on each of four branches per tree and inoculated with 10,000 nematodes or a slurry produced from nematode-free cultures of *Botrytis cinerea* to serve as controls. One half of the trees were removed 10 wks after inoculation. Wounds inoculated with *B. xylophilus* were significantly larger ( $P > 0.01$ ) in length and width than the controls. Approximately 70% of inoculated branches had been girdled at the inoculation site, whereas callus tissue had formed around the control wounds. An average of 26.7 nematodes/gr of wood sampled were extracted from 7 cm branch samples that contained the inoculation sites. After 10 wks, no trees had died and no nematodes were found in the main stems of the inoculated trees or any of the controls.

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BURSAPHELENCHUS XYLOPHILUS ASSOCIATED WITH WEAKENED PINES IN SOUTHERN ONTARIO. Jennifer Juzwik, Pest Control Section, Ministry of Natural Resources, Maple, Ontario, L0J 1E0.

*Bursaphelenchus xylophilus*, the pine wood nematode, was extracted in large numbers from wood chips of branches and main stems of recently killed native *Pinus resinosa* and exotic *P. nigra cebrenensis* in two southern Ontario locations. In the York Regional Forest, Ballantrae, reddening of the tree crown and mortality of five 53-year-old plantation *P. resinosa* occurred within 7 months. *B. xylophilus* was obtained from four of the trees. Examination of three trees found *Armillaria* present and bark beetle damage common. Decline and mortality of 27-year-old *P. nigra cebrenensis* in a research planting, Turkey Point Provincial Park, near St. Williams, had been observed for at least one year. Several pest problems were associated with the decline. A number of other nematode species in addition to *B. xylophilus* were extracted from two recently killed trees. It is suggested that *B. xylophilus* hastened the death of declining trees in both locations.

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DECLINE OF MONTANE BOREAL ECOSYSTEMS IN THE SOUTHERN APPALACHIAN MOUNTAINS. Robert I. Bruck, Dept. of Plant Pathology, N. C. State University, Raleigh, NC 27650.

Over the past 20 years high elevation spruce-fir forests along the Appalachian Mountain crest have begun to exhibit marked dieback and decline symptoms. Mortality on some northern peaks has exceeded 70%. During the summer of 1984 a survey was initiated on eight 6,000+ ft southern Appalachian mountains to characterize the extent and rate of spruce-fir decline. Following the installation of 40 permanent plots it was determined that west-facing slopes exhibit more serious decline symptomatology and a greater incidence of annual ring increment suppression as compared to other aspects. Eighty-two percent of all co-dominant and dominant red spruce sampled (792) exhibited severe increment suppression during the 1958-1962 period. Soil analyses indicate that Pb, Cu, and Zn are loaded to surface horizons in concentrations from 10 to 100 times above ambient levels. Cloud interception (270+ days/yr) suggest a link between decline and anthropogenic pollution.

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EFFECTS OF SIMULATED ACIDIFIED RAIN SOLUTIONS ON ECTOMYCORRHIZAL GROWTH AND DEVELOPMENT OF LOBLOLLY PINE (*PINUS TAEDA* L.) SEEDLINGS. S. Meier, R. I. Bruck and L. F. Grand, Dept. Plant Pathology, N. C. State Univ. Raleigh 27695-7616.

Two-month-old half-sib seedlings of loblolly pine (*Pinus taeda* L.) were hand-watered with simulated rain solutions twice weekly and sampled for ectomycorrhizae at one month intervals for three months. Solutions were prepared using deionized water and the appropriate background ions and adjusted to pH 2.4, 3.2, 4.0 using stock solutions of 1N HNO<sub>3</sub>, 1N H<sub>2</sub>SO<sub>4</sub> or 1N 70 meq SO<sub>4</sub><sup>2-</sup>:30 meq NO<sub>3</sub><sup>-</sup>. Control solutions were prepared at pH 5.6 without acid additions. The percentage of ectomycorrhizal short roots and tips per centimeter of root were determined. There was a lower percentage of ectomycorrhizal short roots and tips as compared with controls at pH 4.0 for solutions prepared with 1N HNO<sub>3</sub> ( $P = .039$ ) and 1N H<sub>2</sub>SO<sub>4</sub> ( $P = .004$ ). Fewer ectomycorrhizal short roots and tips were formed at pH 4.0 using solutions prepared with 1N 70 meq SO<sub>4</sub><sup>2-</sup>:30 NO<sub>3</sub><sup>-</sup> but the effect was not significantly different from seedlings receiving pH 5.6 solutions.

ASSOCIATIONS OF THE PINE TIP MOTH WITH PITCH CANKER OF LOBLOLLY PINE. G.B. Runion and R.I. Bruck, Dept. of Plant Pathology, North Carolina State Univ. Raleigh N.C. 27695-7616

Severe outbreaks of pitch canker, caused by *Fusarium moniliforme* var. *subglutinans* (FMS), have occurred in loblolly pine (*Pinus taeda*) plantations in Eastern North Carolina. The relationship between these outbreaks and severe infestations of the pine tip moth (*Rhyacionia* spp.) was investigated in 1983 and 1984. Successful isolations of FMS on a selective medium were made consistently from a higher percentage of loblolly pine lateral shoots exhibiting tip moth damage than from those undamaged by the moth. In 1984 percentage of tip moth damaged shoots yielding FMS was correlated with host spacing and half-sib pine family. FMS was also isolated from surface sterilized and non-sterilized tip moth larvae and pupae indicating that these stages harbor viable inoculum of the fungus internally and externally. These data suggest that pine tip moth infestations may play a significant role in the epidemiology of pitch canker of loblolly pine in North Carolina.

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ROOT MOVEMENT AND ROOT INJURY ON WIND-EXPOSED SPRUCE AND FIR ON SUBALPINE SITES. D. M. Rizzo and T. C. Harrington, Dept. of Botany and Plant Pathology, Univ. of New Hampshire, Durham 03824.

Growth decline and mortality of red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) on windy subalpine sites in the White Mtns., NH may be due in part to root damage. Roots of exposed overstory trees make vertical and lateral movements that may lead to root breakage and wounding. Thousands of vertical movements, up to 6 cm, were recorded on wind-exposed trees as they swayed in winds exceeding 20 m/s (50 mph). Roots on trees sheltered within dense canopies moved less than 1 cm. Dead and broken fine roots, abrasion wounds and xylem discoloration were more evident on wind-exposed than sheltered trees. Xylem discoloration of woody roots (0.5-2.0 cm diam.), associated with wounding and invasion by hymenomycetes, correlated with growth decline on windy sites. Red spruce appears to be more resistant than fir to invasion of wounds by root rot fungi, consequently, root damage may be less important on spruce than on fir in contributing to growth decline and mortality.

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CHANGES IN WATER POTENTIAL COMPONENTS IN LODGEPOLE PINE SEEDLINGS INOCULATED WITH *ARMILLARIA MELLEAE*. Kazuo SUZUKI, Faculty of Agriculture, University of Tokyo, Tokyo, Japan 113; Kenneth I. MALLET, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6E 2G6; and Yasuyuki HIRATSUKA, Northern Forest Research Centre, Canadian Forestry Service, Edmonton, Alberta, Canada T6H 3S5

Pressure volume relations using the pressure bomb technique have been applied to predict changes in leaf water potential ( $\psi = \psi_{\pi} + \psi_p$ ) in various plants, including conifers. Water potential components ( $\psi_{\pi}$ ,  $\psi_p$ ) in lodgepole pine seedlings were measured for ca. 2 months after inoculation with *Armillaria mellea* and were compared with water-stressed and non-inoculated seedlings. Changes in water potential components were determined before and after visual symptoms appeared. This technique of monitoring disease development before the appearance of visual symptoms may be useful for studying pathogenesis of certain kinds of tree diseases.

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DIFFERENTIATION OF *PERONOSCLEROSPORA* SPECIES BY ISOZYME

ANALYSIS. J. A. Micales<sup>a</sup>, M. R. Bonde<sup>b</sup>, C. L. Peterson<sup>b</sup>, and W. E. Fry<sup>a</sup>. <sup>a</sup>Dept. Plant Pathology, Cornell Univ., Ithaca, NY 14853; <sup>b</sup>USDA-ARS Plant Disease Research Laboratory, Frederick, MD 21701

*Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis* cause important downy mildew diseases of sorghum, sugarcane, and maize; these organisms are difficult to differentiate by morphology. Isozyme analysis was used to detect 28 presumed genetic loci, coding for 26 different enzymes, in 3, 3, and 8 isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis*, respectively. Twenty-five loci were polymorphic and could be used to detect genetic variation. A maize isolate of *P. sorghi* from Thailand had no alleles in common at 25 of 28 loci with maize-sorghum isolates from Argentina and India. *Peronosclerospora sacchari* and *P. philippinensis* shared at least 22 alleles and may be conspecific. These results indicate that isozyme analysis can be used to elucidate genetic and taxonomic relationships among taxa.

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INTERCEPTIONS OF *TILLETIA INDICA* AT THE CALIFORNIA - MEXICO BORDER IN MEXICAN RAILROAD BOXCARS. T.N. Boralyanski<sup>1</sup>, T.T. Matsumoto<sup>2</sup>, and M.R. Bonda<sup>2</sup>. <sup>1</sup>USDA-APHIS-PPD, Calexico, CA 92231. <sup>2</sup>USDA 1720 N St., Sacramento, CA 95814 and <sup>3</sup>USDA-ARS PDRL, Frederick, MD 21701.

*Tilletia indica* (Mitra) Mund. (= *Neovossia indica*), the pathogen causing Karnal bunt of wheat, has been present in Mexico since 1971. The disease has not been reported in the United States; any infestation would result in immediate quarantine action. A Federal Quarantine restricts the importation of wheat from Mexico. On October 19, 1983, USDA inspectors collected wheat kernels from a Mexican boxcar at Calexico, CA. Subsequently, these teliospores were identified as *T. indica*. Laboratory analysis using a centrifuge wash technique of dust and debris resulted in the recovery of teliospores of *T. indica* in 9 of 20 samples. Few of the teliospores were viable and germinated to produce typical sporidia. These boxcars are a potential source of inoculum of *T. indica* into the United States. In December 1984, USDA placed restrictions on the movement of Mexican boxcars into the United States.

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SCANNING ELECTRON MICROSCOPE OBSERVATIONS ON TELIOSPORE SHEATH AND EXOSPORE IN SOME *NEOVOSIA* AND *TILLETIA* SPECIES. D.W. Showers, J.M. Krupp and T.T. Matsumoto. California Dept. of Food and Agriculture, 1220 N St., Sacramento, CA 95814.

The characteristics of the sheath and exospore are important criteria in the taxonomy of Ustilaginales. The sheath surrounding teliospores of certain smut fungi prevents scanning electron microscope observations of the exospore. Controversy exists in the classification of the smuts placing Karnal bunt of wheat and kernel smut of rice into either of two genera, *Neovossia* or *Tilletia*. Three known species of *Neovossia* and *Tilletia* were experimentally treated with various chemical and enzymatic procedures to remove the sheath. Sodium hypochlorite and warm potassium hydroxide in ethanol removed the sheath, but caused a distortion of exospores. Ten enzymes, individually and in combination, were tested. Chitinase appeared to be most effective in removing the sheath with minimal distortion to the exospore. In summary, the teliospores showed a wide range of exospore variation following sheath removal.

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A NEW RACE OF SUNFLOWER DOWNY MILDEW. T. J. GULYA, USDA-ARS and Dept. Plant Pathology, N. D. State Univ., Fargo, ND 58105, and N. V. Rama Raje URS, Dahlgren & Co., Crookston, MN 56716

A new race of sunflower downy mildew, *Plasmopara halstedii*, was identified on the basis of its ability to infect genotypes having the P1<sub>1</sub>, P1<sub>2</sub>, P1<sub>3</sub> or P1<sub>5</sub> resistance genes. The race, designated #4, was initially recovered from a field in north-west Minnesota and has since been confirmed in low proportions from zoosporangial collections from both North and South Dakota. Examination of zoosporangia stored in liquid N<sub>2</sub> has verified its presence as early as 1937. All sources of the P1<sub>5</sub> gene, which governs resistance to race 3, are susceptible to race 4, including USDA germplasm D4-2 and D4-3 (derived from Russian cultivars Novinka and Progress), HA-R4 and R3 (derived from Argentine cultivars Saenz Pena 74-1-2 and Guayacan INTA), and the Romanian inbred RF-5556. Resistance to races 2, 3 and 4 has been identified in selections from crosses of USDA inbred HA-89 with *Helianthus argophyllus*, *H. petiolaris*, and *H. praecox*.



OVERWINTERING MICROBIAL POPULATIONS IN WHEAT STRAW INFESTED WITH *PYRENOPHORA TRITICI-REPENTIS*. W. Pfender and S. Wootke, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Wheat straw from a field with a high incidence of tan spot was collected at grain maturity (July) and either buried in soil or placed on the surface to simulate conservation-tillage residues. Microbial populations were estimated by grinding straw segments, after washing them and removing *Pyrenophora* (Pt) ascocarps, and plating on several media. Pt in surface straw decreased from 1600 to 800 cfu/cm<sup>2</sup> between August and March, and in buried straw from 200 to nondetectable. In March, mature pseudothecia were abundant on surface straw but extremely rare on buried straw. Population of *Septoria nodorum* (Sn) also decreased greatly in buried straw while remaining high in surface straw. A varied community of fungi, including *Trichoderma* and *Pythium*, and high populations of actinomycetes developed in buried straw. In surface straw the actinomycete population was low and the variety of fungi (Pt, Sn, *Alternaria*, *Cladosporium*) changed little from July to March. Bacteria counts did not differ between treatments.

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THE EFFECT OF CULTURAL PRACTICES ON AGGREGATE SHEATH SPOT OF RICE IN CALIFORNIA. P. S. Gunnell and R. K. Webster, Department of Plant Pathology, University of California, Davis, CA 95616

Aggregate sheath spot (AGSS) of rice caused by *Rhizoctonia oryzae-sativae* has become endemic in California rice fields. The impact of cultivar, rate of applied nitrogen, and seeding rate on disease development were assessed over a three year period. Incidence and severity of AGSS were found to be primarily dependent on the cultivar grown. In general, short-statured rice cultivars were found to be more susceptible to disease than tall-statured cultivars. The effect of nitrogen on disease development of AGSS was often cultivar specific, but overall disease incidence and severity was greatest at lower nitrogen levels. The rate of seeding resulted in little or no effect on severity of AGSS. Though AGSS is endemic in California, evidence indicates that the disease is not causing significant yield losses. Stem rot of rice caused by *Sclerotium oryzae* which is also widespread in California rice fields, is more severe at higher nitrogen levels and is usually more aggressive on those cultivars which are least susceptible to aggregate sheath spot.

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HORIZONTAL DISTRIBUTION OF *RHIZOCTONIA SOLANI* (AG-1) SCLEROTIA IN TEXAS RICE SOILS. S.B. Belmar and R.K. Jones, Tx. Agr. Exp. Sta., Dept. of Plant Pathology & Microbiology, College Station, TX, 77843

*Rhizoctonia solani* (AG-1) causes sheath blight in rice and aerial web blight in soybeans. Sclerotia serve to overseason the fungus and are the primary inoculum for these crops grown in rotation. The horizontal distribution of sclerotia was determined for a 0.52 ha site within each of two commercial rice fields. Soil cores, approximately 440 cms, from four concentric 9x9 matrix patterns having 0.33, 1, 3, and 9 m between sampling points were taken. Sclerotia were extracted from soil using elutriation and counted using 10X magnification. Recovered sclerotia from 576 cores ranged from 0-44/core. Variance to mean ratios for the 0.33, 1, 3, and 9 m matrices were: 11.95/11.58, 23.39/12.20, 16.30/9.73, 70.22/16.43, for site A and 1.07/0.95, 10.93/2.33, 4.18/1.52, 3.51/1.23 for site B. The respective estimates of the clustering parameter  $K$  were: 362.42, 13.30, 14.40, 5.02 and 7.52, 0.63, 0.87, 0.67. A disease incidence reading taken at the rice panicle initiation stage for the 9 m matrix showed variance to mean ratios of 65.81/4.96 and 91.00/8.54 with clustering parameter estimates of 0.40 and 0.88, respectively.

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EFFECT OF TEMPERATURE AND PHOTOPERIOD ON RESISTANCE OF ORCHARDGRASS TO *STAGONOSPORA ARENARIA*. R.T. SHERWOOD, C.C. BERG, AND K.E. ZEIDERS. ARS-USDA, U.S. Regional Pasture Research Laboratory, University Park, PA 16802.

Plants of one susceptible and five resistant genotypes of *Lactylis glomerata* were inoculated with *S. arenaria* and incubated at 22 C for 48 hr. Before and after incubation the plants were maintained in cool (16-20 C) or warm (26-30 C) conditions with short (8-9 hr) or long (15 hr) daylengths, in greenhouse and growth chamber trials. Leaves of the susceptible genotype always developed large spots. Leaves of resistant genotypes in cool, long-day conditions developed small spots, and in warm, short-day conditions developed large spots. There were differences among genotypes in cool,

short-day and in warm, long-day treatments. Some genotype X temperature and genotype X photoperiod interactions were significant. Thus resistance to purple leaf spot may be environmentally unstable.

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INFLUENCE OF TILLAGE ON GRAY LEAF SPOT DEVELOPMENT AND NUMBERS OF AIRBORNE CONIDIA OF *CERCOSPORA ZEAEE-MAYDIS*. G. A. Payne and H. E. Duncan, Dept Plant Pathology, North Carolina State Univ., Raleigh NC 27695-7616.

Four tillage treatments (fall plow, fall disk, spring plow, and no-till planting in corn stubble) were compared for their influence on gray leaf spot development and numbers of airborne conidia of *Cercospora zeaee-maydis*. In 1984, lesions appeared earlier (2 days before tasseling) and disease was greater at each evaluation date in the no-till plots than in the other plots. By 9/12, the numbers of lesions on the fifth leaf above the ear averaged 72 and 36 for the no-till plots and for the other tillage plots, respectively. There were no differences in disease severity among the other treatments. Conidia were first trapped 5/23, 5 days after planting, but collection was erratic. Levels of airborne conidia increased beginning 7/27 in all the plots with the greatest number of conidia collected in the no-till plots. Effects of tillage on disease severity and the number of airborne conidia were similar in 1983, but the overall level of disease was less than in 1984.

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SPREAD OF CORN ANTHRACNOSE FROM SURFACE RESIDUE. P. E. Lipps, Dept. of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691.

Anthracnose leaf blight was monitored throughout the growing season to determine the spread of disease from corn residues on the soil surface in the center of two plots maintained under continuous corn or a corn-soybean rotation. The number of leaves with lesions per plant from 28 to 70 days after planting and the percentage of plants with stalk rot at the end of the growing season was negatively correlated (<0.01) with distance from the residues in both plots. The difference (P<0.01) in slopes of regression equations indicated that leaf blight spread more rapidly within rows than across rows. More leaf blight and stalk rot occurred in the continuous corn than in the corn-soybean rotation at various distances from the residue area. Results indicate that disease spread was influenced by orientation of corn rows in relation to the inoculum source and by cropping history.

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MICROORGANISMS ASSOCIATED WITH PECKY RICE. Fleet N. Lee, N. P. Tugwell, G. J. Weidemann and W. C. Smith. University of Arkansas, P.O. Box 351, Stuttgart, Ar 72160.

Bacteria, yeast and fungi were isolated from "pecky" (discolored) rice kernels and tested for pathogenicity. Developing rice caryopses in the dough stage were wounded through a small hole in the glume, inoculated and allowed to mature. Control kernels inoculated with sterile water developed a light brownish discoloration at the wound site. Inoculation with *Nematospora coryli* isolated from soybean resulted in a black discoloration seldom extending 1 mm beyond the wound site and was atypical of pecky rice. Grain inoculated with heat-killed bacteria, yeast or fungi developed similar symptoms. Inoculation with *Curvularia lunata*, *Alternaria alternata*, *Alternaria* (*Trichoconis*) *pedwickii*, *Fusarium oxysporum* or a *Bipolaris* sp. usually resulted in a black brown discoloration extending 2 mm or more from the wound site and in some instances discoloring the entire kernel. *F. oxysporum*-inoculated grain occasionally developed a creamy tint or mycelium above the seed coat. Symptoms in all fungi-inoculated grain resembled those of pecky rice.

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RESPONSE OF COTTON TO TEMIK-TSX AT VARIOUS PLANTING DATES. S. Micinski and P. D. Colyer, Louisiana State University Agricultural Center, Bossier City, LA 71113.

A study was initiated in 1984 at the Red River Research Station to evaluate the response of cotton to in-furrow applications of Temik-TSX at various planting dates. Deltapine 41 was planted with and without Temik-TSX on 15 planting dates from April 4 through May 11, 1984. Generally, application of Temik-TSX had

no effect on stand counts throughout the planting dates. However, treatment with Temik-TSX resulted in fewer thrips, increased bloom counts, and greater yields on most planting dates. The significance of these results on cotton production will be discussed.

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EFFECT OF ACREMONIUM WILT ON SORGHUM IN HONDURAS. G.C. Wall<sup>1</sup>, D.H. Meckenstock<sup>2</sup>, R. Nolasco<sup>1</sup>, and R.A. Frederiksen<sup>1</sup>, <sup>1</sup>Department of Plant Pathology & Microbiology, <sup>2</sup>Dept. Soil & Crop Sciences, TX. Agr. Exp. Stn., College Station, TX 77843, and <sup>3</sup>Recursos Naturales, Region Sur, Choluteca, Honduras.

A survey of sorghum areas in Honduras revealed the presence of Acremonium wilt on 'criollo' land races and on improved sorghum cultivars. Comparisons of healthy and diseased pairs of plants showed highly significant differences for grain weight and number of grains per panicle in a susceptible introduced cultivar (BTx623) and in criollos. Healthy BTx623 plants yielded 36% higher and had 24% more grains per panicle than diseased ones. Healthy criollo sorghum yielded 33% higher and had 26% more grains per panicle than diseased. In addition, BTx623 healthy plants were 5% taller, had 52% longer panicle exertion, and were 16% heavier in 100-grain weight than diseased ones. These differences were all highly significant. Both healthy criollos and BTx623 had significantly longer panicles (11% & 3%, respectively) than diseased plants. Cultivars SC326, 77CS1, GPRI48, SC110, and SC414 were rated resistant.

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SOME EPIDEMIOLOGICAL ASPECTS OF WHEAT LEAF RUST IN MOROCCO. B. Ezzahiri, A. P. Roelfs, and J. R. Burleigh, Dept. de Phytopathologie, I.A.V. Hassan II, Rabat, Morocco, and Cereal Rust Lab., USDA, ARS, Univ. of Minn., St. Paul, MN 55108

Wheat leaf rust was found to overwinter on volunteer wheat in an irrigated area along the Atlantic coast. Rain samplers, spore traps, and multi-location planting of a susceptible cultivar in trap plots were used to detect areas where the increase of primary inoculum occurred. Changes in cultural practices, including an increased area being irrigated thereby permitting continuous cropping and the increased popularity of bread wheats compared to more resistant durum may explain increased importance of leaf rust. One hundred and thirty-two single pustule isolates of the pathogen were used to inoculate twelve host lines with a single gene for resistance. Thirteen virulence combinations of the pathogen were detected. The virulence combination equivalent to the UN race 13, made up 80% of the total isolates. The most popular bread wheat cultivars were susceptible to most isolates.

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DEVELOPMENT AND SPREAD OF COMMON RUST IN MODERATELY RESISTANT AND SUSCEPTIBLE SWEET CORN HYBRIDS. J. M. Headrick and J. K. Pataky. Dept. of Plant Path., Univ. of IL, Urbana 61801.

Effects of host resistance and moisture on the development and spread of common rust of maize (*Puccinia sorghi*) were measured in a disease gradient study that included 2 replications of a 2 x 2 factorial. A single inoculated plant served as an infection focus in each of eight 120 m<sup>2</sup> plots of sweet corn hybrids Florida Staysweet (susceptible) and Sugar Loaf (moderately resistant). Half of the plots were irrigated. Rust incidence was evaluated on a per leaf basis at 3 to 4 day intervals in 5 directions at 5 distances from the focus. Significant differences were observed for hybrid x distance and hybrid x time interactions. The least squares linear regression models of rust development were:  $Y = -35.0 - 30.7D + 2.16D^2 + 8.8T - 0.13T^2 + 0.77DT - 0.05TD^2$  ( $R^2=0.88$ ) and  $Y = 27.7 - 25.5D + 4.30D^2 - 0.21D^3 + 2.1T - 0.15DT$  ( $R^2=0.83$ ) for Florida Staysweet and Sugar Loaf, respectively, where D = distance (m) and T = time (days). The level of resistance in Sugar Loaf appeared to adequately limit rust development and spread.

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DEW POINT MONITORING DEVICE FOR DISEASE FORECASTING. Bailey, J.E., D.K. Thomson, and R. Moates. Dept. of Plant Pathology, NCSU, Raleigh, NC 27695-7616

A microprocessor driven device was developed for implementing Jensen and Boyles' (1966) peanut leafspot advisory model. A dew point sensor consisted of a thermal electric cooler

affixed to the underside of a leaf wetness grid. Water condensed on the surface of the grid and completed the circuit. A temperature sensor imbedded into the printed circuit board on which the grid was silkscreened measured ambient temperature at  $t_0$  and the dew point temperature at  $t_n$ . Dew point was calculated by a preset program and converted to relative humidity for the leafspot model. All calculations, cooler regulation, time keeping, data recording, and information delivery was effected through a central processing unit using CMOS technology which allows for low energy consumption. The unit is portable and runs on nicad batteries recharged via solar cells.

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EVIDENCE FOR LONG-RANGE INOCULUM TRANSPORT OF PERONOSPORA TABACINA. J. M. Davis and C. E. Main, North Carolina State University, Raleigh, NC, and W. C. Nesmith, University of Kentucky, Lexington, KY.

Planetary boundary layer trajectories were calculated to examine pathways and timing of inoculum transport into tobacco areas of west-central Kentucky in 1981 and 1982. First outbreaks of blue mold each year were analyzed using the Atmospheric Transport and Diffusion (ATAD) model. First occurrences and eastward spread of blue mold in these years did not conform to the historical concept of inoculum transport from the southeast. Backward/forward trajectories supported the hypothesis that inoculum originated from wild tobacco (*N. repanda*) in southwest Texas and/or along the trajectory pathway from Texas to west-central Kentucky. A set of guidelines or "blue mold trajectory rules" were developed to identify the most probable trajectories from hundreds calculated. A revised ATAD model, i.e. the Branching Atmospheric Trajectory (BAT) model, is presently being evaluated. This model addresses the problem of restructuring the planetary boundary layer at the day/night and night/day interface.

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OBSERVATIONS ON THE DEVELOPMENT OF RESISTANCE OF FIELD GROWN BURLEY TOBACCO TO BLUE MOLD. W. C. Nesmith, Dept. of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546

Blue mold of burley tobacco, *Nicotiana tabacum*, caused by *Peronospora tabacina*, occurs sporadically in Ky. Occasionally activity is present in most fields unprotected with fungicides. More often blue mold will cause damage in some fields while being absent from others nearby, despite having apparently similar environment and management. Attempts were made to establish infections artificially at 54 of the disease-free sites, following the appearance of blue mold nearby. Inoculations were made with  $5 \times 10^6$  sporangia per/ml of water during periods considered conducive for blue mold development from July 11 to Aug 20, 1980-1984. Significant lesion development occurred at only three sites. At the others, abundant lesions developed only on sucker regrowth and an occasional (less than 2%) random plant. Lesions also developed extensively on greenhouse-grown bait plants placed in the fields. Field-grown leaves from these sites were found to be resistant when inoculated and incubated in a controlled environment along with known susceptible leaves.

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TEMPERATURE TOLERANCE OF PERONOSPORA TABACINA IN THE U.S. M. A. Moss and C. E. Main, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Macroscale epidemics of tobacco blue mold caused major crop losses in 1979 and 1980. Prior to 1979, the disease was mainly confined to plant beds. In the U.S., maximum daily summer temperatures (temp.) of 30C were considered the limiting factor in disease development in the field. During 1979, 1980 and each succeeding year, summer temp. higher than 30C have occurred with expected frequencies, yet widespread field infection, spread and damage have occurred. Recent epidemics may have resulted from a pathogen population adapted to high temp. Computer mapping of temp. during the 1980 epidemic and direct temp. experiments in the phytotron confirm the hypothesis of temp. tolerance. Isolates of *P. tabacina* collected prior to and after 1979-80 sporulated after exposure to 36/25C day/night temp. for two days. Sporangiospores were viable, germinated and caused new lesions at this same high temp. It appears that high summer temp. no longer offers an ecological constraint to blue mold and the disease may be expected to recur in the field each year.

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FACTORS AFFECTING DEVELOPMENT OF COMPUTER-SIMULATED, FOLIAR

EPIDEMICS IN MIXTURES OF RESISTANT AND SUSCEPTIBLE PLANTS. C. C. Mundt and K. J. Leonard, Dept. of Plant Pathology, North Carolina State Univ., Raleigh, NC, 27695-7616.

A modified version of the computer simulation model EPIMUL was used to study the effects of host genotype unit area, spatial distribution of initial disease, and steepness of pathogen dispersal gradients on epidemic development of foliar disease in mixtures of resistant and susceptible plants. The disease-reducing effectiveness of the mixtures relative to pure-line susceptible populations decreased as genotype unit area increased from 0.0025 to 0.56 m<sup>2</sup>. This decline was more pronounced when initial disease was uniformly distributed than when initial disease was concentrated in a single focus. The effectiveness of the mixtures also declined with increasing steepness of dispersal gradients, but more so when initial disease was uniformly distributed than when initial disease was concentrated in a single focus.

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DECISION MODELS TO AID IN CONTROL OF SOOTY BLOTCH AND FLYSPECK DISEASES OF APPLE, Harvey J. Gold and Turner B. Sutton, Biomathematics Program, Statistics, and Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

A model is presented for the optimal timing of chemical sprays to control the diseases sooty blotch and flyspeck of apple. The model accounts, at a simplified level, for dynamics of disease progress, chemical inhibition of disease spread, decay of fungistat, cost of control, and market structure for apple. The model is used, together with the formalism of decision analysis, to determine optimal spray intervals and the way in which uncertainty as to actual system parameter values influences the optimal spray interval. It is concluded that the decision as to chemical treatment is logically divided into two steps: whether or not to treat, and level of treatment. Expected crop quality is the most important determinant for the first step; fungistat decay rate for the second. Because of asymmetries in the economic loss curves, uncertainties lead to levels of treatment considerably above the deterministic optimum.

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DISEASE PROGRESSION OF ALFALFA LEAF SPOT. W. M. Thal and C. Lee Campbell, Department of Plant Pathology, North Carolina State University, Raleigh, 27695.

Progression of alfalfa leaf spot, caused primarily by Leptosphaerulina briosiana, was monitored to compare characteristics of disease development among five alfalfa cultivars and successive harvests within a growing season. Disease severity was assessed visually at 5- or 7- day intervals from March to October in replicated experiments at two locations in Wake Co., NC. Assessments were made on two randomly selected 30-cm sections in each half of the center row of three-row plots. Plant height, plant growth stage, degree of defoliation, and percent coverage of debris on the soil surface were also determined at each sampling date. Rate of disease progression and the shape of the disease progress curve were generally similar among cultivars, but varied among harvests. There appeared to be an effect due to carry-over of inoculum between the first and second, and third and fourth alfalfa harvests. Thus, both environment and previous disease level appeared to affect disease progression over the season.

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FLOODING-DEPENDENT RESISTANCE TO RICE BLAST. Chuong-Hoe Kim, M. C. Rush and D. R. MacKenzie, Dept. Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, La. 70803.

Blast epidemics in replicated plots of the rice cultivars Brazos and M-201, originating from point sources of inoculum of race IH-1 (Pyricularia oryzae Cav.), were studied for the effects of soil flooding. Disease progressed rapidly in unflooded (upland) plots of both cultivars, but was greatly reduced in flooded plots. Draining flooded plots at panicle initiation did not increase the rate of disease development. Flooding upland plots reduced disease development in Brazos but not in M-201. Lesion number per tiller and conidia per lesion were significantly greater under upland conditions. Lesion size was not affected. Relative humidity was higher in flooded plots. Ambient air temperature and leaf wetness duration did not vary significantly between treatments. Available evidence suggests that flooding affects resistance by reducing the number of successful infections.

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CLEISTOTHECIA: THE SOURCE OF PRIMARY INOCULUM FOR POWDERY MILDEW OF GRAPES IN NEW YORK. R. C. Pearson, New York State Agric. Expt. Station, Cornell University, Geneva, NY 14456.

Grape leaf segments bearing many cleistothecia of Uncinula necator (Schw.) Burr were overwintered in the field. These segments were retrieved in spring and soaked in water for 1-6 hr. Segments were then placed over glass slides or detached healthy grape leaves in petri dishes. Spores discharged onto glass slides were found in groups of 3-5 and occasionally germinated. They were identified as ascospores by comparison of their size, shape, and appearance to ascospores in crushed cleistothecia. Germinated spores in the center of 5- to 7-day-old powdery mildew colonies on detached grape leaves exposed to cleistothecia were identified as ascospores. Powdery mildew in the vineyard appeared first as discrete colonies on leaves of 15- to 20-cm shoots arising from the head of cane-pruned vines, always in close proximity to bark. Bark collected from dormant vines in early spring averaged 7 cleistothecia/cm<sup>2</sup>. Vineyard surveys failed to find evidence of perennation in infected buds.

509

EFFECTS OF TEMPERATURE, SOIL WATER POTENTIAL AND TREATMENT DURATION ON PERFORMANCE OF PHOMOPSIS-INFECTED SOYBEAN SEEDS. M. L. Gleason and R. S. Ferriss, Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Soybean seeds inoculated or not inoculated with Phomopsis sp. were incubated in soil for 3 days at 12, 18, 24, 30, or 36 C and soil  $\psi_m = -0.1$  or  $-1.5$  bars, then for 18 days at near-optimum temperature/soil moisture. In the  $-1.5$  bar treatment, inoculated seeds gave significantly lower establishment (ES) than controls at 18 and 36 C for both pasteurized and untreated soil. In the  $-0.1$  bar treatment, ES from inoculated seeds closely resembled controls. Inoculated and control seeds were also incubated for 0, 1, 3, 6 or 10 days at soil  $\psi_m = -5$  or  $-50$  bars, then soil moisture was optimized. For both soil  $\psi_m$  treatments, inoculated seeds generally had lower ES than controls for treatments  $>3$  days. ES was lower in  $-50$  than in  $-5$  bar soil for all treatments  $>3$  days. These data indicate that soil  $\psi_m$ , temperature and duration of dry soil conditions interact to influence ES from Phomopsis-infected seeds.

510

AMOUNT OF DEFOLIATION CAUSED BY PECAN VEIN SPOT DISEASE. R. S. Sanderlin, Louisiana State Univ. Pecan Research and Extension Station, LA Agric. Exp. Sta., LA State Univ. Agric. Ctr., Shreveport, LA 71135.

The amount of defoliation caused by vein spot on pecan, c.v. 'Success', was monitored for a 4 yr. period from 1980-1983. The majority of the leaf loss caused by vein spot occurred from the end of August to the end of October each year. The percent of defoliation that took place during this time period for 1980, '81, '82, '83 was 79.5, 81.9, 64.5, and 69.3, respectively, for trees on which vein spot was allowed to develop unhindered. The mean number of vein spot lesions/leaf for each of these 4 yrs. was 74.9, 75.4, 34.4, and 44.3. Trees that were sprayed with benomyl fungicide had much lower lesion numbers. The treated trees still averaged 21.7 percent defoliation. This was surprisingly high and apparently caused by the inability to completely control vein spot in addition to mite and aphid damage. Although vein spot caused severe late season defoliation, it did not appear to affect nut quality.

511

SPATIAL DISTRIBUTION OF SYSTEMIC DOWNY MILDEW, CAUSED BY PERONOSCLEROSPORA SORGHII, IN SORGHUM FIELDS. W. Schuh and R. A. Frederiksen, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

The spatial distribution of sorghum plants systemically infected by Peronosclerospora sorghii was determined at two locations using Morisita's (Res. Popul. Ecol. IV:1, 1962) index of dispersion. The disease incidence was recorded 5 and 10 weeks after planting. At each location, 1300 contiguous quadrats were assessed. The index showed a statistically significant departure from random distribution for all quadrat sizes at each location. At location A, the index had the highest value with 3.66 at the 1m<sup>2</sup> quadrat size, the lowest with 1.18 at the 64m<sup>2</sup> quadrat size; at location B, 2.38 for the 1m<sup>2</sup> quadrat size and 1.1 for the 128m<sup>2</sup> quadrat size, respectively.

A higher index represents a higher degree of clumping. The plotted index was unimodal at location A, bimodal at location B. Clumps were organized in a hierarchical structure.

#### 512

POTATO YIELD LOSS AND VERTICILLIUM INOCULUM DOSE RELATIONSHIPS FROM WISCONSIN FIELD EXPERIMENTS. S. S. Adams and D. I. Rouse, Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI 53706

Field experiments were conducted in 1984 on loamy sand soils at Hancock and Rhinelander, Wisconsin to determine the relationships between applied and assayed levels of Verticillium dahliae inoculum and potato yield loss measures. A dried and ground fungal-rye mixture, containing microsclerotia from a virulent isolate, was tilled into the soil at planting to give treatment doses. Soil samples were taken immediately, dried for 3 weeks, and assayed by soil dilution plating. Assayed inoculum levels at Hancock and Rhinelander averaged 3% and 7%, respectively, of applied doses over a range of 60 - 6000 applied propagules per gram of soil (ppgs). Recovery efficiencies tended to decrease at the higher doses. At both sites, no significant yield loss was noted below 5 assayed ppgs, and a maximum loss of about 10% of tuber fresh weight occurred at 15 or more ppgs. Slightly higher dry matter losses were recorded at Hancock. Results apply to Russet Burbank potatoes grown at commercial density.

#### 513

ROLE OF ANTIBIOTIC BIOSYNTHESIS IN RHIZOSPHERE DISEASE CONTROL: GENETIC ANALYSIS OF ANTIBIOTIC BIOSYNTHESIS IN A PSEUDOMONAS FLUORESCENS STRAIN. Neal I. Gutterson, Janet S. Ziegler, Tamara J. Layton and Gareth J. Warren, Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., Oakland, CA 94608.

A number of fluorescent pseudomonads isolated from the rhizosphere protect plants against infection by root pathogens and secrete antibiotics. The role of antibiotic biosynthesis in disease protection has not been tested rigorously. To perform such a test, mutants isogenic to the wild type strain must be constructed. A fluorescent pseudomonad, HV37a, produces antibiotic and protects cotton seedlings from Pythium ultimum-induced damping-off. Mutants deficient in antibiotic biosynthesis were isolated using NTG mutagenesis. Mutants were classified based on the ability to cosynthesize antibiotic when plated in pairs. Cosmids containing genes for antibiotic biosynthesis were identified by complementing mutants with an HV37a library. To obtain isogenic mutants, wild type genes in HV37a were replaced by non-revertible mutant alleles constructed in each cosmid.

#### 514

ROLE OF ANTIBIOTIC BIOSYNTHESIS IN RHIZOSPHERE BIOCONTROL POTENTIAL OF PSEUDOMONAS FLUORESCENS STRAINS AND DERIVED MUTANTS ALTERED IN ANTIFUNGAL PROPERTIES. T.V. Suslow. Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., CA 94608

Model systems utilizing Hesperia Fine Sandy Loam (HFSL), cotton, Pythium ultimum, and Pseudomonas fluorescens strains NZ130 and Hv37a have begun to elucidate the role of antifungal compound biosynthesis in biological control. Strains NZ130 and Hv37a produce Fe<sup>+++</sup> regulated and Fe<sup>+++</sup> independent compounds, respectively, highly inhibitory towards P. ultimum and a diversity of other fungi. Significant increases in cotton emergence and survival are observed in HFSL naturally or artificially infested with P. ultimum following seed treatment. Mutants (NTG) of these strains deficient in anti-Pythium biosynthesis did not significantly increase cotton emergence nor differ significantly from parentals in rhizosphere colonization. Genetic analysis of cloned DNA complementing anti-Pythium activity (Gutterson et al, Gill et al) will elucidate the biosynthesis of these compounds and provide defined mutants to more precisely define their role in rhizosphere biocontrol.

#### 515

Efficacy of INA<sup>-</sup> deletion mutant strains of Pseudomonas for biological control of frost injury to strawberry blossoms. Lindemann, J., Suslow, T., Joe, L. and Moayeri, A. Advanced Genetic Sciences, Inc.

Strawberry blossoms from commercial fields in Oregon and California harbored ice nucleation active (INA<sup>+</sup>) Pseudomonas syringae (Ps) and P. fluorescens biotype A (PFA). INA<sup>-</sup> deletion mutant derivatives of each

species were developed using marker exchange techniques (Green, Corotto and Warren, 1985). Blossoms of greenhouse grown strawberry plants were spray inoculated with pure cultures or mixtures of INA<sup>+</sup> and INA<sup>-</sup> bacteria, incubated under mist, assayed for bacterial populations and subjected to freezing temperatures. INA<sup>-</sup> PFA inhibited growth of both INA<sup>+</sup> Ps and INA<sup>+</sup> PFA, and protected 60% of blossoms at -3 C, whereas 100% of control blossoms were frozen at -3 C. INA<sup>-</sup> Ps protected against freezing caused by INA<sup>+</sup> Ps but did not inhibit or protect against INA<sup>+</sup> PFA.

#### 516

A SELECTIVE MEDIUM FOR ATHELIA BOMBACINA. C. S. Young and J. H. Andrews, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

A selective medium (PAB) was developed for quantifying A. bombacina, a basidiomycete antagonist to the apple scab pathogen. It consisted of an infusion from 200g of unpeeled potatoes per liter, 2% agar, 50 ppm Benomyl, 100 ppm chlortetracycline HCl, and 200 ppm streptomycin sulfate. Compact, white colonies of A. bombacina were distinctive on this medium after 6 weeks at 4°C. Sterile and nonsterile apple leaves were inoculated with A. bombacina and recovery was measured by two methods. (1) Nonsterile leaves were imprinted on PAB or potato dextrose agar (PDA). Maximum recovery, expressed as percent inoculated leaves recorded as positive after incubation, was 53% on PDA and 100% on PAB. (2) Leaves were homogenized individually in buffer and samples were plated on PAB and PDA. Maximum recovery, expressed as percent A. bombacina from nonsterile leaves versus sterile leaves, was 0% on PDA and 26% on PAB.

#### 517

BACTERIAL ISOLATES CONTROL PHYTOPHTHORA ROOT ROT OF PERSEA INDICA AND LUPIN. M. K. Kellam and M. D. Coffey, Dept. of Plant Pathology, Univ. of California, Riverside, CA 92521.

An avocado grove was identified where Phytophthora cinnamomi (Pc) was present for 20 yr. yet susceptible rootstocks showed no symptoms of decline. Pc in soil averaged 3.1 propagules per g (ppg) and 25% of roots recovered were infected with Pc. A total count of bacteria on King's B medium averaged 8.7 x 10<sup>4</sup> per g of soil. Bacteria from this site were screened for antagonism to Pc using Persea indica (Pi) and Lupinus angustifolius (blue lupin) as susceptible hosts. Lupin seedlings were dipped into a bacterial suspension, planted into an avocado field soil artificially infested with Pc (~30 ppg), and grown for 3 wk. Pi seedlings were dipped into bacterial suspensions, planted into uninfested soil for 2 wk, then transplanted into infested soil and grown for an additional 2 wk. Immediately after planting both lupin and Pi plants were drenched with 20 ml of bacterial suspension. Root rot was assessed by plating roots onto a selective medium. Six of 30 bacterial isolates reduced root rot by 20-25% and by 25-40% for Pi and lupin, respectively.

#### 518

A COMPUTER MODEL FOR EVALUATING FOLIAR BIOCONTROL AGENTS. G.R. Knudsen and H.W. Spurr, Jr., USDA-ARS, Tobacco Research Lab., Oxford, NC 27565

Foliar disease biocontrol has been hindered by unpredictable field results. Survival of control agents and their interactions with pathogens occur in a dynamic environment. A systems approach helps to understand these complex processes. One such approach (knowledge-based or "expert" systems) uses a set of decision-making rules (shell) coupled to a knowledge base of facts and observations. A computer simulation of Cercospora leafspot of peanut is the shell component of one such system. Disease progress is predicted as a function of weather, pathogen characteristics, plant growth, and control agents. The knowledge base contains epidemiological parameters, weather data, and results from controlled-environment studies using different biocontrol agent candidates. The model is used to optimize spray timing and dosage, and to predict field results.

#### 519

MONOCULTURE OF CRIMSON SWEET WATERMELON PROMOTES SOIL SUPPRESSIVENESS TO FUSARIUM WILT. D. L. Hopkins, Agricultural Research and Education Center, IFAS, University of Florida, Leesburg, FL 32749-0388.

In a study of effect of watermelon monoculture on Fusarium wilt, Crimson Sweet seemed to have a unique type of resistance to *Fusarium oxysporum* f. sp. *niveum* that was more stable in a monoculture than that of other cultivars. In previous greenhouse and field tests, Crimson Sweet had been classified as moderately resistant to Fusarium wilt. However, in every year after the second of a 6-year monoculture, Crimson Sweet had less wilt and higher yields than the highly resistant Calhoun Gray. In greenhouse bioassays for wilt on the susceptible cultivar Florida Giant, there was less wilt in soil from monoculture plots of Crimson Sweet than from those of Calhoun Gray. When fumigated and nonfumigated Crimson Sweet plot soil were infected with *F. oxysporum* f. sp. *niveum* ( $1.5 \times 10^3$  propagules/g), there was 20% wilt of Florida Giant in the nonfumigated soil and 100% in the fumigated soil.

## 520

VARIABILITY IN SUPPRESSIVENESS OF CONTAINER MEDIA AMENDED WITH COMPOSTED MUNICIPAL SLUDGE TO RHIZOCTONIA AND PYTHIUM DISEASES. Hoitink, H. A. J. and Kuter, G. A. Dept. Plant pathology, OARDC-OSU, Wooster, OH 44691.

Suppressiveness of peat (CP) and peat container media amended with composted municipal sludge (CMS) to Rhizoctonia (R) and Pythium (P) diseases was determined under production conditions. CP media, if conducive initially to R and P, remained conducive throughout the production cycle of crops. Some CP batches were suppressive to either P, R or both diseases immediately after formulation. However all were conducive within 4 wk after planting. CMS media varied in suppressiveness from batch to batch, but all were suppressive within 4 wk after planting in outdoor production systems but not under greenhouse practices. A direct relationship existed between disease suppression bioassay values and actual disease severity observed in industry.

## 521

USE OF COMBINATIONS OF MICROBIAL ANTAGONISTS TO SUPPRESS RHIZOCTONIA AND PYTHIUM DAMPING-OFF IN COMPOST-AMENDED CONTAINER MEDIA. Kuter, G. A. and Hoitink, H. A. J. Dept. of Plant Pathology, OARDC-OSU, Wooster, OH 44691.

A variety of fungal and bacterial antagonists induced suppression to damping-off caused by *Rhizoctonia solani* and *Pythium ultimum* in peat media amended with composts. Results achieved with single antagonists were inconsistent; one pathogen was suppressed but not the other, or suppression was obtained in media amended with one batch of compost but not another. Combinations of *Trichoderma* spp. with various bacterial antagonists were more effective in consistently reducing disease. The same combinations were not effective in peat media without compost amendments. Suppression of Rhizoctonia damping-off was greatest when compost-amended media inoculated with antagonists were replanted. The efficacy of the various combinations obtained in plant bioassays could not be predicted from *in vitro* tests.

## 522

BIOLOGICAL ACTIVITY OF SOILBORNE MICROORGANISMS AGAINST FUSARIUM WILT OF CHINA ASTER. T. D. Cavileer and J. L. Peterson, Plant Pathology Dept., Rutgers University, New Brunswick, NJ 08903.

Four soilborne microorganisms (*Trichoderma harzianum*, *T. viride*, *Trichoderma virens*, and *Leuconium cepacia*) were tested for biological activity against Fusarium wilt (*Fusarium oxysporum* f. sp. *celastri*) of China aster. Biocontrol effectiveness was measured in fumigated and non-fumigated field soil planted with three aster varieties. Biocontrol activity was also tested in the greenhouse by applying the antagonists to plants in cell packs prior to transplanting to Fusarium-infested soil. A second experiment tested biological activity when the antagonists were added directly to Fusarium-infested peat-sand mix. *P. cepacia* significantly increased plant fresh weight in the field test. *G. virens* and *T. harzianum* significantly increased plant fresh weight and reduced disease incidence in the susceptible variety 'Single Rainbow'. Antagonists were not too effective when added only to the plant cell packs but were more effective when incorporated into Fusarium-infested mix prior to transplanting 2-week old seedlings. N.J. A.E.S. Publ. No. K-11270-2-85.

## 523

TARGETING ROOT-KNOT NEMATODE DEVELOPMENTAL STAGES WITH FUNGAL ANTAGONISTS. J. T. Gaspard and R. Mankau, Dept. of Nematology, University of California, Riverside, CA 92521.

## 1344 PHYTOPATHOLOGY

Small field plots of tomato (*Lycopersicon esculentum*) cv. Tropic heavily infested with *Meloidogyne javanica* were treated in the fall, post-harvest, with the nematode egg parasites *Paecilomyces lilacinus* (PL) and *Verticillium chlamydosporium* (VC) to target the large number of eggs present at that time. Fifty ml of PL or VC from 7 day V8 broth shake cultures was applied/plant. After treatment plants were killed with a 2% glyphosate solution to encourage saprophytic colonization of the roots by these fungi. Four weeks post-treatment egg populations were > 80% lower in the PL and VC treatments than fungus free controls. In the spring VC and PL plots were treated with the nematode trapping fungus *Monacrosporium ellipsosporum* cultured on a vermiculite + bran medium, at a rate of 454 kg/acre. Three weeks post-treatment surviving juveniles were > 95% lower in the PL+ME and VC+ME treatments than the untreated control.

## 524

THE EFFECT OF MOTILITY ON WHEAT ROOT COLONIZATION BY FLUORESCENT PSEUDOMONADS ANTAGONISTIC TO TAKE-ALL OF WHEAT. W. Howie and R. J. Cook, Dept. of Plant Pathology, Washington State Univ., Pullman, Wa 99164.

Several *Pseudomonas fluorescens* strains antagonistic to take-all of wheat were treated with NTG to obtain mutants which were non-motile. All nonmotile strains were nonflagellated as determined by transmission electron microscopy. Root populations were determined 10 days after planting at 1-3, 5-7, or 7-9 cm below the seed from wheat plants grown in a Thatuna silt loam and a Quincy loamy fine sand. The two soils were adjusted to -0.2 bars, where motility should function, and at -2.0 bars, where motility should not function. There were no significant ( $P=0.05$ ) differences between root populations of nonmotile mutants and their motile parents for a given soil or matrix potential treatment. Likewise, nonmotile strains suppressed the development of *Gaeumannomyces graminis* var. *tritici* as well as their parentals. These results indicate that root elongation is the primary means by which bacteria introduced onto the seed are spread, in terms of cm distances, in the absence of downward movement of water.

## 525

SYMBIOTIC ORGANISMS ASSOCIATED WITH PLANT PARTS OF MULTI-ADVERSITY RESISTANCE (MAR) AND NON-MAR COTTONS. K. M. El-Zik, L. S. Bird, M. Howell, and P. M. Thaxton, Dept. of Plant Pathology and Microbiology, Texas Agric. Exp. Stn., College Station, TX 77843

Twelve MAR and non-MAR cultivars differing in their resistance to pathogens and insects were grown in the field for two years. Leaves, squares, and terminals were harvested. The MAR cultivars had higher populations of symbiotic bacteria (*Bacillus* spp.) with rough white (RW) and smooth white (SW), and unidentified translucent (T) colonies on Allen's soil extract agar than non-MAR cottons. Tamcot CAMD-E and LEB0-3 had the highest concentration of SW bacterial colonies in all plant parts. Populations of yeast with red (R) colonies were high in non-MAR cultivars. The ratio of SW/R and T/R was much higher for the MAR than the non-MAR cottons. The highest concentrations of symbionts were isolated from squares, followed by terminals and leaves. The results support the hypothesis that MAR is caused, in part, by specific symbiotic microorganisms of the host tissues and that their concentrations are under genetic control.

## 526

AN APPROACH TO SCREENING FOR BIOCONTROL AGENTS BASED UPON DESIRED AGENT ATTRIBUTES. C. M. Kenerley and J. P. Stack, Dept. Pl. Path. & Micro., Texas Agric. Expt. Stn., College Station, TX 77843.

The attributes a hyperparasite must have to be an effective biocontrol agent (BA) depends upon the target propagule and the task the hyperparasite must accomplish. For hyperparasites of soil-borne pathogens producing nonmotile propagules such as sclerotia, the following attributes were considered desirable: 1) an ability to adversely affect propagule germination and/or viability; 2) growth potential through soil; 3) activity over a range of environmental conditions; and, 4) survival and/or reproductive potential. A technique was developed to evaluate these attributes. When applied directly to sclerotia, some hyperparasites rendered 75-100% of sclerotia inviable. However, when introduced on a carrier into a sclerotia-infested soil, the hyperparasites were much less effective; 0-75% sclerotia rendered inviable. Successful screening for BA may need to incorporate an assessment of attributes in addition to parasitism.

## 527

GROWTH POTENTIAL IN SOIL OF HYPERPARASITES OF SCLEROTIA. J. P.

Stack, C. M. Kenerley, and R. E. Pettit. Dept. Pl. Path. & Micro., Texas Agric. Expt. Stn., College Station, TX 77843.

One important attribute a biological control agent of sclerotia of soil-borne pathogens must possess is an ability to reach the target propagule. To assess the growth potential in nonsterile soil of various mycoparasites, lignite granules impregnated with different nutrient substrates were colonized by isolates of *Chaetomium globosum* (Cg), *Gliocladium roseum* (Gr), *Trichoderma hamatum* (Thm), *T. harzianum* (Thr), and *Trichoderma* sp. (Tsp), and laid in soil under nylon screens. Over time the top layer of soil was removed and the screen lifted to expose the granules. Hyphal extension from the granules was measured microscopically. Cg, Gr, and Tsp grew 1.3, 0.8, and 0.9 mm respectively, in 24 hr at -1.0 bars matric potential. Thm and Thr did not extend from the granules more than 1-2 mm. The influence of soil moisture and temperature and substrate composition (carbon and nitrogen source and C/N ratio) on the growth potential of these mycoparasites has been studied with the aim of maximizing growth potential.

## 528 Withdrawn

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INTEGRATED CONTROL OF PHYTOPHTHORA CINNAMOMI WITH AVOCADO REPLANTS IN SOUTHERN CALIFORNIA. M. D. Coffey, S. D. Campbell, and F. B. Guillemet, Dept. of Plant Pathology, University of Riverside, CA 92521.

Currently, the major thrust of our chemical control program on avocados is concerned with the difficult problem of replanting *P. cinnamomi*-tolerant rootstocks, such as Duke 7, in soils with a history of root rot. Fumigation with methyl bromide has proved to be a relatively unreliable method of control, due variously to the difficult soil profiles, sloping terrains, and unsuitable soils encountered in avocado culture. Field trials using the fungicides metalaxyl, oxadixyl, and fosetyl-Al, as an alternative to fumigation, have been in progress for several years. In a strategy involving preplant treatment of the rootstock containers and postplant applications, generally three times a year at approximate 8 wk intervals, all three fungicides have given good efficacy against root rot and allowed successful replanting of avocados.

## 530

BIODEGRADATION OF METALAXYL IN AVOCADO SOILS. A. M. Bailey and M. D. Coffey, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Using a sensitive bioassay involving *Phytophthora boehmeriae* as the test organism, enhanced rates of biodegradation of metalaxyl were detected in some avocado soils with a history of treatment. The average half-life of metalaxyl in these soils was 28 days. The composition and level of microbial populations in active and inactive soils did not differ. Active microbial populations were recovered from metalaxyl treated and untreated soils and were capable of degrading metalaxyl. The average percentage degradation of  $^{14}\text{C}$ -metalaxyl with individual organisms at 25 days of incubation was ~ 50% and with groups of organisms ~ 80%. Four compounds, in addition to metalaxyl, were detected using two-dimensional thin layer chromatography and later characterized by mass spectral analyses. None of the breakdown products were fungicidal. In addition, the ability of the organisms to degrade the chloroacetanilide herbicide metolachlor was tested both in liquid culture and soil. The percent degradation of metolachlor with individual organisms varied from 4-86%.

## 531

COMPARISON OF APPLICATION TECHNIQUES WITH IPRODIONE FOR CONTROLLING ONION WHITE ROT. Peter Oudemans and L. V. Edgington, Dept. of Plant Pathology, University of California, Riverside, CA 92521 and University of Guelph, Ontario N1G-2W1.

Iprodione was tested for control of white rot in a plot infested with lab-grown sclerotia of the pathogen *Sclerotium cepivorum*. Three methods of application were compared: drenching or seed treatment with a 50 wettable powder and an in-furrow treatment with a granular formulation. Residues of iprodione were determined approximately 2 wk after infection was first noted and onions were harvested 2 wk later for disease assessment. Disease incidence was closely correlated with the level of iprodione residues on the onion bulbs. Drenching with 0.125 g ai/m of row was found to be the most effective treatment. In addition, iprodione was found to dissipate from onion bulbs quite rapidly, having a half-life of 7-12 days.

## 532

ALFALFA CULTIVAR RESISTANCE TO PHYTOPHTHORA ROOT ROT AND RESPONSE TO METALAXYL. S. L. Nygaard and C. R. Grau. University of Wisconsin, Madison, WI 53706.

Relative root rot resistance based on a metalaxyl equivalency scale and rate of metalaxyl response were studied using 5 alfalfa cultivars with various levels of *Phytophthora megasperma* f.sp. *medicaginis* (Pmm) resistance. One day old seedlings were drenched with 0, 0.01, 0.05, 0.25, 1.25, or 6.25 ppm metalaxyl, inoculated with ca. 500 zoospores Pmm per seedling 3 days later and incubated at 24C. Seedling mortality was recorded at two day intervals for three weeks. Relative resistance, based on metalaxyl equivalency, was determined for the cultivars Vernal, Armor, Trident, and Apollo II by plotting seedling death rate on a metalaxyl-response regression line derived for the highly susceptible cultivar Iroquois which has only 1% resistant plants. The regression line was  $Y = -1.50 - 0.18X$ , where  $Y = \log(\text{rate of seedling death})$  and  $X = \log(\text{ppm metalaxyl})$ . Inherent varietal resistance for the cultivars Vernal (5%), Armor (4%), Trident (7%), and Apollo II (5%) were equivalent to 0.09, 0.22, 0.25, and 1.63 ppm metalaxyl, respectively. Cultivar response to metalaxyl amendment, as measured by rate of seedling mortality, was greater for susceptible as compared to the more resistant varieties.

## 533

INTERACTIONS OF METALAXYL AND RESISTANCE IN PHYTOPHTHORA ROOT ROT OF SOYBEANS. P.J. Larson, L.A. Neese, D.R. Ivers and J.S. Baumer, Land O'Lakes, RR 2, Webster City, IA 50595.

Interactions between metalaxyl and combinations of specific resistance (SR) or tolerance (T) for the control of *Phytophthora* root rot of soybeans were studied in the field in 1984. The design was a split-plot with six replications with cultivars as main plots and fungicide treatments as sub-plots. Low, moderate and high T cultivars (Pike, Corsoy, Max) were chosen, along with their SR isolines (Pike II, Corsoy 79, L4104), plus nonisolines L4404 and L1771. Metalaxyl was applied as a seed and/or soil treatment at 0.0484 g a.i./kg and 0.0279 g/m of row, respectively. Stand counts, percentage of healthy plants, plant height and yield all revealed similar trends. Soil treatment outperformed seed treatment, and in-furrow was better than banded. Low T cultivars benefitted most from treatment, but were still below acceptable yield levels. High T cultivars with SR were highest yielding with or without fungicide. Moderate T with no or partial SR to the races present averaged a 6.2 bu/ac increase with soil treatment.

## 534

THE USE OF SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY ANALYSIS TO STUDY THE DEPOSITION AND DISTRIBUTION OF FUNGICIDES APPLIED TO GREENHOUSE CROPS VIA SMOKES OR FOGS. C. C. Powell and C. R. Krause, Dept. of Plant Pathology, OARDC and The Ohio State Univ., Columbus 43210 and \*USDA-ARS, 359 Main Rd., Delaware, OH 43015.

Specimen stubs were mounted upright or inverted on ring-stands and placed in greenhouses within the leaf canopy of bench grown crops. Fungicides were applied as either a self-dispersing smoke or with a thermal pulse-jet fogger. Specimens were examined with a scanning electron microscope (SEM) and an energy dispersive X-ray analyzer (EDXA). Fungicide particles were identified on the basis of their morphology and the presence of chlorine. Particles generated by the smoker application were amorphous or globular, intermittent, 5 to 15  $\mu\text{m}$  in length and found only on upright stubs. Particles produced by fogging were granular, numerous, 1 to 18  $\mu\text{m}$  in diameter and found on both upright and inverted stubs, depending on their position relative to the applicator. Preliminary investigations on leaf surfaces indicate similar deposition patterns.

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EVALUATION OF TIMING OF TWO FUNGICIDES IN THE CONTROL OF RIPE ROT OF PEPPERS. J. F. Hadden, L. L. Black and J. M. Gatti. Dept. Plant Path. & Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803.

A study was conducted to evaluate the timing of fungicidal sprays to control ripe rot (*Colletotrichum* spp.) of pepper. Cayenne 'LSU 16' and bell 'Bell Boy' peppers (*Capsicum annuum*) were planted in an infested field. Maneb (Manex at 1.875 ml/liter) and chlorothalonil (Bravo 500 at 3.75 ml/liter) were sprayed on foliage to run-off at 1-4 wk intervals, beginning at first bloom and continuing throughout the season. Peppers were harvested at the ripe, red stage of maturity and disease losses were estimated by calculating the percentage of diseased pods. Seasonal ripe rot disease losses averaged 76% in unsprayed control plots. Disease losses in weekly sprayed chlorothalonil and maneb plots averaged 25 and 37 percent, respectively. Ripe rot incidence was significantly decreased by both weekly and biweekly fungicide treatments.

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INVESTIGATIONS ON THE EFFECTS OF TILT AGAINST *EXSEROHIUM TURCICUM*. Kira L. Bowen and W.L. Pedersen. Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

The effects of propiconazole (Tilt<sup>R</sup>, Ciba-Geigy) on infection by *Exserohilum turcicum* were studied on corn lines in the glass-house. Inoculation resulted in an average deposition of 8.2 conidia/cm<sup>2</sup> of leaf. Protectant effect (reduction of lesion numbers) of Tilt, at 62 and 115 g ai/ha, was not significantly greater than mancozeb (Dithane M-45<sup>R</sup>, Rohm & Haas) at 220 g ai/ha, but rate of lesion expansion was significantly lower with Tilt. Tilt applied at 160 and 209 g ai/ha resulted in complete protection, although some phytotoxicity was observed on corn inbreds, Mt42, A632 and Mo17. Tilt applied to expanding lesions significantly reduced the rate of lesion expansion and sporulation. Protectant and eradicator properties of Tilt also were investigated over time.

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FUNGICIDES AND APPLICATION TIMING IN RELATION TO LEAF SPOT DISEASE AND YIELD OF ALFALFA. S. C. Broscious and H. W. Kirby. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Benomyl (1 lb/A), copper hydroxide (2 lb/A), and mancozeb (2 lb/A) were applied to the first three harvests of 'Phytor' alfalfa using the following schedules: 26 or 10 days before harvest, 26 and 10 days before harvest, or 26 and 18 and 10 days before harvest. All treatments except copper hydroxide reduced defoliation compared to the control. Final disease severity was lower for most treatments but differences existed among harvests. Single-degree-of-freedom contrasts between application schedules indicated the occurrence of favorable infection periods which varied with harvests. Comparisons of early, late, and 2 applications revealed important infection periods early in the 2nd and 3rd harvests which corresponded to significant yield increases from single early applications of mancozeb. No 1st harvest treatments increased yields. The magnitude of yield increases from 1 or 2 applications of mancozeb on the 2nd or 3rd harvest indicate that fungicide application may be economical.

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CONTROL OF CORN GRAIN STORAGE FUNGI WITH POSTHARVEST FUNGICIDE TREATMENTS. D.G. White, D.C. Burnette, and B.J. Jacobsen. Dept. of Plant Pathology, Univ. of Illinois, Urbana, 61801.

Benomyl (Benlate, E.I. DuPont and Co.), thiabendazole (Merfect 340F, Merck Chemical Co.) and A9248 (Abbott Laboratories) were evaluated as potential grain protectants under accelerated aging conditions (26C, 90% RH) and in modified grain bins. In accelerated aging experiments the fungicides controlled *Aspergillus* spp. and *Penicillium* spp. In other experiments, two grain bins (685 quintals ea.) equipped for low temperature drying were constructed using dividers to partition each bin into 16 equal compartments. Benomyl (0, 1, 5 and 10 ug/g) and

thiabendazole (0, 5, 10 and 20 ug/g) were applied at harvest to 25% moisture corn to determine if fungi could be controlled during low temperature drying and storage. In a separate experiment, A9248 (0, 5, 10 and 20 ug/g) was also evaluated. Kernel infection with *Aspergillus* spp. and *Penicillium* spp. was lower with all fungicide treatments. The use of low rates of fungicides is an effective control of these fungi in low temperature drying and storage at 15-18% moisture.

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CHEMICAL CONTROL OF POWDERY MILDEW ON SUNFLOWER. Kon-taxis, D. G., Univ. of California Cooperative Extension, 1700 Oak Park Blvd., Pleasant Hill, CA. 94523.

Two hand sprays at a 20-day interval of triadimefon (Bayleton 50W) and propiconazole (Tilt 3.8F) at .350 kg. and 0.336 kg. a.i., respectively, in 935 liters of water per hectare suppressed powdery mildew, *Erysiphe cichoracearum* D.C. on sunflower *Helianthus annuus* L. Similar sprays with mancozeb at 0.280 kg. a.i., per hectare were not as effective. The first spray was applied when plants were about 90cm tall and free of disease. The chemicals did not control rust, *Puccinia helianthi* Schw., which was prevalent. Leaves of plants sprayed with triadimefon, propiconazole or mancozeb were respectively 0, 1.3, and 5.0 percent infected 46 days after the last spray. Bayleton sprayed plants were powdery mildew free 51 days after last spray. The leaves of non-treated plants were 55 percent infected. The data were statistically significant.

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INFLUENCE OF TRANSPLANTING DATE AND CHEMICAL TREATMENT ON TOBACCO BLACK SHANK AND ROOT KNOT NEMATODES. A. S. Casinos and N. A. Minton, Department of Plant Pathology, University of Georgia, and USDA, SEA, Coastal Plain Experiment Station, Tifton, GA 31793.

*Nicotiana tabacum* L., 'NC 2326' was transplanted on April 6, April 26, May 15 into a field infested with *Phytophthora parasitica* var. *nicotianae* and *Meloidogyne* spp. Treatments were: metalaxyl at 2.24 kg ai/ha; phenamiphos at 6.72 kg ai/ha; the two materials together; and an untreated control. Yields were highest and disease was lowest in the metalaxyl-phenamiphos treatment for all plantings. Metalaxyl and phenamiphos alone increased yield and reduced disease in the first and last planting. Yields were higher in the first planting than the last only for the metalaxyl-phenamiphos treatment. Black shank was decreased across all treatments as planting was delayed. Root knot damage was reduced by phenamiphos treatments in each planting date and was lowest in the last.

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A NEW TECHNIQUE FOR DETECTION AND QUANTIFICATION OF *PHYTOPHTHORA CACTORUM* IN NATURALLY INFESTED SOIL. M. K. Rahimian and J. E. Mitchell, Dept. of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

Release of zoospores of *Phytophthora cactorum* *in vitro* occurs over a range of temperatures from 4-24C with an optimum of 8C. The common *Pythium* sp. detected in soils naturally infested with *P. cactorum* did not produce zoospores at temperatures below 12C. A selective procedure was developed for quantifying *P. cactorum* in soil that involved suppression of zoospore production by *Pythium* spp. by incubating soil at 8C. Soil was air dried, moistened (4 ml water/10 g soil) for 3 days, and flooded with distilled water (DW) for 2 days at 24C and 5D  $\mu\text{Em}^{-2}\text{sec}^{-1}$  light intensity. The excess water was then removed and replaced with DW cooled to 8C and the soil was incubated at 8C for 2 h during which zoospore release occurred by *P. cactorum*. The zoospore suspension was plated on Schmitthenner's selective medium (PBNC). *P. cactorum* counts of up to 56 zoospores/g dry soil were obtained for naturally infested soils from ginseng gardens.

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*PHYTOPHTHORA* SPP. ISOLATED FROM SURFACE-WATER IRRIGATION SOURCES IN CALIFORNIA. S. M. Mireccich, G. T. Browne, W. Krueger and W. Schreuder. USDA, ARS, Univ. of Calif., Davis, CA 95616

Surface-water irrigation sources (Sacramento River, Glenn-Colusa, Tehama-Colusa, and Orland Water Users canals, Glenn County;

Stanislaus and Calaveras rivers, Mormon Slough and Woodbridge Canal, San Joaquin County) were surveyed for *Phytophthora* spp. in 1982, 1983, and 1984. Among the 1633 isolates recovered from the surface-water irrigation sources, we recognized *P. cambivora*, *P. cactorum*, *P. citricola*, *P. citrophthora*, *P. megasperma*, *P. cryptogea*, *P. drechsleri*, *P. syringae* and seven other different unidentified *Phytophthora* spp.. Selected isolates of the *Phytophthora* spp. from the irrigation water sources were pathogenic to walnut, cherry, almond, peach, plum, and apple seedlings in greenhouse tests. The isolates of *Phytophthora* spp. from the irrigation water sources are morphologically identical or similar to isolates of the same *Phytophthora* spp. recovered from orchard fruit and nut trees naturally affected with *Phytophthora* root and crown rot or trunk and scaffold branch cankers.

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INOCULUM PRODUCTION BY *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANAE* IN ROOTS OF FLUE-CURED TOBACCO. H. D. Shew, Dept. Plant Pathology, N. C. State Univ., Raleigh 27695-7616.

Eight-wk-old seedlings of four flue-cured tobacco cultivars ranging in resistance to *Phytophthora parasitica* var. *nicotianae* from none to high were inoculated with 50 ml of a zoospore suspension ( $10^3$ /ml). Root systems of the seedlings were then incubated in two soil types (Norfolk sandy loam and Grantham silt loam) at soil matric potentials of -0.1, -1.0, and -10.0 bars at 24 C. After 4 wks, soil was assayed for *P. parasitica* var. *nicotianae* on a selective agar medium. Inoculum density of the fungus pathogen varied with soil type and cultivar. Inoculum densities were similar within a cultivar at all matric potentials tested. Sporangia constituted a higher percentage of the propagules recovered at -0.1 and -1.0 bars. Inoculum densities ranged from 2 to 14 propagules per mg root fresh weight.

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IMPLICATION OF *PHYTOPHTHORA* SPP. IN A RASPBERRY DECLINE SYNDROME. W. F. Wilcox and J. R. Nevill, Department of Plant Pathology, New York State Agr. Expt. Station, Geneva, NY 14456.

Seven different *Phytophthora* spp. were isolated from dead or declining raspberry plants in 7 of 9 New York sites surveyed in 1984. Affected plants were frequently associated with poorly-drained soils and showed typical root and crown rot symptoms. *Phytophthora megasperma* was recovered from 3 sites, *P. cactorum* from 2 sites, and *P. citricola* from 1 site; unidentified isolates appear to represent four additional species. Of these, *Phytophthora* spp. #1 (3 sites) and #2 (2 sites) are homothallic, whereas *Phytophthora* spp. #3 (2 sites) and #4 (1 site) are heterothallic. In preliminary pathogenicity tests with 'Heritage' and 'Taylor' red raspberries, *P. citricola* and *P. sp.* #1 caused plant death, *P. sp.* #2 caused extreme plant stunting, and the remaining *Phytophthora* spp. caused moderate to severe stunting. These results suggest that infection by *Phytophthora* spp. may be a significant factor in a previously-unexplained decline syndrome of raspberries in New York.

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*PHYTOPHTHORA* SPECIES CAUSING ROOT AND CROWN ROT OF CHERRY TREES IN NEW YORK. W. F. Wilcox, S. N. Jeffers, J. E. K. Hayes, and N. S. Aldwinckle, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

During 1980-83, *Phytophthora megasperma*, *P. cryptogea*, and *P. cambivora* were consistently isolated from dead and dying cherry trees in New York. In a 1984 survey of 7 symptomatic cherry orchards, *P. megasperma*, *P. cactorum*, *P. cambivora*, and an unidentified *Phytophthora* sp. similar to that recently reported from cherry in California (PHYTOPATHOLOGY 73:221-225) were recovered from 5, 2, 1, and 2 orchards, respectively. All species were isolated from necrotic root and crown tissue except *P. cactorum*, which was recovered only from soil. All species caused root and crown rot on Mahaleb and Mazzard cherry seedlings grown in artificially infested potting medium in greenhouse tests. These results indicate that *Phytophthora* species are common causes of root and crown rots on cherry trees in New York.

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DISTINCT VEGETATIVE COMPATIBILITY GROUPS (POPULATIONS) OF *FUSARIUM OXYSPORUM* COLONIZING CELERY ROOTS FROM

CALIFORNIA. James C. Correll, J. E. Puhalla, and R. W. Schneider, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

*Fusarium oxysporum* was isolated from celery roots showing symptoms of Fusarium yellows disease and roots that were asymptomatic. Isolates were classified by colony size, virulence on green celery, and vegetative compatibility group (VCG) based on pairing tests with nitrate reductase mutants (Puhalla, Can. J. Bot. 1985). All isolates from diseased celery roots were small colony types, virulent on celery, and in the same VCG (*F. oxysporum* f. sp. *apii* (Foa) race 2). Isolates colonizing symptomless celery roots included typical Foa race 2 and isolates that were primarily large colony types and avirulent on celery. The isolates that were nonpathogenic (np) on celery belonged to at least 10 VCG's. One population (VCG-2001) of the np isolates was recovered throughout one field and in 5 additional fields in Ventura and Santa Barbara counties. A second population was recovered in 2 fields. The data indicate that pathogenic populations of Foa race 2 can be positively identified using rapid laboratory procedures. Moreover, there may be specific np populations of *F. oxysporum* associated with certain crops and/or soils.

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DIFFERENTIATING RACES OF *FUSARIUM OXYSPORUM* F. SP. *PISI* BASED ON VEGETATIVE COMPATIBILITY. J. C. Correll, J. E. Puhalla, R. W. Schneider, and J. M. Kraft, Department of Plant Pathology, University of California, Berkeley, CA 94720, and USDA-ARS, Prosser, WA 99350.

A collection of *Fusarium oxysporum* f. sp. *pisi* (Fop) was differentiated into Fop race 1, 2, 5, and 6 based on pathogenicity tests on differential pea cultivars. Complementary nitrate reductase mutants were generated within each race and paired in all combinations to determine the heterokaryon or vegetative compatibility groups (VCG's) among them. All isolates identified as Fop race 1, 2, and 5 in virulence tests belong to 3 distinct VCG's. These races apparently represent 3 distinct populations of the pea wilt pathogen. However, all race 6 isolates were vegetatively compatible with race 1 and therefore in the same VCG. Heterokaryon formation was more rapid and robust between race 6 than between race 1 and race 6. Race 1 and 6 are in the same VCG, but can be differentiated in virulence tests. This suggests that there is genetic heterogeneity within this VCG.

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A SORBOSE BASE SELECTIVE MEDIUM FOR ENUMERATING PROPAGULES OF *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2 IN ORGANIC SOILS. R. T. Awaah and J. W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A selective medium incorporating L-sorbose (20 g), Bacto agar (20 g), DL-asparagine (2 g), chloramphenicol sulfate (600 mg), PCNB (120 mg ai), and Dexon (120 mg ai) in 1 L distilled water distinguished four main types of *Fusarium* colonies on soil dilution plates after 5-6 days. Colonies of *Fusarium oxysporum* f. sp. *apii* race 2 on this medium exhibited creamy to white color, smooth margins, compact mycelium, and slightly raised centers. The efficiency of recovery of the medium was 78% and 85% at populations of 10,000 and 5,000 propagules of *F. oxysporum* f. sp. *apii* race 2, respectively, per g dry soil. Populations of this pathogen at five sites in one field determined with this medium ranged from 1,751 to 2,469 propagules/g dry soil. In another field the vertical distribution of the pathogen was 1,125 and 900 propagules/g dry soil in the top 0-20 cm and 20-40 cm, respectively.

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FACTORS AFFECTING *FUSARIUM* WILT DEVELOPMENT IN FOUR COTTON SELECTIONS. D. P. Jeffers, Department of Plant Pathology, University of California, Davis, CA 95616; R. H. Carber, U.S. Cotton Research Station, Shafter, CA 93263; P. A. Roberts, San Joaquin Valley Agricultural Research and Extension Center, Parlier, CA 93648.

Cotton (*Gossypium hirsutum*) cultivars Acala SJ-2 and Acala SJ-1, and cotton lines N6072 and N8577 which differ in reaction to *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) and/or *Neovossia incognita* (Mi) were used in replicated split plots with varying levels of Fov and Mi for comparisons of *Fusarium* wilt development. Data obtained were preplant soil populations of Fov and Mi, Fov location in serial sections of stem and leaf tissue, foliar symptoms, and postharvest root discoloration and gall ratings. Results showed that colonization of host tissue by Fov, and the expression of wilt symptoms occurred in all cotton selections under both high and low Mi populations. However, the distribution of Fov in the vascular tissue of stem and side branches was greatest for Acala SJ-2.



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RELATION OF SOILBORNE INOCULUM DENSITIES OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* AND *MELOIDOGYNE INCOGNITA* TO THE PHENOLOGY OF COTTON PLANTS AND THE DEVELOPMENT OF *FUSARIUM* WILT. J.E. DeVay, \*R.H. Garber, R.J. Wakeman, D. Jeffers, S.N. Smith, and \*\*P. Roberts. Dept. of Plant Pathology, Univ. of California, Davis, CA 95616; \*ARS-USDA, Shafter, CA 93263 and \*\*San Joaquin Valley Agric. Res. and Ext. Center, Parlier, CA 95648.

This study concerned the quantitative relationships between inocula of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and *Meloidogyne incognita* (MI) and the incidence of *Fusarium* wilt of cotton (*Gossypium hirsutum*, cultivar Acala SJ-2). Among 19 field sites sampled, the interactions of cotton with populations of FOV and MI showed that approximately 1500 FOV propagules/g soil associated with 50-80 MI/250 g soil were the lowest inoculum densities causing a maximum incidence of foliar symptoms. The incidence of vascular discoloration was 2 to 4 times greater than the incidence of foliar symptoms and always preceded foliar symptoms. Earliness of foliar symptoms was associated with decreased plant height, reduced numbers of nodes and bolls, and lower lint yields.

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STEPWISE REGRESSION MODEL FOR PREDICTING MICROSCLEROTIAL NUMBERS OF *MACROPHOMINA PHASEOLINA* IN MISSOURI SOILS. Rosenbrock, S. M., and T. D. Wyllie, University of Missouri, Columbia, Mo. 65211.

Six areas, 14-23 fields per area, in Missouri were sampled twice in 1984 for numbers of microsclerotia of *M. phaseolina* /gram soil (MS), soil fertility, and %sand, silt, and clay. Temperatures in three categories (expressed as degree days (DD) <0 C, <18 C, and >18 C) were collected from Oct. 1983 to May 1984 for each area. Area locations (LOC) were determined by miles south and west of a point 35 miles east of the southeast corner of the state. Stepwise regression analysis of combined samplings showed % silt, LOC, DD<0, and pH were significant ( $r^2 = 0.45$ ,  $P = 0.0001$ ). Response surfaces for MS as a function of % silt and pH (with LOC and DD<0 held constant) showed highest MS were found in soils having pH 6.8-7.5 and silt 50-70%. There was a significant ( $P = 0.0001$ ) decrease in MS as DD<0 increased.

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CROP ROTATION AS A MEANS OF CONTROLLING POPULATIONS OF *MACROPHOMINA PHASEOLINA* IN MISSOURI SOILS. T.D. Wyllie and S.M. Rosenbrock, University of Missouri, Columbia, MO 65211.

*Macrophomina phaseolina*, the causal agent of charcoal rot, has a wide host range (ca. 400 spp.). It has generally been considered that crop rotation would be ineffective as a control or management procedure. Three studies in Missouri in 1971-75, 1978-81, and 1982-84 indicate that soil populations of microsclerotia (ms) are significantly ( $P=0.05$ ) affected by different host crop species, including soybeans, corn, sorghum, fescue, wheat, and cotton. Monocropping soybeans generally result in the highest ms populations when compared to the other crops. A two-year rotation from soybeans is required to significantly reduce ms numbers. Numbers were reduced from 45 to 81 and 42 to 20 ( $P=0.05$ ) with two-year rotations out of soybeans in two of these studies.

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ENHANCEMENT OF *VERTICILLIUM* WILT ON SOLANACEOUS VEGETABLES BY TREFLAN. Jack Altman, Dept. of Plant Path. and Weed Sci., Colorado State Univ., Ft. Collins, CO 80523.

*Verticillium dahliae* Kleb. inoculum from dry ground infested potato stems incorporated into Treflan [Trifluralin (1,1,1-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine)] treated soil (800-1000 microsclerotia/Kg soil) resulted in a marked increase in disease in eggplant, 'black beauty' in greenhouse tests in comparison with plants infested but non-Treflan treated soil that had mild symptoms of disease. *Verticillium* symptoms were evident after 6 wk and became more severe after 9 wk. Disease increased in infested soil with increasing concentrations of Treflan from 1.25 to 5.0  $\mu\text{g/g}$  soil. Plants in this soil were severely stunted. Leaves, stems and flowers in the upper one-third of the plants became brown and necrotic. Tomatoes and peppers grown in Treflan treated and infested soil were stunted but did not exhibit necrosis.

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GERMINATION OF PROPAGULES OF *VERTICILLIUM DAHLIAE* IN SOIL TREATED

WITH METHIONINE AND OTHER SUBSTANCES AFFECTING ETHYLENE PRODUCTION. E. Kapulnik, \*J. Quick and J. E. DeVay. Department of Plant Pathology, and \*Department of Land, Air, and Water Resources, University of California, Davis, CA 95616 U.S.A.

This study was made to determine the effect of soil treatments on ethylene (ET) production and the viability of propagules of *Verticillium dahliae*. Amending soil with methionine or other substances greatly increased ET levels in non-sterile soil infested with *V. dahliae*. ET production was enhanced more in soil with low nitrate (10-20  $\mu\text{g/g}$  soil) than in soil with high nitrate content (10,000  $\mu\text{g/g}$  soil). The addition of methionine to moist soil at 23°C and 33°C (imposed fungistasis) increased the number of germinating propagules of *V. dahliae* by 2-3 times in soil assays suggesting that some propagules remained dormant and undetected in untreated soil. These results suggest that the rate of ET production in soil varies with nitrogen content and that methionine may induce the germination of dormant propagules of *V. dahliae*.

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PREVALENCE OF PRUNUS NECROTIC RINGSPOT VIRUS AND ITS CORRELATION WITH PEACH TREE CANKERS IN SOUTHEASTERN PENNSYLVANIA. C. A. Powell, Pennsylvania Department of Agriculture, Harrisburg, PA 17110 and B. A. Jaffee, Dept. of Plant Pathology, Pennsylvania State University, Fruit Research Lab., Biglerville, PA 17307.

Peach trees with severe, moderate, or no canker in each of 8 orchards (4 'Loring', 3 'Redhaven', 1 'Garnet Beauty') in SE PA were assayed for Prunus necrotic ringspot virus (NRSV) by ELISA and Immuno-Blot (IBA) using antiserum to NRSV-G. Thirty of 206 (15%) of the trees were infected with NRSV. By variety, the number of infected trees was 1 of 37 (3%), 3 of 40 (8%), and 1 of 32 (3%) for 'Loring' with severe, moderate, or no canker, respectively; 0 of 10 (0%), 2 of 20 (10%), and 4 of 44 (9%) for 'Red Haven' with severe, moderate, or no canker, respectively; and 10 of 10 (100%), 8 of 10 (80%), and 1 of 3 (33%) for 'Garnet Beauty' with severe, moderate, or no canker, respectively. Thus, with the possible exception of the one 'Garnet Beauty' orchard, viruses detected by antiserum to NRSV-G were not correlated with peach tree canker. ELISA and IBA gave identical results.

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EFFECT OF AN APHID-INFECTING VIRUS ON BARIETY YELLOW DWARF VIRUS TRANSMISSION. F. E. Gildow and C. D'Arcy. Plant Pathology Departments, Penn State University, University Park, PA 16802, and University of Illinois, Urbana, IL 61801.

Cereal grain aphids of the species *Rhopalosiphum padi*, *R. maidis*, *Sitobion avenae*, *Schizaphis graminum*, and *Metopolophium dirhodum* were allowed a 24 hr acquisition feeding on parafilm membranes containing purified preparations of a previously described (Virology 112:346) aphid virus (AV). Seven and 14 days later individual aphids were assayed for AV by ISEM or fixed for thin-sectioning. Results of 3 experiments indicated only *R. padi* and *S. graminum* became infected. Virus-free aphids of either species became infected when fed on plants with infected aphids or when allowed to feed on sucrose following feeding by infected aphids. Infection with AV had no effect on BYDV vector-specificity or transmission efficiency when compared to healthy aphids. Ultrastructural studies showed no cytopathology or AV accumulation in the accessory salivary gland of actively feeding AV-infect aphids at 7 days post inoculation, even though AV was identified in the hemocoel.

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EFFECT OF SOYBEAN PUBESCENCE ON APHID PROBING BEHAVIOR AND TRANSMISSION OF SOYBEAN MOSAIC VIRUS (SMV). U. B. GUNASINGHE,

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS, 61801 AND M. E. IRWIN, ILLINOIS NATURAL HISTORY SURVEY, 607 E. PEABODY, CHAMPAIGN, ILLINOIS, 61820.

The probing behavior of three species of aphids - *Myzus persicae* (Sulzer), *Aphis citricola* Van der Goot, and *Rhopalosiphum maidis* (Fitch) - was observed under a dissecting microscope on soybean isolines with different pubescent properties. Significant differences were observed in number of probes, time spent not probing, and time to first probe for all 3 species of aphids on these isolines. Transmission efficiency of these species of aphids was studied on these isolines by allowing them access to an infected host for a specific time period. Significant differences were observed in aphid transmission of SMV to these isolines, given the same access period on the host plant.

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VECTOR RELATIONSHIPS OF TWO STRAINS OF SOYBEAN DWARF VIRUS. V. D. Damsteegt and A. D. Hewings, USDA-ARS Plant Disease Res. Laboratory, Fort Detrick, Bldg. 1301, Frederick, MD 21701.

Strains of soybean dwarf virus have been grouped into two types, dwarfing (SDV-D) and yellowing (SDV-Y), which are persistently transmitted by *Acyrtosiphon (Aulacorthum) solani*. Aphid populations from New Brunswick, Canada (C), California (US), New Zealand (NZ), and Japan (J) can be differentiated by morphology, food host preferences, host response to feeding, and feeding location on soybean seedlings. Transmission efficiency of the J population was greater than C, US, or NZ. All populations transmitted SDV-D more efficiently than SDV-Y; nymphs appeared to transmit both strains as or more efficiently than late-instar morphs. Latent periods for both strains were shorter and the viruses persisted longer in J aphids than in US aphids. The optimum temperature for transmission was 20C; high (>29C) and low (5 and 10C) temperatures decreased the infection rate and delayed the onset of symptoms in both strains but especially with SDV-D.

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POTATO LEAFROLL VECTORS IN THE SAN LUIS VALLEY OF COLORADO. R.E. Klein\* and C.H. Livingston\*\*, Department of Plant Pathology Washington State University, Pullman, WA, 99164\* and Department of Plant Pathology, Colorado State University, Fort Collins, CO, 80523\*\*.

A heat unit accumulation (HUA) model was developed which accurately predicted the appearance of alate green peach aphids (GPA) (*Myzus persicae*) in yellow pan traps in the San Luis Valley (SLV) of Colorado. HUA was correlated with potato leafroll (PLR) disease increase in seed potatoes prior to, but not after, the implementation of an integrated pest management program to control the GPA, thought to be the sole PLR vector. However, PLR continued to be a major problem in the SLV. Field collections of aphids from PLR-affected potato plants indicated that the potato aphid (POA) (*Macrosiphum euphorbiae*) could also transmit PLR. PLR infection of sequential sets of bait plants exposed during the 1984 growing season was correlated with POA counts on nearby sticky traps, but not with GPA numbers. Thus, POA now appears to be a major vector of PLR in the SLV.

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THE EFFECTS OF SUPPRESSIVE VIRUS AND APHID RESISTANCE ON THE EPIDEMIOLOGY OF WATERMELON MOSAIC VIRUS 2. Gray, S. M.<sup>1</sup>, Moyer, J. W.<sup>1</sup>, Kennedy, G. G.<sup>2</sup>, and Campbell, C. L.<sup>2</sup> Dept. of Plant Pathology<sup>1</sup>, and Dept. of Entomology<sup>2</sup>, North Carolina State University, Raleigh, NC 27695-7616

The spatial and temporal characteristics of epidemics induced

by watermelon mosaic virus 2 (WMV 2) were determined in one *Cucumis melo* genotype resistant to *Aphis gossypii* and suppressively resistant to WMV 2, to another genotype resistant to *A. gossypii*, and a third genotype susceptible to both. Final disease incidence in the aphid resistant and the aphid/virus resistant genotype ranged from 8% to 72% lower than in the susceptible genotype during the spring planting. The final disease levels during the summer planting were not significantly different among genotypes. The increased incidence was attributed to an increase in the number of alighting aphids and the amount of WMV 2 inoculum in the surrounding area during the summer. Only the infected plants of the susceptible genotype were consistently observed in a clustered pattern.

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COMPARATIVE REACTION OF RESISTANT AND SUSCEPTIBLE PEPPER CULTIVARS TO CALIFORNIA ISOLATES OF POTYVIRUSES. O. A. Abdalla and P. R. Desjardins, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

Delray and Tamber-2, bell pepper cultivars and a chili cultivar, Tam mild chili-2 (TMC-2), reported resistant to potato Y (PVY), tobacco etch (TEV), and pepper mottle (PeMV) in other states, were singly inoculated with California isolates of the viruses in the greenhouse to evaluate their resistance to California isolates. Two susceptible cultivars, Yolo Wonder and Anaheim Chili were similarly inoculated. Plant height, fresh weight and yield were compared. Resistant cultivars were affected by California virus isolates, but statistical analyses showed they were significantly superior in all parameters to susceptible cultivars. Virus titer was determined by ELISA at 21, 82 and 89 days. Delray was the most resistant to the three viruses. TMC-2 was resistant to all 3 viruses initially, but was less resistant as it matured. Tamber-2 response was similar to TMC-2 when inoculated with PVY and TEV, but was less resistant to PeMV.

#### 565

REACTION OF SOMACLONAL VARIANTS OF RUSSET BURBANK AND OF A DIHAPLOID POTATO TO POTATO VIRUS X (PVX). H. H. Murakishi and R. R. Harris. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Four hundred somaclones of Russet Burbank potato were regenerated from leaf protoplasts and screened for reaction to a ringspot strain of PVX by ELISA and indexing to *Gomphrena globosa*. Eighty-two percent of the plants became infected two weeks after sap inoculation. The survivors were reinoculated after which only one of the somaclones remained uninfected. Repeated assays showed that this somaclone was virus-free. However, three cuttings rooted from this plant all became infected when inoculated. A dihaploid (2n=2x=24) potato, a clone of a *Solanum phureja*-*S. tuberosum* cross, was resistant to the ringspot strain of PVX but developed necrosis, mosaic and high virus titer when inoculated with a necrotic strain of PVX. One of 100 dihaploid somaclones regenerated from leaf protoplasts of the hybrid clone exhibited a complete reversal of reaction: susceptibility to the ringspot strain but resistance to the necrotic strain of PVX.

#### 566

EFFECT OF SOYBEAN MOSAIC VIRUS ON PLANT GROWTH AND PRODUCTIVITY. R. Lastra & S. El Ashuh. DPV, CATIE, Turrialba, Costa Rica and Lab. Virus Plantas, IVIC, Apdo. 1827, Caracas, Venezuela.

Absolute growth and productivity of soybean var. Jupiter were severely affected by SMV. Ten-day old soybean plants mechanically inoculated with SMV showed 37% reduction in foliar area at 30-day old and 56% at 60 days. In field inoculated plants absolute growth was reduced by 62% at the beginning of the flowering stage. All plant parts were affected (leaves 62%, shoots 64% and roots 83%). Early infection with the virus greatly affects productivity. Ten-day old infected plants showed 80% pod reduction. Pods were small, with 99% of brown spotted seeds. Infection of 25-day old plants resulted in 70% pod reduction with 89% spotted seeds. Infection of 40-day old plants showed 32% pod reduction with 44% of spotted seeds. Seeds from spotted pods had an average weight reduction of 28% less than the checks. Seed transmission of SMV was 90-100% tested after harvested. However, this percentage of transmission was steadily reduced when the seeds were stored at room temperature.

#### 567

PURIFICATION AND PARTIAL CHARACTERIZATION OF WHEAT SPINDLE

STREAK MOSAIC VIRUS (WSSMV). K. Zagula Haufler and D. W. Fulbright. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312

A previously reported purification protocol for WSSMV (Haufler and Fulbright, 1983. *Phytopathology* 73:789) utilizing phosphate buffer yielded a partially-purified preparation with  $A_{260}/A_{280}=1.3-1.5$ . To eliminate precipitation of host DNA with virus particles, HEPES buffer was substituted for the phosphate buffer. A modified purification protocol consisting of extraction with 0.02M HEPES, pH 7.0, containing 1.0M urea, 0.01M NaDIECA and 0.1% Na<sub>2</sub>SO<sub>3</sub>, clarification with chloroform, concentration with 4% PEG (MW 8,000) and 0.25M NaCl, resuspension in 0.02M HEPES containing 4% Triton X-100, and sucrose-cesium sulfate density gradient ultracentrifugation yielded a purified preparation with a mean  $A_{260}/A_{280}=1.25$ . Using 10% SDS-PAGE of total plant proteins and partially-purified virion proteins, a protein with an estimated molecular weight of 36 Kd was present in preparations from infected tissue but not in those from healthy tissue.

#### 568

ULTRASTRUCTURE OF WHEAT LEAF CELLS INFECTED WITH WHEAT SPINDLE MOSAIC VIRUS AND LEAF RUST. Caleb H. Shrier and Wayne E. Gardner. Department of Plant Science, South Dakota State University, Brookings, SD 57007

Wheat streak mosaic virus (WSMV) and *Puccinia recondita* urediospore infection of wheat leaves was characterized by formation of viral inclusions and rust hyphae, haustoria and urediospores. Wheat cytoplasm contained large masses of WSMV virions but very few other inclusions suggesting that the rust infection reduced the number of cylindrical inclusions. Virus-like particles, most of which were different from WSMV, were observed in haustoria and intercellular hyphae. The hyphae were packed with organelles including dicaryotic nuclei. In the septal pore region mitochondria, microbodies and glycogen appeared to be bounded by a membrane. Nuclei were observed passing through septal pores. The haustorial mother cell was situated intercellularly, penetrated the host wall, joined the intracellular haustorial neck which was connected to the haustorium which contained nuclei, mitochondria, microbodies, ribosomes, lipid and vacuoles.

#### 569

OCCURRENCE OF WHEAT SPINDLE STREAK MOSAIC VIRUS ON WINTER WHEAT IN GEORGIA. Bays, D. C., Cunfer, B. M., and Demski, J. W., University of Georgia Experiment Station, Experiment, GA 30212

A virus disease not previously reported in Georgia was observed in winter wheat during the spring of 1984. The virus was identified as wheat spindle streak mosaic (WSSMV) using symptomatology, mode of transmission, enzyme-linked immunosorbent assay and serum specific electron microscopy. WSSMV was observed in a number of locations around the state and in Hartsville, South Carolina, but was primarily concentrated in an area surrounding Plains, Georgia. Symptoms of WSSMV were found on two wheat cultivars, Coker 797 and Florida 301, and on rye. Field studies showed a significant yield decrease between healthy and diseased areas with Florida 301 at two out of three locations (22 and 35%). Yield reduction was due primarily to a decrease in tillering. Other yield components that were reduced significantly were kernel weight, straw weight and total biomass. This is the first report of WSSMV in the Southeastern U. S.

#### 570

PRODUCTION OF DISEASE IN HELMINTHOSPORIA GROWN WITH *ESCHERICHIA COLI*. G. D. Lindberg and M. Price, Dept. of Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr. and Dept. Biochem., Louisiana State Univ., Baton Rouge, LA 70803.

We reported production of disease in all *Helminthosporium turcicum* but no *H. maydis* grown with 1-3-wk-old colonies of a *Pseudomonad*. Diseases were produced in both *H. turcicum* and *H. maydis* grown with 10-day-old colonies of *E. coli* obtained from feces of the American cockroach. All subcultures of *H. turcicum* grown with *E. coli* produced diseased colonies; symptoms of lysis developed first at the colony centers and moved outward whereas symptoms in the *Pseudomonad*-diseased *H. turcicum* isolate were most severe at the colony edge. Three distinct diseases were produced in *H. maydis* grown with *E. coli*, a stunted, "powdery" edged isolate, a black-blotched isolate and a brown, water-soaked isolate. The *E. coli*-*H. turcicum* and *H. maydis* diseases were transmitted readily to their healthy isolates. Viruslike particles and virus crystalline arrays were found in thin sections of the *Pseudomonad*-diseased *H. turcicum* isolate and isometric virus particles were demonstrated with lysozyme treated *Pseudomonad*.

#### 571

WATER RELATIONS OF *VITIS VINIFERA* L. INFECTED WITH PIERCE'S DISEASE BACTERIA. P. H. Goodwin, J. E. DeVay, and C. P. Meredith, Department of Plant Pathology and \*Department of Viticulture and Enology, University of California, Davis, CA 95616

Stomatal resistance ( $R_s$ ) of *Vitis vinifera* L. 'Chardonnay' leaves infected with Pierce's Disease bacteria (PDB) was measured in the green area of leaves exhibiting marginal chlorosis and necrosis.  $R_s$  of diseased leaves increased from 22.0 s cm<sup>-1</sup> on July 20 to 55.4 s cm<sup>-1</sup> on Sept. 22, 1984. Uninfected grapevines from the same variety and vineyard had an  $R_s$  of 0.2 s cm<sup>-1</sup> on July 20 increasing to 0.7 s cm<sup>-1</sup> on Sept. 22. The  $R_s$  of symptomless leaves from infected vines was 1.4 s cm<sup>-1</sup> on Sept. 22 and was not statistically different from that of uninfected vines. The water potential of infected leaves declined sharply from the leaf area near the petiolar junction to the marginal necrotic area. PDB also causes almond leaf scorch, and infected almond leaves had a higher  $R_s$  than leaves from uninfected trees. The results suggest that infection of grapevines by PDB causes water stress which may result in the typical symptoms of Pierce's Disease.

#### 572

COMPARISON OF MONOCLONAL ANTIBODIES AND POLYCLONAL ANTIBODIES IN THE DETECTION OF ASTER YELLOWS AGENT BY ELISA AND IMMUNOFLUORESCENT STAINING. C. P. Lin and T. A. Chen, Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903.

Monoclonal antibodies (MA) against aster yellows (AY) MLO produced by hybridoma techniques, and polyclonal antibodies (PA) from serum collected after splenectomy were used in the detection of AY agent. Using ELISA, the MA reacted to the AY-infected plants and discriminated between the AY and other MLOs specifically. Both the plant antigen-absorbed and the unabsorbed PA cross reacted to plant antigens and thus were unsuitable for distinguishing different MLOs. In immunofluorescent staining, cross and longitudinal sections of leaf midribs of healthy and AY-infected plants were made. Sections were stained with FITC-conjugated anti-mouse IgG. MA bound specifically to the AY-MLO in the sieve tubes of diseased plants, while PA treatment showed fluorescence in both healthy and diseased plants suggesting non-specific binding to plant cells.

#### 573

EFFECTS OF GLUTARALDEHYDE FIXATION ON ANTIGENICITY AND SURFACE LABELING PROPERTIES OF SPIROPLASMA CITRI. T.M. Moury, K. Klomparens-Baker\*, and M.E. Whalon, Departments of Entomology and \*Botany and Plant Pathology, \*Center for Electron Optics, and \*Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

The horseradish brittle root isolate of *S. citri* was fixed in 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, 0.25, and 0.10 percent glutaraldehyde. Spiral morphology was maintained at all concentrations. Antigenicity declined with increasing concentration as indicated by ELISA. Cell surface antigens, however, were labelled equally well at all concentrations with an IgG-colloidal gold conjugate. Sheep red blood cells treated in the same manner did not label. *S. citri*-specific IgG applied before the conjugate blocked the label, but nonspecific IgG and buffer controls did not. Apparently, membrane-bound *S. citri* antigens are less affected by glutaraldehyde fixation and probably comprise only a small proportion of the total antigen complex.

#### 574

COMPARISON OF METABOLIC CHANGES IN PERIWINKLE INFECTED WITH SEVERAL MYCOPLASMA-LIKE ORGANISMS. S. M. Douglas, The Connecticut Agricultural Experiment Station, Box 1106, New Haven, CT 06504.

The effects of mycoplasma infection of periwinkle (*Catharanthus roseus* L.) on total protein and activity of several enzymes were studied in relation to symptom expression and disease development. Mycoplasma-like organisms (MLOs) associated with Aster Yellows (AY) and Elm Phloem Necrosis (EPN) and *Spiroplasma citri* were maintained under controlled conditions by graft transmissions within a clone of periwinkle. Plants of different ages were examined at intervals after grafting. The total protein of healthy plants was significantly greater than of plants infected with MLOs. Soluble peroxidase and polyphenol oxidase activities of AY- and EPN- infected plants were systemically enhanced. Peroxidase activity was often four times greater in MLO-infected

compared to healthy plants. These metabolic changes were observed well in advance of external symptoms of disease or evidence of phloem necrosis.

#### 575

DETECTION OF X-DISEASE MYCOPLASMA-LIKE ORGANISMS IN PLANT AND INSECT HOSTS USING CLONED, DISEASE SPECIFIC DNA. B. C. Kirkpatrick, D. C. Stenger, T. J. Morris, and A. N. Purcell, University of California, Berkeley, CA 94720.

DNA was extracted from MLO enriched extracts derived from *Colladonus montanus* leafhoppers infected with the peach yellow leafroll (PYLR) strain of X-disease. Equilibrium centrifugation in CsCl/ethidium bromide gradients produced two distinct bands. The upper DNA band was removed and sequentially digested with Hind III and Eco RI. The resulting fragments were inserted into the plasmid UC8 and cloned in *E. coli*. Recombinant clones were screened by colony, dot and Southern blot hybridization using <sup>32</sup>P-nick translated DNA extracted from healthy and PYLR infected *C. montanus* and celery. Cloned, disease specific DNA hybridizes with DNA extracted from *Vinca major* or celery infected with either the PYLR or Green Valley strain of X-disease but not with healthy plant DNA. No hybridization has been detected with aster yellows infected *Vinca*.

#### 576

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO SPIROPLASMA CITRI AND CORN STUNT SPIROPLASMA. Ramon Jordan<sup>1</sup>, Meghnad Kona<sup>2</sup>, Ing-Ming Lee<sup>3</sup>, and Robert E. Davis<sup>2</sup>, USDA, ARS, <sup>1</sup>Florist and Nursery Crops Lab. and <sup>2</sup>Plant Virology Lab., Beltsville, MD 20705, and <sup>3</sup>Department of Botany, University of Maryland, College Park, MD 20742.

Spleen cells from mice immunized with a mixture of *S. citri* (M200H) and three strains of corn stunt spiroplasma (F32, PUB-17, I747) were fused with NS1 myeloma cells for the production of monoclonal antibody-secreting hybridomas. Hybridoma supernatants were initially screened in various direct and indirect ELISA tests against untreated and sodium dodecyl sulfate (SDS)-treated cells. Of 34 out of 47 spiroplasma-specific hybridomas, 7 secrete monoclonal antibodies (McAbs) that react with all four spiroplasma immunogens, 13 secrete McAbs specific for one or more of the three corn stunt spiroplasmas, and 14 produce McAbs specific to *S. citri*. Results of ELISA, immunofluorescence, and growth and metabolic inhibition assays with these and other spiroplasmas will also be presented.

#### 577

SEROLOGICAL DOT-IMMUNOBINDING FOR DETECTION OF SPIROPLASMA CITRI. J. Fletcher, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078

The dot-immunobinding assay (S. Haber, personal communication) was adapted for detection of *Spiroplasma citri*. Filter paper discs blocked with ovalbumin were spotted with the sample, incubated in antiserum (90 min), protein-A-peroxidase (30 min), and substrate, chloro-naphthol (10 min). The test was completed in under 4 hr. Detection limit for *S. citri* isolate BR3 averaged  $4 \times 10^{10}$  CFU/ml. Limits for other spiroplasmas of the same serogroup were higher, while strain 23-6 (different serogroup) was not detected. *S. citri* was detected in 46 of 53 single leafhoppers (*Circulifer tenellus*) fed on diseased plants, but not in control insects. The pathogen was detected in plants at peak of infection (when titer is highest), but not at early or late stages, though spiroplasmas were detected by ELISA or by isolation at all these times. Though less sensitive than ELISA, this assay may be useful in situations where economy and rapidity are desired.

#### 578

CALIFORNIA STRAINS OF ASTER YELLOWS DISEASE ENHANCE SURVIVAL OF CORN LEAFHOPPER ON ASTER. Alexander H. Purcell and Karen Gonot Suslow, Department of Entomological Sciences, University of California, Berkeley, CA 94720.

We confirmed previous reports (Sci. Am. 203:138) that the corn leafhopper *Dalbulus maidis* survives well on asters with aster yellows (AY) disease but not on healthy aster, and that corn leafhoppers could be "conditioned" by previous exposure to AY-aster to survive on healthy aster. Three California AY strains (DAY, SAY, TLAY) greatly improved survival of *D. maidis* on aster. AY also enhanced survival of *D. maidis* on celery or

*Plantago major* but not on periwinkle (*Vinca major*). Adult females "conditioned" by exposure to AY-aster survived on healthy aster much longer than did "conditioned" adult males. Exposure to corn stunt-diseased corn did not improve the survival of *D. maidis* on aster, nor did "AY-conditioning" prevent transmission of corn stunt spiroplasma to corn.

#### 579

DETECTION OF SPECIFIC PROTEIN OF SPIROPLASMAS WITH MONOCLONAL ANTIBODIES TO *Spiroplasma citri*. J. D. Lei and T. A. Clea, Department of Plant Pathology, Rutgers University-NAES, New Brunswick, NJ 08903.

The electrophoretic blots of SDS-PAGE protein patterns of spiroplasmas were probed with monoclonal antibodies (MCA) to *Spiroplasma citri* (SC) strain R8A2. All twelve MCA's, previously produced in this lab, reacted with a major band (30,000 M.W.) in the protein pattern of R8A2. The protein patterns of SC strains Algeria, Israel, Iran and ASP-1 were similar to the other SC strains except that the major band equivalent to the 30,000 M.W. protein of the strain R8A2 had slightly higher mobility in SDS-PAGE and did not react with the MCA IIE8-23. Trace amounts of the 30,000 M.W. protein were also detected by MCA IIE8-23 in proteins of SC strains Algeria and Iran, green leaf bug spiroplasma LB-12, corn stunt spiroplasma I-747, and *S. plumbea* 23-6. These MCA's are useful tools for the analysis of the protein profile of spiroplasmas.

#### 580

SEMISELECTIVE AGAR MEDIA FOR ISOLATION OF PSEUDOMONAS SYRINGAE PV. SYRINGAE PATHOGENIC TO BEANS. S. K. Mohan and N. W. Schaad, Dept. of Plant, Soil & Ent. Sci., Univ. Idaho, Moscow, ID 83843.

Two semiselective agar media, KBBC and SNBC, were developed for isolation of *Pseudomonas syringae* pv. *syringae* (Ps) from beans. Dilute cell suspensions of Ps strains and washings of bean seeds were used to determine the rate of recovery and degree of inhibition of saprophytic bacteria, respectively, in comparison with King et al.'s medium B (KB). KBBC was made by supplementing KB with (g/l) boric acid (1.5), cycloheximide (0.2) and cephalixin (0.08). Colonies of Ps were flat and translucent with blue fluorescence. Recovery of 10 strains varied from 80-140% and inhibition of saprophytes ranged from 90-99%. SNBC contained (g/l) sucrose (20.0), KNO<sub>3</sub> (1.0), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.3), K<sub>2</sub>HPO<sub>4</sub> (3.0), NaH<sub>2</sub>PO<sub>4</sub> (1.0), boric acid (1.5), cycloheximide (0.2) and cephalixin (0.08). Colonies of Ps were raised, globose and white with blue fluorescence. Recovery of strains varied from 82-145% and inhibition of saprophytes ranged from 77-99%.

#### 581

CHLOROPHYLL ALTERATIONS IN HELIANTHUS ANNUUS INFECTED BY PSEUDOMONAS SYRINGAE PV. IAGETIS. L. Fucikovsky, B. Kennedy, Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108 & W. Koukkari, Dept. of Botany, Univ. of Minn., St. Paul, MN 55108.

Bacteria introduced into 5 day old greenhouse grown sunflower (*Helianthus annuus*) seedlings produces bleaching of young developing tissues. Cotyledons of diseased plants do not bleach, but persist, resist senescence and retain chlorophyll. Area of developing leaves is reduced by half in 7 day-old plants, diminishing to a third or less after 9, 11 and 16 days. Concomitantly the chlorophyll concentration in the first leaves diminishes more than eight fold and such leaves never recuperate from toxemia. Growth of new apical tissue without bleaching occurs on the fourth set of new leaves.

#### 582

PHOTOMORPHOGENIC EFFECTS OF PSEUDOMONAS SYRINGAE PV IAGETIS ON HELIANTHUS ANNUUS. L. Fucikovsky, B. Kennedy, Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108, & W. Koukkari, Dept. of Botany, Univ. of Minn., St. Paul, MN 55108.

The pathogen produces a severe toxemia on apices of sunflower seedlings in the greenhouse. Leaf angles, leaf dry weight, leaf expansion, leaf texture (brittleness), leaf chlorophyll content and rhythmic movements are altered; cotyledons are not noticeably affected and they are functional longer than on control plants. Bleached leaves emerging from the apex 4-14 days after stem inoculation below cotyledons never recover, but eventually new upper leaves (4th leaf stage) will become green. The plant is stunted; stems are shorter and bleached above the inoculation point. Plants maintained in total darkness and

with exposures to 15 min of red or far-red light every 12 hours were stunted and depicted typical phytochrome associated changes such as red light inhibition of stem elongation.

583

DELINEATION OF *XANTHOMONAS CAMPESTRIS* PV. *CITRI* STRAINS WITH MONOCLONAL ANTIBODIES. A.A. Benedict, A.M. Alvarez, E.L. Civerolo, C.Y. Mizumoto. University of Hawaii, Honolulu, HI 96822. USDA-ARS, Beltsville, MD.

Monoclonal antibodies (MCA) were generated to identify *Xa* pv. *citri* strains, Types A, B, and C. Antibody A1 reacted with Type A reference strains (XC62, XC63) and 12 Type A strains from Argentina. Antibody A2 separated the A reference strains. Antibody B1 reacted only with a Type B strain (XC64) and not with 10 B strains from Argentina. Antibody B2 reacted with all Type B strains from Argentina, a Type C reference strain (XC70) from Brazil, and a *Xanthomonas* strain associated with bacteriosis of Mexican lime. B2 did not react with Type B reference strains (XC64, XC69). On the basis of tests with 448 other *Xanthomonas* strains and 102 non-xanthomonads of plant and animal origin, these MCA were specific for pv. *citri*, except that A1 reacted with 2 strains and B2 reacted with 4 strains of other xanthomonads.

584

LESION DEVELOPMENT AND POPULATION GROWTH OF *XANTHOMONAS CAMPESTRIS* PV *PHASEOLI* ON THE LEAF SURFACE OF BEAN PLANTS EXPOSED CONTINUOUSLY OR INTERMITTENTLY TO HF. K. L. Reynolds and J. A. Laurence. Dept. Plant Pathology, Cornell U., Ithaca, NY 14853.

Four week old *Phaseolus vulgaris* cv 'California Light Red Kidney' plants were exposed to 0 or 1  $\mu\text{g F/m}^2$  continuously, or 3 or 5  $\mu\text{g F/m}^2$  intermittently for 15 days after inoculation with *X. campestris* pv *phaseoli*. Intermittent exposures were conducted such that the total dose of HF in each case was equal to the dose resulting from continuous exposure to 1  $\mu\text{g F/m}^2$  (ie, 15  $\mu\text{g F/m}^2$ -days). Bacterial suspensions were sprayed on each plant to establish a leaf-surface population on the first trifoliate leaf and a lesion on the second trifoliate leaf. Lesion diameters were measured when first visible and again at the end of the experiment. At 5 day intervals leaves were collected, measured and washed. Washings were serially diluted and plated to estimate leaf-surface populations of the bacterium. Leaves were harvested at the end of the experiment to determine F accumulation. Lesion expansion and final size increased linearly with foliar F content while leaf-surface populations were apparently unaffected by HF.

585

DIFFERENTIAL REMOVAL OF IMMUNOREAGENTS FROM INDIRECT ELISA COMPLEXES. M. Akius, G. I. Mink, and W. -Y. Wang. Washington State Univ., IAREC, Prosser, WA 99350.

The effect of low pH Glycine-HCl buffer on dissociation of immunoreagents in indirect Elisa was examined using two flaviviruses, prune dwarf virus (PDV) and necrotic ring spot virus (NRSV) and a potyvirus, bean common mosaic virus (BCMV). After the initial indirect test virtually all goat anti-rabbit or anti-mouse conjugated antibodies could be dissociated by two hr soak at pH values below 2.5. In the same pH range most, but not all, virus specific antibodies dissociated from virus attached to the solid phase. Little, if any, PDV or BCMV dissociated from the polystyrene at these pH's. Significant amounts of NRSV, however, were removed at all pH values below 3.25. Potential use of low pH treatments for multiple tests with attached viruses will be discussed.

586

A BROAD SPECTRUM MONOCLONAL ANTIBODY PREPARED AGAINST BEAN COMMON MOSAIC VIRUS. Wei-Young Wang, G. I. Mink and M. J. Silbernagel. Dept. of Plant Pathology, Wash. State Univ., IAREC, Prosser, WA, 99350.

A hybridoma cell line bc197 which secretes a monoclonal antibody (BC-1) of the IgG2a isotype was prepared by fusion between mouse myeloma cell line P3X63Ag8.653 and BALB/c mouse spleen cells. Ascitic fluid produced from BALB/c mice had a titer of  $10^7$  in indirect ELISA. Twenty-two BCMV strains in either serogroup A or B reacted with BC-1 to a similar extent. The sensitivity of BC-1 was comparable to those of polyclonal antibodies. However, no precipitating lines were formed in agar double-diffusion plates. Potyviruses other than BCMV also

reacted with BC-1. These included some isolates of bean yellow mosaic, peanut mottle, blackeye cowpea mosaic and watermelon mosaic (type 2) viruses. This broad spectrum monoclonal antibody is valuable not only as a diagnostic tool but also useful to study the conserved epitope present on these potyviruses.

587

THE USE OF ENZYME-LINKED IMMUNOBLOT ASSAY (EIBA) TO DETECT BEAN COMMON MOSAIC VIRUS IN INDIVIDUAL BEAN SEEDS. Wei-Young Wang, G. I. Mink and M. J. Silbernagel. Dept. of Plant Pathology, Wash. State Univ., IAREC, Prosser, WA, 99350.

A simple and sensitive technique was developed to detect BCMV in bean seeds using a nitrocellulose membrane as solid support. Pieces of cotyledon from individually soaked seed were either directly placed on the membrane or ground in buffer and the extract blotted onto the membrane. Virus infected seeds were identified by incubating the membrane first with virus-specific rabbit antiserum or a mouse monoclonal antibody and then with a goat anti-rabbit (or anti-mouse) IgG horseradish peroxidase conjugate. The remaining part of the tested seed was planted and the germinated seedlings examined for infection. This method was more sensitive than conventional ELISA and appeared to be useful to rapidly screen seeds prior to planting. However, due to the uneven distribution of virus in seed parts, whole seeds need to be tested to estimate actual virus incidence in seed lots.

588

THE INHIBITORY EFFECT OF BEAN OR PEA SEED EXTRACTS IN ELISA DETECTION OF PEA SEED-BORNE MOSAIC VIRUS. Wei-Young Wang, G. I. Mink and M. J. Silbernagel, Dept. of Plant Pathology, Wash. State Univ., IAREC, Prosser, Washington, 99350.

The absorbance values at 405 nm (A405) in both double-sandwich and indirect ELISA was reduced up to 95% when buffered extracts of pea or bean seeds were mixed with PSBMV-infected leaf samples prior to placement in polystyrene plates. The inhibition was specific to PSBMV as seed extracts had little effect on A405 values obtained with bean common mosaic virus. Little or no inhibition was observed if virus samples were added to the wells a few sec before the extract was added. Seed extracts appeared to interfere with the adsorption of virus to the polystyrene. A linear relationship was found between ELISA readings and dilutions of the seed extract. Because of these inhibitors detection of PSBMV in individual pea seeds by ELISA was difficult.

589

BIOPHYSICAL CHARACTERIZATION OF TWO PRUNUS NECROTIC RINGSPOT VIRUS ISOLATES FROM SWEET CHERRY TREES. Ching-Ang Ong and G. I. Mink. Washington State Univ., IAREC, Prosser, WA 99350.

Two biotypes (QD and QM) of prunus necrotic ringspot virus that caused severe rugose mosaic or no symptoms were isolated repeatedly from sweet cherry trees in WA. Although serologically similar, they differed considerably in pathogenicity, symptomatology, sedimentation patterns and electrophoretic mobilities. Isolate CH38, representative of biotype QD, incited primary lesions on *Chenopodium quinoa* followed by systemic mottling, necrosis and dieback. Isolate CH39, representative of biotype QM, produced only systemic mottling. When centrifuged in 10-40% rate zonal sucrose density gradients, virions of CH38 separated into 2 major zones while those of CH39 formed 3 zones. Isolate CH38 resolved into 4 and isolate CH39 into 3 bands of different electrophoretic mobilities in agarose gel electrophoresis. However, after proteinase digestion, the RNA of both isolates resolved into 3 bands.

590

MINI DOUBLE IMMUNODIFFUSION TEST FOR DIRECT PROJECTION AS SLIDES. Ching-Ang Ong and G. I. Mink. Washington State Univ., IAREC, Prosser, WA 99350.

A simple and inexpensive procedure to prepare slides directly from double immunodiffusion tests without special photographic techniques is described. Agarose (1.5%) is cast on a polyester based film (4 x 3 1/2 mm). The film support is then placed on a template and wells of 1.6 mm diameter are

punched using a capillary tube, a microscope and in-house vacuum system. Typically about 3-5  $\mu$ l of reactants is introduced into each well. Upon completion of the test, the agarose gel is washed, stained, destained and dried down directly onto the film support. Dried gel thus prepared can be mounted on a slide mount for projection or stored in a file. Precipitation lines stained with crocein scarlet in combination with coomassie blue were more distinct than those stained with coomassie blue alone or imido black stains.

#### 591

USE OF A MONOCLONAL ANTIBODY REACTIVE WITH SEVERAL POTYVIRUSES FOR DETECTION AND IDENTIFICATION IN COMBINATION WITH VIRUS-SPECIFIC ANTISERA. John Hammond, Roger H. Lawson, and H. T. Hsu, USDA, Agricultural Research Service, Florist and Nursery Crops Laboratory, Beltsville, MD 20705 and American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

A monoclonal antibody produced against tulip breaking virus antigen has been found to react with some other potyviruses, including bean yellow mosaic, iris mild mosaic and iris severe mosaic viruses. By use of a Heterologous Double Antibody Sandwich ELISA in which plates were coated with the monoclonal antibody, and using either virus-specific rabbit antisera followed by goat anti-rabbit conjugate, or virus-specific rabbit antibody conjugates, plants infected with these viruses were clearly identified. By this means, the sensitivity of the double antibody sandwich form of ELISA is maintained without the disadvantage (for field screening) of strain specificity, and there is no need to prepare virus-specific conjugates.

#### 592

THE USE OF CLONED cDNA TO DETECT POTYVIRUS INFECTIONS. John Hammond and Rosemarie W. Hammond, USDA, Agricultural Research Service, Florist and Nursery Crops Laboratory, Beltsville, MD 20705.

Single-stranded complementary DNA (ss cDNA) was prepared by oligo-dT priming of RNA prepared from an isolate of bean yellow mosaic virus (BYMV) from gladiolus. The ss cDNA was found to hybridize to heterologous isolates of BYMV and some other potyviruses, including potato virus Y and tobacco etch virus. Double-stranded cDNA was then prepared and fragments cloned into plasmid pUC9 after restriction endonuclease digestion. Bacterial colonies containing viral-specific inserts were selected by colony hybridization using ss cDNA as a probe. The cloned viral-specific fragments have been examined to determine their relationships to RNA of heterologous BYMV isolates and of other potyviruses, including potato virus Y, tulip breaking virus, and tobacco etch virus. The relative sensitivities of detection of BYMV by dot-blot hybridization and various serological assays has also been examined.

#### 593

THE USE OF ANTISERA AGAINST CYLINDRICAL INCLUSION PROTEINS TO DETECT AND IDENTIFY POTYVIRUSES. John Hammond and Roger H. Lawson, USDA, Agricultural Research Service, Florist and Nursery Crops Laboratory, Beltsville, MD 20705.

The potyviruses induce characteristic cytoplasmic inclusion bodies, composed of a protein encoded by the viral genome which is not serologically related to the viral coat protein. The cylindrical inclusion protein (CIP) may be serologically related or distinct from the CIPs of other potyviruses. The CIPs of three potyviruses infecting bulb crops have been extracted and distinguished electrophoretically (Alper et al, *Phytopathology* 74:960-962). In a modified procedure, purified CIP was prepared for use as antigen. Antisera to CIPs of bean yellow mosaic, clover yellow mosaic and pea mosaic virus prepared in this manner have been useful in the sensitive detection of bean yellow mosaic virus, and in the differentiation of isolates. The method is recommended especially for potyviruses of limited host range, from which purification is difficult.

#### 594

REACTIVITIES OF APPLE MOSAIC VIRUS (ApMV)/PRUNUS NECROTIC RINGSPOT VIRUS (NRSV) MONOCLONAL ANTIBODIES (McAbs) WITH ApMV, NRSV AND OTHER ILARVIRUSES. Ramon Jordan, Joan Aebig and H. T. Hsu, USDA, ARS, Florist and Nursery Crops Lab., Beltsville, MD 20705 and American Type Culture Collection, Rockville, MD 20852.

Seven McAbs made to ApMV and/or NRSV were evaluated in various direct and indirect ELISA and Immunodot-blot assays. Virus treatments included 0.2-2% sodium dodecyl sulfate with and without 5% mercaptoethanol, and pH 9.5/56°C. Generally, McAbs 1, 2, and 3 gave stronger reactivities with treated ApMV than with untreated virus. Conversely, McAbs 4, 5, and 6 reacted stronger with untreated ApMV. McAbs 5, 6, and 7 reacted strongest with untreated NRSV, and McAb 4 reacted well with both treated and untreated NRSV. In other indirect ELISA tests, McAbs 2, 3, and 6 were found to react with several other ilarviruses, specifically tobacco streak virus, citrus variegation virus, prune dwarf virus, as well as alfalfa mosaic virus. In Western-blot analysis with intact coat protein (CP) subunits and enzyme-digested CP subunit fragments, only McAbs 1, 2, and 3 react with ApMV and only McAb 4 reacts with NRSV.

#### 595

ELISA DETECTION OF RELATIONSHIPS AMONG THREE LUTEOVIRUSES. Adrianna D. Hewings, USDA-ARS, Plant Disease Research Lab., Fort Detrick, Bldg. 1301, Frederick, MD 21701 and Cleora J. D'Arcy, Dept. of Plant Pathology, U. of IL, Urbana, IL 61801

Three luteoviruses, barley yellow dwarf virus from Illinois (BYDV PAV-IL), beet western yellows virus from California (BWVY-CA), and soybean dwarf virus from Japan (SDV-D) were studied by direct double antibody sandwich ELISA (DDAS ELISA) and by indirect ELISA on unprecoated plates. DDAS ELISA detected 1.5ng/ml purified virus in homologous tests but failed to detect virus in heterologous tests up to 800 ng/ml. In DDAS ELISA with sap extracts from infected plants, the SDV-D system detected BYDV-PAV-IL and BWVY-CA, the BYDV-PAV-IL system detected BWVY-CA, and the BWVY-CA system occasionally detected BYDV-PAV-IL. All heterologous reactions were weak but significant ( $P = .05$ ). Indirect ELISA detected reciprocal relationships among all three viruses at 100ng/ml or less. These preliminary data (one bleed from one rabbit/virus) suggest that all relationships are distant.

#### 596

REACTION OF A BROAD SPECTRUM OF PVY ISOLATES TO MONOCLONAL ANTIBODIES IN ELISA. E.N. Fernandez-Northcote and P. Gugerli. Universidad Nacional Agraria, La Molina - International Potato Center, Aptdo. 5969, Lima-Peru, and Swiss Federal Agricultural Research Station of Changins, CH-1260, Nyon, Switzerland.

A collection of PVY isolates from geographically distinct locations has been used to compare by direct ELISA the effectiveness in detecting PVY of a broad spectrum monoclonal antibody (MA) and standard polyclonal antibodies (PA) to PVY<sup>n</sup>. The reaction of other potyviruses was also tested. All 26 Andean isolates from PVY<sup>o</sup>, PVY<sup>n</sup>, and PVY<sup>c</sup> group of strains reacted with both MA and PA. Isolate PVY<sup>c</sup> Arran from Holland reacted only with PA. With MA the reaction of isolates was generally better than with PA, and minimum background values due to nonspecific reactions were obtained after 20 h. Potato virus A, wild potato mosaic virus, potato virus V (PVY<sup>c</sup>-GL, PVY<sup>c</sup>-AB, and UF isolates), Peru tomato virus, and tobacco etch virus did not react significantly with both MA and PA to PVY. However PVY isolates reacted weakly with Peru tomato virus PA.

#### 597

A RAPID AND SIMPLE REVERSE PASSIVE HAEMAGGLUTINATION ASSAY (RPH) FOR PLANT VIRUS DETECTION. E.Sander, R.G.Dietzgen, B.Köhm, M.P. Cranage\* and R.R.A.Coombs\*. Institut Biologie II, Universität Tübingen, D-7400 Tübingen, Fed. Rep. Germany; \*Dept. of Pathology, Div. of Immunology, University of Cambridge, Cambridge, U.K.

In the reverse passive haemagglutination assay chymotrypsin-treated sheep erythrocytes coupled with monoclonal or polyclonal virus-specific antibodies by CrCl<sub>3</sub> can be agglutinated by the appropriate virus. This one step method reached a sensitivity and specificity comparable to the direct ELISA. About 1 ng of tobacco mosaic, arabis mosaic, pea enation mosaic, potato leafroll and potato virus X were detected in sap of hop, cucumber, tobacco and tubers and leaves of different potato varieties. Haemagglutinating potato lectin was blocked with N-acetyl-glucosamine oligomers. The results can be read with the unaided eye within 90 min or 15 min when centrifuged briefly at low speed. Temperatures between 4° and 37°C were suitable. Antibody-coupled erythrocytes can be stored ready for use when glutaraldehyde-stabilized and subsequently freeze-dried.

#### 598

THE EFFECT OF SUCROSE ON SPOT HYBRIDIZATION ASSAYS FOR

SUGARCANE MOSAIC VIRUS. K. S. Derrick, S. H. Chang, R. C. French and C. A. Clark, Dept. of Plant Pathology and Crop Physiology and Dept. of Biochemistry, La. Agric. Exp. Sta., La. State Univ. Agr. Ctr., Baton Rouge, LA 70803.

The addition of sucrose to extracts of sugarcane mosaic virus (SCMV) infected tissue or purified SCMV RNA preparations was found to significantly increase the binding of viral RNA to nitrocellulose (NC) and charge modified nylon (CMN) membranes. Hybridization assays for SCMV on NC, NC pretreated with high salt or CMN were about 8 times more sensitive when the extracts or RNA preparations used for spotting contained 0.4 M sucrose. In contrast, sucrose had no effect on the binding of potato spindle tuber viroid to CMN. Since the RNA of some viruses in the potato virus Y group have been shown to contain polyadenylate sequences, the effect that sucrose has on the binding of SCMV RNA to NC and CMN may be due to a polyadenylate sequence.

#### 599

A somaclonal variant of tomato resistant to race 2 of *Fusarium oxysporum* f.sp. *lycopersici*. S. A. Miller, G. R. Williams, H. Medina-Filho and D. A. Evans, DNA Plant Technology Corp., 2611 Branch Pike, Cinnaminson, NJ 08077.

A somaclonal variant resistant to race 2 of *Fusarium oxysporum* f.sp. *lycopersici* (Fol) has been selected from plants regenerated from callus cultures of the processing tomato (*Lycopersicon esculentum* L.) cultivar UC82B. UC82B is resistant to race 1 of Fol but susceptible to race 2. Leaf explants were cultured on modified MS medium in the absence of selective agents (i.e. Fol culture filtrates or toxins), then regenerated shoots were rooted and transferred to the greenhouse. R<sub>1</sub> generation seedlings were evaluated for resistance to Fol race 2 after inoculation by the root-dip method. Resistance is governed by a single, dominant gene. The Fol race 2 resistant variant has maintained the resistance to Fol race 1 expressed in its progenitor UC82B.

#### 600

MODE OF INHERITANCE OF RESISTANCE TO THREE RACES OF *FUSARIUM OXYSPORUM* ON COWPEA. K.S. Rigert and K.W. Foster, Department of Agronomy and Range Science, Univ. of Calif., Davis, CA, 95616.

We have previously described the development of a root dip inoculation technique and scoring system for evaluation of *Fusarium* wilt resistance in cowpea. With this technique, the standard differentials failed to distinguish the three races of *Fusarium* wilt. However, three races can be distinguished using PI 162925 and PI 115683 as differentials. The objective of this study was to determine the inheritance of wilt resistance in the cultivars CB5, CB3, and 7964 using the root dip technique. These cultivars were crossed in all combinations and F<sub>1</sub>, BC, F<sub>2</sub>, and F<sub>3</sub> progeny were evaluated for resistance to the three wilt races. Segregation ratios showed that CB5 and CB3 possess different dominant genes which confer intermediate and high levels of resistance to Race 1, respectively. Different dominant genes conditioning resistance to Races 2 and 3 were found in CB3 and 7964. F<sub>3</sub> family cosegregation indicated that the Race 2 and Race 3 resistance genes are linked.

#### 601

SCREENING WORLD APPLE GERMPLASM COLLECTION FOR RESISTANCE TO *PHYTOPHTHORA CACTORUM*. R.S. Dethlefs, Agriculture Canada Research Station, Summerland, British Columbia, Canada, V0H 1Z0

Five hundred thirty five accessions from the USDA world apple germplasm collection were screened for resistance to *Phytophthora cactorum*, causal agent of apple crown rot. In vitro, dormant excised twigs were inoculated with *P. cactorum*. Length of necrosis was used as a measure of relative resistance. Sixteen plant introduction lines were very resistant, 50 showed intermediate resistance, and 469 lines were very susceptible to *P. cactorum*. These results indicate genetic variation in resistance and suggest the possibility of breeding resistant rootstocks to control the disease.

#### 602

VARIATIONS IN MAIZE INBRED RESPONSE TO MAIZE DWARF MOSAIC VIRUS INOCULATION TREATMENTS. Raymond Louie, USDA-ARS, Dept. of Plant Pathology, OARDC, Wooster, OH 44691

Evaluation of resistance of 12 maize inbreds to maize dwarf

mosaic virus (MDMV) was influenced by inoculation treatments, method of data analyses, and genetic determinants. Treatments included four ages of test plants; as many as four repeated inoculations; 1, 3, or 6 rubs/inoculation; virus dilutions of 1:10; 1:40; 1:80; 1:160, or 1:320; and MDMV-A or MDMV-B. Tests results were subjected to ANOVA and the means separated by Scott-Knott cluster analyses. The treatment involving four inoculations with two rubs each at four day intervals produced the most infected plants in all inbreds. MDMV-B was not generally more infectious than MDMV-A. Pa405 and Oh28 were most and least resistant, respectively, in all tests. KY61:2335, M14, and Va35 displayed great variability in different tests and were classified as moderately resistant or susceptible depending on inoculation treatment.

#### 603

THE EFFECT OF SEPTORIA TRITICI BLOTCH AND CHEMICAL DESICCATION ON SOURCE-SINK RELATIONS IN SPRING WHEAT CULTIVARS. Z. Eyal, Dept. of Botany, Tel Aviv University, 69978, Israel.

Spring wheat cultivars subjected to *Septoria tritici* blotch, post-anthesis chemical desiccation and mechanical defoliation expressed differential response in kernel weight losses. Positive, significant correlations were obtained across cultivars between loss in kernel weight and loss in total biomass in *Septoria* and desiccant affected plants. Pre-anthesis mechanical reduction in sink size in stressed plants resulted in significant compensation in kernel weight across cultivars as compared to intact spikes. Glumes and awns markedly contributed to kernel weight in intact and halved spikes in desiccated plants. Abiotic and biotic stresses affected the rate of accumulation and depletion of stem dry weight and soluble carbohydrates. Lower losses in kernel weight in the tolerant cultivar Miriam were associated with greater losses in non-structural carbohydrates and fructosans.

#### 604

PATHOGENIC RACES OF *CERCOSPORA ORYZAE* IN THE SOUTHERN UNITED STATES. D. N. Sah and M. C. Rush, Dept. of Plant Path. and Crop Physiol., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803

Leaf and sheath samples of rice with typical narrow brown spot and purple brown blotch symptoms were collected from seven major rice-producing parishes in Louisiana during the 1983 and 1984 growing seasons. Single spore isolates of *C. oryzae* were obtained. The differential cultivars Mira, Mars, Kamrose, Rexoro, Zenith, Bluerose, Shoemed, and Fortuna were inoculated with conidia from each isolate. Inoculated plants were maintained in a humidified chamber in the greenhouse and evaluated for disease after 3 weeks. Thirty-six races in six physiologic race groups were differentiated among 54 isolates from Louisiana, Arkansas, and Texas. The cultivar Mars, recently released with vertical resistance to *C. oryzae*, was found to be susceptible to 11 of the 36 races. These findings suggest that *C. oryzae* is highly variable in pathogenicity. Race reducing resistance may be more effective for control of this disease than monogenic resistance.

#### 605

SOME REACTIONS OF A TOLERANT BARLEY COMPOSITE CROSS POPULATION AND BARLEY TOLERANT-INTOLERANT ISOLINES TO BARLEY YELLOW DWARF VIRUS. J.M. Crosslin\*, T.W. Carroll\*, E.A. Hockett\*\*, and S.K. Zaske\*, \*Plant Pathology Department, and \*\*USDA-ARS, Plant and Soil Science Department, Montana State University, Bozeman, MT 59717

A male sterile facilitated recurrent selection population of barley, designated Composite Cross (CC) XLIV, was field evaluated in Montana for reaction to barley yellow dwarf virus (BYDV). CC XLIV was inoculated with BYDV in 1983 and 1984 and severely chlorotic or intolerant plants were rogued to increase the percentage of green or tolerant plants in succeeding generations. In replicated trials, yields of the 1983 and 1984 populations in 1984 were reduced approximately 50% and 30%, respectively. Four barley isolines, with or without the Yd2 gene for BYDV tolerance, were also inoculated and evaluated. Isolines with or without Yd2 showed yield reductions of less than 5% and over 90%, respectively.

#### 606

MOLECULAR CHARACTERIZATION OF A RACE SPECIFIC INCOMPATIBILITY GENE. C. Napoli, J. Swanson, D. Dahlbeck, and B. Staskawicz, Dept. of Plant Pathology, Univ. of Calif., Berkeley, CA 94720.

A gene cloned from *Pseudomonas syringae* pv. *glycinea* Race 6 has been demonstrated to confer a change in race specificity from virulence to avirulence when mobilized into *P. s. glycinea* Race 5 and assayed on soybean cultivar Harosoy (Staskawicz et al., 1984, PNAS 81:6024-6028). This gene has been designated as the race specific incompatibility A (*rsiA*) gene. Deletion mapping and transposon mutagenesis have been used to locate the structural gene and promoter region of *rsiA*. A subclone of *rsiA* has been inserted into a *trp* expression vector and the protein product visualized on SDS-PAGE gels utilizing the maxi-cell procedure. Hybridization studies (Southern analysis) show an internal region of the *rsiA* structural gene is unique to Race 6, with no cross hybridization to Races 0, 1, 4 or 5. However, initial experiments indicate there are conserved regions with varying homology at both the 3' and 5' ends of the gene. Dideoxy sequencing has been initiated to establish the nucleotide sequence of this gene.

#### 607

CLONING OF A FACTOR FROM *PSEUDOMONAS SYRINGAE* PV. *TOMATO* RESPONSIBLE FOR A HYPERSENSITIVE RESPONSE ON SOYBEAN. D. Y. Kobayashi and N. T. Keen, Dept. of Plant Pathology, University of California, Riverside, CA 92521.

*Pseudomonas syringae* pv. *tomato* (*Pst*) is the causal agent of bacterial speck of tomato and forms a hypersensitive response (HR) on soybean. To investigate the specific DNA sequences of *Pst* that are involved in initiating the HR on soybean, a genomic library of *Pst* was constructed using the cosmid cloning vector pLAFR3. Each clone of *Pst* DNA was mobilized from *E. coli* into *Pseudomonas syringae* pv. *glycinea* (*Psg*) using the helper plasmid pRK2013. Transconjugants were then inoculated into the soybean cultivar Harosoy and screened for a phenotypic change from a compatible to a hypersensitive response. One out of 600 *Psg* transconjugants elicited a hypersensitive response which was similar to that formed in response to *Pst*.

#### 608

VIRULENCE MUTANT IN *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* SIMILAR TO HYPERSENSITIVE RESPONSE MUTANTS. Marilyn J. Stapleton, Mary C. Deasey, Ann G. Matthyse, Biology Department, Coker Hall 010-A, University of North Carolina, Chapel Hill, NC 27514.

Transposon Tn5 was used to generate virulence mutant NC12 of *Pseudomonas syringae* pv. *phaseolicola* ATCC strain 19304. Exconjugants of spot matings between *P. syringae* pv. *phaseolicola* (*Psp*) strain 19304 and *E. coli* 5m 10 pSUP1011 were selected on neomycin-containing minimal media for transposition of Tn5 from pSUP1011. A radiolabeled probe containing 3.3 kb of Tn5 DNA hybridized to a 15.9 kb *EcoRI* fragment from a genomic blot of mutant strains NC12, RR25 and HR37 (hypersensitive response mutants) but not to parent strain Psp19304. The probe hybridized to an additional 5.7+0.6 kb band in strain HR25. Other neomycin-resistant Tn5 insertion mutants with apparent wild-type phenotypes hybridized to different sized bands. Mutant strain NC12 completely lost the ability to induce wild-type virulence symptoms on the leaves of *Phaseolus vulgaris* and to elicit a hypersensitive response in tobacco leaves (a non-host plant).

#### 609

CLONING AND ANALYSIS OF GENES ASSOCIATED WITH PATHOGENICITY AND HYPERSENSITIVITY FROM *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA*. P. B. Lindgren, R. C. Peet, and N. J. Panopoulos, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The isolation of 8 independent prototrophic mutants of *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) with altered host responses by Tn5 mutagenesis has been reported (Lindgren et al. 1984, Phytopathology 74:837). These mutants which were selected for their inability to elicit the hypersensitive response on tobacco, are also nonvirulent or have reduced virulence on bean and have varying abilities to elicit HR on other heterologous hosts. Tn5 was contained in 3 different *EcoRI* target fragments of 17, 8 and 6 Kb. A total genomic library of *Sau3A* partially digested DNA from wild-type *Psp* was constructed in the cosmid vector pLAFR-3. Six colonies showing homology to 7 Kb of DNA subcloned from the 17 Kb *EcoRI* target fragment was identified by colony hybridization. One of these clones could complement 7 of the 8 mutants. This clone contains two *EcoRI* fragments of approximately 17 and 6 Kb, and restores the wild-type phenotype to mutants with insertions in similar sized *EcoRI* fragments.

#### 610

CLONING AND ANALYSIS OF GENES INVOLVED IN PHASEOLOTOXIN PRODUCTION BY *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA*. R. C.

Peet, P. B. Lindgren and N. J. Panopoulos, Department of Plant Pathology, University of California, Berkeley, CA 94720.

The isolation of 6 independent prototrophic *Tox<sup>-</sup>* mutants of *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) through Tn5 mutagenesis has been reported (Peet et al. 1984, Phytopathology 74:837). Tn5 was contained in 5 different *EcoRI* target fragments of approximately 1.0, 1.3, 2.3, 4.5 and 6.6 Kb. The Tn5 from one mutant (NPS4336) contained within an 8.0 Kb *EcoRI* fragment was cloned in pUC8. A total genomic library of *Sau3A* partially digested DNA from wild-type *Psp* (NPS312L) was constructed in the mobilizable cosmid vector pLAFR3 and maintained in *E. coli* HB101. Colonies showing homology to the cloned *EcoRI* target fragment from NPS4336 were identified by colony hybridization. Two cosmids, when mobilized from *E. coli* by plasmid pRK2013 in individual conjugations to the 6 *Tox<sup>-</sup>* mutants, restore OCTase-specific toxin production in a total of 5 of the mutants as determined by a quantitative microbiological assay.

#### 611

FUNCTIONAL COMPLEMENTATION OF A *TOX<sup>-</sup>* MUTANT OF *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA*. David E. Clements and C.J. Romeo, Department of Biochemistry and Biophysics, H.V. Kandar and Suresh S. Patil, Department of Plant Pathology, University of Hawaii, 96822.

A genomic library of *P. s. pv. phaseolicola* PDDCC 4612 was constructed by ligating size selected partial *EcoRI* restricted DNA into pLAFR1 and transfecting *E. coli* HB101. A partial screen of the library with one U.V. mutant of *P. s. pv. phaseolicola* using tri-parental mating resulted in the complementation of the mutant. The complementing insert is about 23 kb. A partial restriction map has been made using Bam HI, Hind III and KpnI. The smallest segment capable of complementation is being determined using  $\lambda$  ::Tn5 mutagenesis and subcloning. Other *Tox<sup>-</sup>* mutants are being screened to determine the number of genes involved in the production of phaseolotoxin.

#### 612

CONSTRUCTION AND CHARACTERIZATION OF A *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* *recA<sup>-</sup>* STRAIN. D. K. Willis and N. J. Panopoulos, Dept. of Plant Pathology, Univ. of Calif., Berkeley, CA 94720.

We have reported the cloning of the *P. s. pv. syringae recA* gene which was isolated by the ability of this gene to complement the recombination deficiency (*Rec<sup>-</sup>*) and UV sensitivity (*UV<sup>S</sup>*) of a *recA* deletion mutant of *Escherichia coli* (Willis et al. 1984 Phytopathology 74:837). We have mutagenized the *P. s. syringae* (*PSS*) *recA* gene (contained in the pLAFR1 recombinant pCUV8) with Tn5 to give pCUV802. The *EcoRI* fragment containing the *PSS recA:Tn5* mutation was sub-cloned from pCUV802 into a vector with a *ColE1* replicon to give pKw11. We have been able to exchange this mutation into *PSS* by utilizing the formation of a cis-merodiploid intermediate in which pKw11 has integrated recombinationally into the *PSS* chromosome. The phenotype of *PSS recA:Tn5* mutants is similar to that displayed by *E. coli recA<sup>-</sup>* mutants in that they are *UV<sup>S</sup>* by plate test. The potential uses of *recA<sup>-</sup>* derivatives of *PSS* will be discussed.

#### 613

CHARACTERIZATION OF CALIFORNIA ISOLATE OF DASHEEN MOSAIC VIRUS. W. Kosiralana, L. G. Weathers, and D. J. Gumpf, Department of Plant Pathology, University of California, Riverside, CA 92521.

Dasheen mosaic virus (DMV) in Chinese evergreen plants (*Aglaonema commutatum*) was reported in California in 1983. A California isolate (DMV-CA) was purified from infected greenhouse grown *Philodendron selloum* seedlings and physicochemical properties of the virions determined. Purified virions formed a single UV-absorbing infectious band with a density of 1.31 and 1.245 g/cc in cesium chloride and cesium sulfate equilibrium density gradients, respectively. Analysis of virion protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed only a single band of coat protein with a molecular weight of ~ 32,000. Virions contained infectious single stranded RNA with polyadenylated sequences. Utilizing denaturing agarose gel electrophoresis, viral RNA migrated as a single band, identical to tobacco etch virus RNA, indicating that the molecular weight of DMV-RNA was about 3.2 x 10<sup>6</sup>.

#### 614

FLEXUOUS ROD-SHAPED PARTICLES ASSOCIATED WITH BLUEBERRY MOSAIC DISEASE. K. S. Kim and R. C. Gergerich, University of Arkansas,



Ultrastructural studies of mosaic-diseased highbush blueberry (*Vaccinium corymbosum* L.) revealed the consistent presence of filamentous viruslike particles in leaf cells. The particles were present both in the cytoplasm and nucleus. The particles, approximately 12 nm in diameter with an undetermined length, occurred as bundles of various sizes, numbers and shapes. In the cytoplasm the particles were associated with a large number of ribosomes and rough endoplasmic reticulum. These bundles were often arranged to form a stellate configuration, each bundle radiating from the center in a different direction. The particles within the bundles appeared to occur as a unit of 4 filaments bonded tightly together along their long axis exhibiting a square when such a unit was sectioned transversely. In longitudinal sections the units appeared as two parallel particles separated by an electron-lucent space resulting in a tubule-like appearance. No pinwheel inclusions were observed and the particles were not limited to phloem tissue.

## 615

SEED AND GRAFT TRANSMISSIBLE ROD-SHAPED VIRUS-LIKE PARTICLES ASSOCIATED WITH STRIPED CHLOROSIS OF MIMOSA: ULTRASTRUCTURAL AND CYTOCHEMICAL ASPECTS. E. M. Martin and K. S. Kim, University of Arkansas, Fayetteville, AR 72701.

Rod-shaped particles, approximately 35 x 95 nm, were associated with a chlorotic stripe symptom in mimosa, *Albizia julibrissin*, leaves. They were seed (20%) and graft (approach) transmitted, but were not transmitted by sap inoculation. The particles were associated with granular and fibrillar viroplasmic inclusions. The granular inclusions were similar to caulimovirus inclusions and cytochemical studies indicated that the inclusions and the particles contained DNA. Expanding leaves with typical symptoms from seedlings as young as three months exhibited various developmental stages of the particles and associated inclusions. The granular inclusions in early stages of infection occurred adjacent to the nucleus as small electron-dense patches of circular profiles with scattered virus-like particles at their exterior. Individual particles occurred outside the nuclear pores with their short axis towards the pores. No plant viruses having a DNA genome and the morphology of the mimosa particles have been reported.

## 616

Purification of virions and RNA of an Illinois barley yellow dwarf virus isolate transmitted by *Rhopalosiphum padi* L. J. F. Murphy and Cleora J. D'Arcy, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801.

An Illinois isolate of barley yellow dwarf virus, transmitted specifically by *R. padi* (BYDV-RPV-IL), was increased in oats (*Avena byzantina* C. Koch 'Coast Black') and tissue stored at -80C. Purifications with 1.5% Rohment P extraction yielded more BYDV-RPV-IL than liquid nitrogen extraction. Virus yields obtained from root tissue were consistently higher than from shoot tissue. Root tissue preparations stirred rapidly (ca. 160 rpm) on a magnetic stirplate during enzyme incubation yielded significantly less virus (570 µg/kg) than those stirred slowly (ca. 80 rpm; 1130 µg/ml). Highest yields (1330 µg/kg) were obtained from preparations not subject to stirring during enzyme incubation. In contrast, yields from shoot preparations, stirred (380 µg/kg) or not stirred (720 µg/kg), did not differ significantly. Intact BYDV-RPV-IL RNA was isolated by treatment with 100 µg/ml proteinase K, 1.0% SDS and 0.5% 2-mercaptoethanol, or by using water-saturated phenol.

## 617

PRODUCTION OF MONOCLONAL ANTIBODIES TO WOUND TUMOR VIRUS (WTV) USING EXTRACTS FROM CULTURED VIRUS-INFECTED LEAFHOPPER CELLS. Ramon Jordan, Donald Nuss, and H. T. Hsu, USDA, ARS, Florist and Nursery Crops Laboratory, Beltsville, MD 20705, New York State Dept. of Health, Albany, NY, and American Type Culture Collection, Rockville, MD 20852.

Balb/c mice were immunized with a clarified cytoplasmic extract of WTV acutely infected AC-20 leafhopper cells. Hybridoma supernatants were assayed in various direct and indirect ELISA tests using clarified extracts of WTV-infected sweet clover root tumors, purified WTV, and synthetic double-stranded RNAs as antigens. Ascitic fluids produced from eleven hybridoma cell lines gave reciprocal ELISA titers ranging from 0.5 to 6.0 x 10<sup>6</sup> against clarified tumor extracts and purified virus. None of the McAbs react in ELISA or immunodot-blot assays (IDBAs) with WTV treated with sodium dodecyl sulfate (SDS), or pH 9.5/56°C. Several McAbs do react to SDS/mercaptoethanol-treated extracts 'renatured' with 8M urea before IDBAs. Results of immunofluorescence, immunoprecipitation and modified Western-blot analysis will also be presented.

## 618

SPECIFICITIES OF MONOCLONAL ANTIBODIES TO STRAINS OF POTATO VIRUS Y. S.K. Yao, S.H. Cai, S.R. Jia, Vegetable Research Inst., Chinese Academy of Agricultural Sciences, Beijing, China and H.T. Hsu, American Type Culture Collection, Rockville, MD, USA

Monoclonal antibodies (McAb) of hybridomas derived from mice injected with a tomato strain of potato virus Y (PVY) were evaluated with three previously well characterized strains of PVY. Six of the 16 hybridomas produced antibodies reactive to PVY<sup>O</sup>; four cell lines secreted antibodies reactive to PVY<sup>N</sup>; and the other five produced antibodies specific to PVY<sup>C</sup>. Only one cell line produced antibodies reactive to all three strains of PVY. No noticeable changes in antibody properties by the hybridomas were observed after 20 *in vitro* subcultures. Antibody titers of ascitic fluids were about 100 to 200 times those of rabbit antisera when measured by enzyme-linked immunosorbent assays (ELISA). The reaction of McAb with PVY antigens could be blocked by the presence of polyclonal rabbit antiserum. In ELISA tests, PVY specific McAb did not react with tobacco mosaic, turnip mosaic, cucumber mosaic, and potato X viruses.

## 619

THE USE OF ENZYME-LINKED IMMUNOSORBENT ASSAY TO ASSESS THE PURIFICATION OF MAIZE DWARF MOSAIC VIRUS. H.K. Palomar, S.G. Jensen. Dept. of Pl. Path., Univ. of Nebraska, Lincoln, NE 68583.

The ELISA double antibody sandwich method was used to quantitatively determine yields and losses during each step in the purification of maize dwarf mosaic virus (MDMV, strains A and B). Storage, extraction, clarification and concentration techniques were compared over a range of dilutions using both ELISA and infectivity assays. Strain A was more stable than strain B in leaves frozen at -80 F. The best of four extraction methods gave twice the yield of the poorest. The pH of the extraction buffer (pH 6.0 to 8.5) was of limited importance. Polyethylene glycol at 6% was more effective than 2, 4 or 8% for precipitating the virus. Serologically reactive but noninfectious material, presumably incomplete virus or capsid protein, was identified in certain fractions during the purification. Results from the two assay methods were similar in most respects but ELISA was much more accurate.

## 620

SOIL-BORNE WHEAT MOSAIC VIRUS INCLUSION BODIES ARE POSITIVE FOR SOIL-BORNE WHEAT MOSAIC VIRUS PROTEIN WITH IMMUNOGOLD. H.K. Brakke and W.B. Langanburg, ARS-USDA, Dept. of Plant Path., Univ. of Nebr., Lincoln, NE 68583-0722.

Ultrathin sections of root and shoot tissues of soil-borne wheat mosaic virus-infected wheat (*Triticum aestivum* L. cv. Scout 66) were stained by an indirect immunogold procedure using antibody to capsid protein. The antibody reacts with the 90kd protein found in extracts of infected leaves as well as with capsid protein. Inclusion body proteins were labeled with gold in ultrathin sections as were virus particles and virus inclusions. Inclusion bodies were frequently adjacent to nuclei. This could reflect functional attachment or consequences of cytoplasmic streaming. The immunogold staining procedure could not distinguish viral capsid protein from inclusion body protein in ultrathin sections.

## 621

PURIFICATION AND ANALYSIS OF THE H PROTEIN OF TOBACCO MOSAIC VIRUS BY IMMUNOLOGICAL TECHNIQUES. Ralf G. Dietzgen and Milton Zaitlin, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

H protein, associated with virions of TMV, contains sequences of the viral coat protein to which a non-coat polypeptide of host or viral origin is linked (Virology 126,429-448,1983). Antisera developed by immunizing rabbits with H protein-containing gel bands showed reactivity with H protein, coat protein and host plant proteins in the indirect ELISA and by Western blotting analysis. The IgG fraction of these antisera was obtained by chromatography on protein A-Sepharose. Most of the antibodies to coat protein and plant proteins were removed by cross absorption. Antibodies to the unique H protein portion were coupled to Sepharose and H protein purified by affinity chromatography. For the detection and analysis of cleaved peptides of H protein, and to localize H protein in infected tissue, monoclonal antibodies to the unique moiety are being generated.

PROPERTIES OF ALFALFA MOSAIC VIRUS SPECIFIC MONOCLONAL ANTIBODIES. Edward L. Halk and Lynette M. Burhop, Agrigenetics Corp., 5649 E. Buckeye Rd., Madison, WI 53716

Monoclonal antibodies specific for alfalfa mosaic virus (AMV) or AMV coat protein were characterized by their reaction with intact virus or coat protein in various types of ELISA and by binding to AMV peptide fragments in western blots. Antibody A1 reacts with intact or glutaraldehyde fixed virus in direct or indirect ELISAs but not with coat protein. Antibody A1 does not react with AMV protein in western blots. Antibodies A3, A4, A5 and A6 preferentially react with coat protein and not intact virus in ELISAs. These antibodies can be differentiated on the basis of the specific AMV peptide fragments to which they bind in western blots, and by differential reactions with a panel of AMV strains in indirect ELISA. Monoclonal antibodies detected purified AMV to an endpoint of 12 ng/ml in sandwich type ELISA and in dot blots on nitrocellulose.

## 623

Serological relationships among six isolates of red clover necrotic mosaic virus: Identification of a new serotype. A.L.N. Rao, S.T. Ohki, D.K. Lakshman and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5.

The antigenic relationships of three red clover necrotic mosaic virus (RCNMV) isolates, E (England), A (Australia) and C (Canada) have been compared by immunodiffusion, intragel crossabsorption, and immune electron-microscopy to three established serotypes of the virus: serotype I (RCNMV-34), serotype II (RCNMV-48), serotype III (RCNMV-Sw). These tests differentiated isolate C from serotype I and III but not from serotype II. Similar tests established that isolates F and A constitute a new serotype, IV, since they were serologically indistinguishable from one another and differed from the three serotypes. No major differences was observed in the electrophoretic mobilities of the corresponding coat proteins from each serotype when analysed by SDS-Polyacrylamide gel electrophoresis.

## 624

CONCENTRATION OF MAIZE CHLOROTIC MOTTLE VIRUS (MCMV) AND MAIZE DWARF MOSAIC VIRUS (MDMV-B) IN CORN LETHAL NECROSIS. K.-B. Boldberg and M.K. Brakke, Department of Plant Pathology and USDA-ARS, University of Nebraska, Lincoln, NE 68583-0722.

The concentration of MCMV was 1.5-2.0 fold higher in maize infected with both MCMV and MDMV-B than in singly infected plants, but the concentration of MDMV-B remained unchanged or decreased in doubly infected plants. The concentration of a 66 kd MDMV-B inclusion body protein also decreased or remained unchanged. Doubly infected plants showed comparable increases of MCMV virus protein as determined by PAGE and ELISA and of virions by density gradient centrifugation. However, assay of MCMV RNA by density gradient centrifugation gave a 1.5-4.0 fold increase in doubly infected compared with singly infected plants and also indicated a higher concentration of virus than did assays of virions. There were no detectable differences in rRNAs as a result of mixed or single virus infections even though the MCMV RNA of doubly infected plants accounted for up to one-third the total RNA. Dry weight absorbance measurements gave a specific absorbance,  $A_{268}$ , 1  $\mu\text{g/ml}$ , of 6.7 for MCMV.

## 625

A MOLECULAR WEIGHT ANALYSIS OF THE CAPSID PROTEINS FROM MAIZE DWARF MOSAIC VIRUS STRAINS. M. A. Langham and R. W. Toler, Department of Plant Pathology & Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843

Maize dwarf mosaic virus (MDMV) is commonly subdivided into six strains (A,B,D,E,F, and O); however, the identification of these strains is often difficult due to their close relationship and intense serological interactions. The determination of molecular weight of the capsid protein from these strains is one part in the differentiation of strains by protein characterization. Molecular weights were determined by utilizing discontinuous SDS-PAGE with a 4% stacking gel and 12.5% running gel. Isolates of MDMV-A from johnsongrass exhibited variation in average molecular weight ranging from 34,675 to 38,200 daltons. The average molecular weights of the strains are as follows: MDMV-A--36,777 $\pm$ 1,700; MDMV-B--36,313 $\pm$ 254; MDMV-D--34,367 $\pm$ 1,371; MDMV-E--34,123 $\pm$ 2,623; MDMV-F--34,928 $\pm$ 1,852; and MDMV-O--37,888 $\pm$ 606.

DETECTION OF MAIZE DWARF MOSAIC VIRUS BY IMMUNO-BLOT. M.A. Langham, R.W. Toler, and L.M. Giorda, Dept. of Plant Pathology & Microbiology, Tx. Agric. Exp. Stn., College Station, TX 77843.

A rapid assay (5 hr) for maize dwarf mosaic virus (MDMV) was made by the immuno-blot technique. Sap from infected plants was diluted in a two fold series. The samples (200  $\mu\text{l}$ ) were dotted using a microfiltration apparatus onto nitrocellulose and incubated for 30 min as was each following step. After blocking with TRIS buffered saline with 1% bovine serum albumin (TBS-BSA), the membrane was removed from the apparatus, and the remaining steps were completed in trays. After a second blocking with TBS-BSA, the membrane was incubated in MDMV-A antiserum (1:5600) and washed with TBS. Goat anti-rabbit IgG-horseradish peroxidase (1:300) was used as a secondary antibody. Following washing, the dots were developed using 4-chloro-1-naphthol. The greatest dilution detected for strains A,D,E, and F of MDMV was 1/8192, 1/4096, 1/2048, and 1/8192 respectively. These dilutions were each three fold greater than those detected by double antibody sandwich ELISA. MDMV-A antiserum failed to detect strains B and O.

## 627

DOUBLE-STRANDED RNA FROM PLANTS INFECTED WITH ROD SHAPED VIRUSES WITH UNDIVIDED GENOMES. R. A. Valverde, J. A. Dodds, and J. A. Heick, Department of Plant Pathology, University of California, Riverside, CA 92521.

Double-stranded RNA (dsRNA) of several members of the poty-, carla-, potex-, and tobamoviruses was purified by chromatography on CF-11 cellulose from 7-30 g of infected tissue. The size and number of expected dsRNA replicative forms (RF) obtained was predictable and diagnostic for each virus group analysed. Additional dsRNAs also detected were characteristic of each particular virus. The diagnostic value of the dsRNAs for plant virus identification was evaluated. The effect of host, temperature, and age of infection on the quality of dsRNA detected by gel electrophoresis was studied. Infected plants kept at 27°C and harvested 10 days after inoculation gave the highest dsRNA yields. Regardless of the host, dsRNAs of similar quality were consistently obtained. Some uninoculated plants also contained dsRNAs with molecular weights similar to the dsRNAs of plant viruses.

## 628

ELECTRON MICROSCOPY AND LOW MOLECULAR WEIGHT dsRNA ANALYSIS OF SPOT MOSAIC-INFECTED WHEAT. S. T. Ohki, D. K. Lakshman and C. Hiruki, Department of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada.

Since the etiology of wheat spot mosaic (WSM) remains to be elucidated, electron microscopy and low molecular weight dsRNA analysis were conducted. Vesicles and lipid-like bodies of 2-20  $\mu\text{m}$  in diameter were observed in the infected cells, but no typical virus-like particles were found. As dsRNA analysis is a well established procedure for diagnosing many unknown virus- or viroid-like diseases, dsRNA species from WSM-infected, wheat streak mosaic virus-infected, mite-infested, and healthy wheat leaves were extracted. Analysis in non-denaturing 6% polyacrylamide gels revealed two mite-specific RNA species. However, WSM-specific RNA species have so far not been found.

## 629

USE OF RADIO-LABELLED cRNA AND BIOTIN-LABELLED cDNA FOR THE DETECTION OF POTATO SPINDLE TUBER VIROID. D.K. Lakshman, X. Wu, W.C. Leung and C. Hiruki, Dept. of Plant Science and Dept. of Medicine, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2P5.

With the development of potato spindle tuber viroid (PSTV) cloning and dot-spot hybridization assay with nick translated probe, PSTV detection has become more accurate. In the present investigation two additional dot-spot assays with either radio-labelled cRNA or biotin-labelled cDNA has been utilized. For this, the complete DNA copy of PSTV, originally cloned in pBR 322 (Van Wezenbec et al., 1982), was isolated by Bam III digestion. A riboprobe plasmid pSP 64 was linearized with Bam HI and ligated with the PSTV DNA copy. Transcription of the subcloned plasmids resulted into either PSTV cRNA or PSTV RNA, depending on the orientation of the insert. Thus, the clones will be useful for detection of both PSTV (+)RNA and (-)RNA in the plants. The relative efficiencies of the radio-labelled cRNA and biotin-labelled cDNA probes from the subcloned riboprobe plasmids in the dot-spot detection of PSTV will be presented.

Analysis of double-stranded RNAs from plants infected with five isolates of red clover necrotic mosaic virus. A.L.N. Rao and G. Hiruki, Dept. of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

Double-stranded RNAs (dsRNAs) were isolated from plants infected with five isolates of red clover necrotic mosaic virus (RCNMV), belonging to four serotypes, by CF-11 cellulose chromatography and analysed by polyacrylamide gel electrophoresis. The electrophoretic mobilities of dsRNA segments were distinct for each serotype and also for two isolates of serotype IV, which otherwise were indistinguishable. Two major dsRNAs, dsRNA 1(3X10<sup>6</sup>d) and dsRNA 2(0.8-1.3X10<sup>6</sup>d), representing the two genomic single stranded RNAs and one subgenomic dsRNA(1-1.3X10<sup>6</sup>d) were detected in all serotypes, while two additional minor subgenomic dsRNAs(2.3X10<sup>6</sup>d and 2.1X10<sup>6</sup>d) were detected only in samples extracted from plants infected with serotype IV. Differences both in number and electrophoretic mobilities of dsRNAs support the division of RCNMV isolates.

## 631

COMPARISON OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) AND A FILTER PAPER DOT-IMMUNOBINDING ASSAY FOR DETECTION OF WHEAT SOILBORNE MOSAIC VIRUS. Z. Mahrni AND J. L. Sherwood, Plant Pathology Dept., Oklahoma State University, Stillwater, OK 74078

Direct sandwich ELISA and a filter paper dot-immunobinding assay were compared for detection of wheat soilborne mosaic virus WSBMV in purified or crude sap preparations from wheat. The filter paper dot-immunobinding assay, originally developed for detection of seed proteins (Anal. Biochem. 132:462), has been adapted for detection of plant viruses (Haber, S., Agriculture Canada, Winnipeg, Manitoba). With purified preparations, 10 ng/ml of WSBMV was detected by ELISA and the degree of response was linear to 200 ng/ml. With dot-immunobinding, 64 ng/ml of WSBMV was detected and the degree of response appeared similarly linear. In crude sap, ELISA was more reliable in discerning differences in virus concentrations than the dot-immunobinding assay. The dot-immunobinding assay could readily differentiate virus infected and uninfected plants and can be completed in less than 2 hours.

## 632

DIFFERENTIAL DETECTION OF MAIZE STRIPE VIRUS CAPSID AND NON-CAPSID PROTEINS IN INFECTED PLANTS AND INSECTS. B. W. Falk<sup>1</sup>, J. H. Trail<sup>2</sup> and S. A. Lommel<sup>3</sup>. University of Florida, Belle Glade, 33430; <sup>2</sup>University of Florida, Fort Lauderdale, 33314; and <sup>3</sup>Kansas State University, Manhattan, 66506.

The maize stripe virus (MStpV) capsid (32K) and noncapsid (16K) proteins were detected in extracts of MStpV-infected plants by Western blotting. However, only the 32K protein was detected in MStpV-viruliferous Periclypterus maidis, the planthopper vector. Based on transmission and serological data, MStpV replicates in P. maidis. The 32K protein was not detected in P. maidis immediately after acquisition access on MStpV-infected plants, but was detected after an 8-10 day incubation period. Both the 16K and 32K proteins are viral-coded as each was specifically immunoprecipitated from MStpV RNA in vitro translation products by using antisera to each respective protein. It appears that although both the 32K and 16K proteins are virus-coded, the 16K protein is only readily detectable from plant hosts.

## 633

A POSSIBLE SEED-TRANSMITTED LATENT VIRUS OF CORIANDER. I. I. Hoefert, M. R. Johns, and J. E. Duffus, USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905

A rod-shaped virus-like particle was isolated from symptomless plants grown from four seed lots of coriander (Coriandrum sativum L.). The presumed virus is found in relatively small concentrations in infected plants. Similar particles were found in anthers of coriander prepared for transmission electron microscopy. Rod-shaped particles were detected in tapetal cells of developing anthers, where the particles showed a spatial relationship to nuclei. An antiserum is now being prepared that will allow detection of the virus in coriander plants and will provide information that may be utilized in the study of transmission and host range of the virus.

## 634

WHEAT STREAK MOSAIC VIRUS IS ASSOCIATED WITH SCROLLS THAT CROSS CELL WALLS IN INFECTED WHEAT PLANTS. William G. Langenberg, ARS-USDA, Dept. of Plant Path., Univ. of Nebr., Lincoln, NE 68583-0722.

Antibodies to wheat streak mosaic virus (WSMV) capsid protein stained some pinwheel and scroll inclusions in the cytoplasm of infected wheat cells and some that were attached perpendicularly to or that crossed cell walls. Inclusions containing virions were always heavily stained. Goat anti-rabbit IgG bound to colloidal gold was used as a secondary stain to visualize the primary antibody in ultra thin sections. Anti-virion antibodies did not react in vitro with the major inclusion body polypeptide. Staining of pinwheels and scrolls with anti-virion antibody thus indicates the presence of virions or capsid protein entrapped in the inclusions. Pinwheel and scroll inclusions may also serve to align virus with plasmodesmata to facilitate cell-to-cell spread.

## 635

PRODUCTION OF MONOCLONAL ANTIBODIES TO PEANUT MOTTLE VIRUS AND WHEAT STREAK MOSAIC VIRUS. J. L. Sherwood<sup>1</sup>, M. R. Sanborn<sup>2</sup> AND G. C. Keyser<sup>2</sup>. Plant Pathology Dept.<sup>1</sup>, and Botany and Microbiology Dept.<sup>2</sup>, Oklahoma State University, Stillwater, OK 74078.

Stable hybridoma cell lines that produced monoclonal antibodies against peanut mottle virus (PMV) or wheat streak mosaic virus (WSMV) were produced by fusing spleen cells of mice immunized with a mixture of the two viruses to mouse myeloma cell line P3x63Ag8.653. None of the hybridomas produced antibody that reacted with both viruses. The antibodies characterized to date produced by anti-PMV secreting hybridomas have all been IgG, while those against WSMV clones have been IgM. In Western blots most of the anti-WSMV clones reacted with the 48,000 and 43,000 dalton coat proteins and one clone reacted with the 32,000 dalton coat protein in addition to the other two coat proteins. None of the clones against PMV or WSMV reacted with host plant material.

## 636

VIROID-LIKE RNAs ASSOCIATED WITH THE CITRUS EXOCORTIS DISEASE REACTION IN CITRON. N. Duran-Vila and J. S. Semancik, IVIA, Valencia, Spain and Department of Plant Pathology, University of California, Riverside, CA 92521.

The citrus exocortis viroid (CEV) has been well characterized as a 371 nucleotide transmissible RNA that produces stunting and leaf epinasty and rugosity in Gynura aurantiaca and citron (Citrus medica). Field isolates of the exocortis disease designated as mild or moderate by the reaction on the citron indicator, which in most cases are not transmissible to Gynura, display 1-3 sub CEV-RNA species which migrate in PAGE under denaturing conditions in the region of viroid RNA. Two of the isolated RNA species are characterized as viroid-like by Cf-11 cellulose chromatography properties, the resolution of circular and linear molecular forms and transmission to citron. These data suggest that the citrus exocortis disease reaction may be induced by a complex of either distinct or CEV-related viroid-like RNAs.

## 637

BARLEY YELLOW DWARF VIRUS IN OATS: EFFECT ON FEEDING BEHAVIOR OF TWO GRAIN APHIDS. C. B. Montllor and F. E. Gildow, WRRG, USDA-ARS, Berkeley, CA 94710 and The Pennsylvania State University, University Park, PA 16802.

The feeding behavior of Schizaphis graminum and Rhopalosiphum padi was electronically monitored on healthy and BYDV-infected oats. Both aphids are vectors of BYDV, but only R. padi vectors the RPV strain used in this study. Five characteristics of feeding behavior were measured. For S. graminum, the number of probes leading to the phloem were fewer, the time taken to establish phloem ingestion was less, and the time spent ingesting from the phloem was greater on infected plants. Similar changes in feeding of S. graminum have been shown to be correlated with improved growth and fecundity. R. padi showed the same trends, but the differences in behavior on healthy compared to infected oats were not significant. The time taken to reach the phloem, and total number of probes into plant tissue were not different on infected vs. healthy plants for either species. Physiological and epidemiological implications are discussed.

AN ENZYME-LINKED IMMUNOSORBENT ASSAY TO DETECT BARLEY STRIPE MOSAIC VIRUS IN BARLEY FOR USE IN THE MONTANA SEED CERTIFICATION PROGRAM. S.K. Zaske, T.W. Carroll, and S.K. Sipes, Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717

A direct enzyme-linked immunosorbent assay (ELISA) to detect barley stripe mosaic virus (BSMV) in barley has been adapted for use in the Montana seed certification program. The assay replaces a double immunodiffusion technique facilitated by sodium dodecyl sulfate (SDS-disk test) which could detect single infected embryos when a seed lot of 200 individual embryos was tested. This ELISA meets the current needs and capabilities of the program. Some sensitivity was sacrificed to conserve immunoglobulin and reduce processing time. It detects about 0.5 ug/ml BSMV or 1 infected seedling/250 seedlings in a composite sample using p-nitrophenyl phosphate as substrate. Four replicates of 250 seedlings/seed lot are homogenized and used as antigen in Dynatech Immunolon 2 flat-bottom plates. Plates are incubated at 37C for 1 hr during each step, coated with 1/1000 dil (0.1ug/ml) BSMV immunoglobulin, and 1/200 dil conjugate.

## 639

A PHYSIOLOGICAL DISORDER OF SOME PRUNUS LINES RESEMBLING VIRUS SYMPTOMS. C. C. Reilly, W. R. Okie and P. L. Pusey, USDA-ARS, S. E. Fruit & Tree Nut Res. Lab., Byron, GA 31008.

A physiological disorder of leaves was first observed in 1980 in commercial nectarine varieties (Columbia, Pochantas and Cherokee), selections and unimproved peach varieties and studied for the next 4 years. Symptoms which appeared similar each year were well-defined yellow-green to light orange-red blotch areas on mature leaves. These areas were large, irregular and scattered over the leaf blade with an orientation from the midrib outward toward the margin. Symptoms appeared in early summer on mature leaves and remained throughout the season. Symptoms were not graft transmissible to scion from affected rootstock nor in the reciprocal graft. All seedlings from some parents had blotch symptoms, whereas, in other cases only a low percent of the seedlings were affected. Blotch-affected plants usually gave negative reactions on Shirofugen cherry or ELISA against Prunus necrotic ring spot virus.

## 640 Withdrawn

## 641

ISOLATION OF TOBAMOVIRUS FROM DOGWOOD (*Cornus florida*) IN TENNESSEE. B. B. Reddick, Dept. of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37901-1071.

Eight Tennessee nursery stock blocks (145 dogwood trees) used for seedling production were assayed for virus using *Chenopodium quinoa* and *Nicotiana glauca* as assay hosts.

Initially three trees were found to be virus infected, but no virus could be reisolated later from two of these trees. The third virus isolate (DWV-T) was reisolated from dogwood and identified as a tobamo-like virus. Electron microscope observations of fresh sap and purified preparations showed virus particles 300 x 18 nm. The coat protein MW equalled 18 x 10<sup>3</sup> in SDS-PAGE. The virus did not react with TMV antisera in Ouchterlony gel double diffusion tests and did not infect *Nicotiana tabacum* 'Xanthi', 'Burley 21', or 'Samsun NN', or *C. quinoa*. It did infect *C. amaranticolor* and *Gomphrena globosa* causing both local and systemic symptoms, and *N. glauca* causing systemic symptoms. Further tests are underway to determine DWV-T's relationship to other tobamoviruses.

## 642

COINCIDENCE OF VIRUS DISEASES AND DECLINE OF WHITE CLOVER IN A MISSISSIPPI PASTURE. M. R. McLaughlin and G. A. Pederson, U. S. Department of Agriculture, Agricultural Research Service, Forage Research Unit, P. O. Box 5367, Mississippi State, MS 39762

White clover (*Trifolium repens* L. cv Regal) and tall fescue (*Festuca arundinacea* Schreb. cv Ky 31) were seeded together at recommended rates in a prepared seedbed on 10 ha at the Prairie Experiment Station, Prairie, MS in October 1982. Grazing was begun in late Spring 1983. From August 1983 to March 1985, 100 counts and collections of white clover were made on each of six dates. During that time the clover stand (stolons per meter) declined 50% while the incidence of virus-infected plants

(determined by ELISA) increased to 35% of the surviving plants. Peanut stunt virus infected 25% of the surviving plants and accounted for 55% of cumulative total infections, followed by alfalfa mosaic virus (15%), white clover mosaic virus (10%), cucumber mosaic virus (7%), clover yellow vein and red clover vein mosaic viruses (5% each), and bean yellow mosaic and clover yellow mosaic viruses (less than 1% each). Virus diseases may play a significant role in white clover decline.

## 643

TOMATO SPOTTED WILT VIRUS ASSOCIATED WITH A LETHAL DISEASE OF ANEMONE CORONARIA. S.S. Hurtt, S.C. Trees, and J.C. Watterson. USDA-ARS-FNCL, Beltsville, MD, 20705; Pan Amer. Seed Co., West Chicago, ILL, 60185; Petoseed Res. Center, Woodland, CA, 95695.

Virus-like symptoms were observed in F<sub>1</sub> seedling anemone grown for cut flower production. Symptoms included severe stunting, chlorosis, leaf rosetting and twisting, purple discoloration, and death. A virus with a host range indicative of tomato spotted wilt virus (TSWV) was isolated from anemone by mechanical inoculations of crude sap to petunia and tobacco. Brown necrotic local lesions, diagnostic for TSWV, appeared on petunia in 2-4 days. TSWV-like particles were numerous in ultrathin sections of inoculated *N. clevelandii*. Periwinkle inoculated with the virus gave a strong positive reaction in ELISA with TSWV antiserum. Virus-inoculated anemone seedlings showed chlorotic local lesions in 3 wk that became necrotic and expanded. Systemic symptoms resembled those on naturally-infected anemone. This is the first report of natural TSWV infections in commercial productions of anemone. The virus may have been transmitted to anemone by thrips.

## 644

LEAF REGENERATION AND VIRUS SCREENING OF SOMACLONES OF ISOGENIC LINES OF TOMATO. K. A. Barden and H. H. Murakishi, Department of Plant Pathology, Michigan State University, E. Lansing, MI 48824-1312.

Leaf disks (6 mm) of five isogenic lines of *Lycopersicon esculentum* Mill. cv. Craigella (GCRI, Littlehampton, U.K.), differing in genes for resistance to tomato mosaic virus (ToMV), formed shoots on Murashige and Skoog medium containing 1.7uM IAA and 13.3uM benzyl adenine. The genotypes represented were fully susceptible (+/+), tolerant (Tm-1/Tm-1), or resistant (Tm-2<sup>2</sup>/Tm-2<sup>2</sup>; Tm-1/+, Tm-2/+; Tm-1/+, Tm-2/Tm-2<sup>2</sup>) to ToMV-0 (common strain). Over 200 rooted somaclones of the susceptible line (+/+) and 12 of a resistant line (Tm-1/+, Tm-2/+) were rub-inoculated with a yellow strain of ToMV which has a host range similar to ToMV-0. Neither symptom expression nor virus multiplication (as detected by ELISA) occurred in somaclones of the resistant line. Also, virus multiplication was not detected in several somaclones of the susceptible line one month after inoculation.

## 645

A QUANTITATIVE METHOD FOR THE SPATIAL RELATIONSHIP OF VIRUS INFECTED PLANTS USING PAIRED DISTANCE MEASUREMENTS. Gray, S. M.<sup>1</sup>, J. W. Moyer<sup>1</sup>, and P. Bloomfield<sup>2</sup>, Dept. of Plant Pathology<sup>1</sup>, and Dept. of Statistics<sup>2</sup>, North Carolina State University, Raleigh, NC 27695-7616.

The spatial pattern of virus infected plants arranged on a variable-sized lattice was analyzed by a two-dimensional, distance-class method. Using one infected plant as the origin, the distance between it and every other infected plant on the lattice was defined in horizontal (X) and vertical (Y) units. Pairs of infected plants are then categorized into two-dimensional, [X,Y], distance classes. The number of pairs of infected plants in each distance class was counted and divided by the total number of pairs in that distance class. The process was repeated using each infected plant on the lattice as the origin. Computer simulations generated expected standardized count frequencies under the assumption of random pattern on the lattice. Theoretical and empirical sampling are used to demonstrate the power of the test and to compare the two-dimensional analysis with ordinary runs and doublet tests.

## 646

A POSSIBLE NEW VIRAL DISEASE OF *MANDEVILLA* SP. Keiler, R.D. and Weathers, L.W., Department of Plant Pathology, University of California, Riverside 92527.

A disease, characterized by leaf mottling and puckering, was found in all

plants of two species of *Mandevilla* examined at several commercial nurseries in southern California. No particles were observed in electron microscope examinations of thin sections and leaf dips, and all attempts to purify or transmit the "virus" were unsuccessful. Double stranded RNA (dsRNA) purified from leaf, stem, and root tissues suggests that the disease is of viral origin. Double stranded RNA was purified from plant tissue, treated with RNAase and DNAase, and electrophoresed on a 6% polyacrylamide mini-gel for 4 hr at 30 mA. After treatment with ethidium bromide, either 1 or 3 closely-spaced bands (depending on the species of *Mandevilla*) were observed on the gel at a position indicating a dsRNA size of approximately  $7 \times 10^6$  daltons (fungal virus was used for a standard). Electron microscopy of Kleinschmidt spreads of purified dsRNA revealed molecules of about 3.4  $\mu$ m.

647

EFFECT OF ROOT SURFACE AGGLUTININ ON THE MICROBIAL ACTIVITIES IN THE RHIZOSPHERE. I. MOVEMENT AND BIOLOGICAL CONTROL ACTIVITY.

W. L. Chao and R. K. Li, Dept. of Microbiology, Soochow Univ., Shin Lin, Taipei, Taiwan, R. O. C.

Organisms which can be agglutinated by root exudate were isolated from the pea rhizosphere. Their vertical movement in the rhizosphere was studied by introducing the organisms as seed treatment. In natural soil, bacterial isolates which can be agglutinated by pea root exudate can be detected in most of the root segments. However, the degree of colonization was strongly influenced by the presence of some indigenous microorganisms and sometimes complete inhibition of colonization was observed. Organisms which can not be agglutinated by the root exudate can only be detected at the upper portion of the rhizosphere. Conidia obtained from different isolates of *Trichoderma* and *Gliocladium* were shown to be able to be agglutinated by pea root exudate but not by seed exudate. Its significance in the biocontrol activities of *Trichoderma* and *Gliocladium* were discussed. The attempt to try to eliminate this agglutination by using various carbohydrates has failed.

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MXP, A SEMI-SELECTIVE MEDIUM FOR *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI*. L. E. Claflin<sup>1</sup>, M. D. Sasser<sup>2</sup>, and A. K. Vidaver<sup>1</sup>, <sup>1</sup>Dept. of Pl. Path., Univ. of Nebraska, Lincoln, NE 68583, and <sup>2</sup>Univ. of Delaware, Newark, DE 19711, respectively.

A semi-selective medium (MXP) was developed for isolation of *X. campestris* pv. *phaseoli* from common blight infected bean tissue and infested soil. Other pathogens and *Xanthomonas* spp. tested also produced typical yellow, mucoid, smooth, convex colonies on MXP. Growth rate and plating efficiencies of *X. c.* pv. *phaseoli* strains on MXP varied with the strain and were usually less than those on yeast extract-dextrose-calcium carbonate (YDC). MXP medium contained, in (g/L),  $K_2HPO_4$  (0.8),  $KH_2PO_4$  (0.6), yeast extract (0.7), soluble potato starch (8.0) potassium bromide (20.0), glucose (1.0), and agar (15.0). Zones of starch hydrolysis were enhanced by 3  $\mu$ l of methyl violet 2B (1% solution in 10% ethanol) and 6  $\mu$ l methyl green (1% aqueous solution) per L. After autoclaving, chlorothalonil, cephalixin, kasugamycin, and gentamycin (Sigma) were added at rates of 14, 20, 20, and 2  $\mu$ g/ml, respectively.

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CLONING AND EXPRESSION OF A *SERRATIA MARCESCENS* GENE ENCODING CHITINASE. R. L. Fuchs, S. A. McPherson and D. J. Drahos. Biological Sciences, Monsanto Company, CR&DS, Chesterfield Village Parkway, Chesterfield, MO 63189.

Insects, fungi and nematodes contain chitin as integral structural components at one or more stages of their life cycles. Engineering plant specific rhizosphere or phyloplane colonizing bacteria to express and secrete chitinase may result in the production of biocontrol agents targeted at specific plant pathogens. A cosmid bank was constructed in pLAFRI with *Serratia marcescens* chromosomal DNA to clone one or more of the genes encoding chitinase. Four independent chitinase-positive clones were isolated, characterized and shown to share a common 9.5 kb EcoRI fragment apparently encoding the same 57 kd chitinase protein, the most abundant chitinase produced by *S. marcescens*. The low level of chitinase expression obtained with the initial cosmid clones was significantly enhanced by inserting a 2.4 kb EcoRI "star" fragment generated from the 9.5 kb fragment downstream of the Pbla promoter.

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RELATIONSHIPS OF MYCOPHAGOUS COLLEMBOLA AND RHIZOCTONIA SOLANI POPULATIONS IN BIOCONTROL. E. A. Curl, J. D. Harper\*, C. M. Peterson, and R. T. Gudaszkas. Dept. of Botany, Plant

Pathology, and Microbiology and Dept. of Zoology-Entomology\*, Auburn University, AL 36849.

Mycophagous springtails (Insecta: Collembola) can reduce the inoculum density (ID) of *Rhizoctonia solani* in soil and thereby effectively lower the disease severity index (DSI) of cotton seedlings. In growth chamber tests, a sandy loam was supplemented with *R. solani*-oat inoculum at 0.5 g/kg soil, then populated with collembolan species (*Proisotoma minuta* and *Oncy-chirus encarpatus*) at 0 to 2000/kg soil. The presence of Collembola increased cotton seedling emergence, but emergence in soil with 2000 insects/kg was not significantly different from that with 1000/kg. The DSI in pathogen-infested soil without Collembola was higher than in soil with 1000 or 2000 insects/kg. When a fixed insect population of 1700/kg soil was used with varied pathogen ID, the DSI was reduced in soil with an ID of 0.05 or 0.1 g/kg but not when the ID was 0.5 or 1.0 g/kg.

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THE BIOLOGY AND EPIDEMIOLOGY OF *CRYPTOMYCELLA PTERIDIS* ON BRACKEN FERN (*PTERIDIUM AQUILINUM*). G. L. Andersen, D. Moscow and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

*Cryptomycella pteridis* (Kalchbr.) Bohn. (*Pteridium aquilinum* (L.) Kuhn) infects bracken fern in several widely separated locations in Northern California. Acervuli produce abundant conidia (over  $10^9$  conidia per frond) which fill subepidermal cavities and erupt as cirri on abaxial pinnule surfaces and on rachises. Most fronds arising from infected rhizomes exhibited puckering, curling, stunting, and eventual extensive necrosis typical of this disease. Bracken fern was susceptible to infection only after the crozier stage and while fronds remained succulent. At least 48 hr of continuous free moisture was required for infection. Only *P. aquilinum* exhibited symptoms after inoculation with *C. pteridis*; none of 12 other plants inoculated with *C. pteridis* exhibited disease symptoms. Lateral rhizome extension and above ground biomass of *C. pteridis* infected bracken fern clones were significantly less than healthy clones on each of 3 sampling dates.

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AMMONIA EVOLUTION AS AN ANTIFUNGAL MECHANISM IN VITRO. B-F Huang and B. J. McMaster, Crop Science Lab., Allied Corp., P.O. Box 6, Solvay, N.Y. 13209.

A simple defined medium was developed to detect antibiotic production by strains of *Pseudomonas fluorescens* against *Rhizoctonia solani* and *Pythium ultimum*. The medium composition was (g/l): 0.5g  $KH_2PO_4$ , 1.0g  $NaHCO_3$ , 5.0g asparagine, 0.2g  $FeCl_3 \cdot 6H_2O$ , 0.1g  $MgSO_4 \cdot 7H_2O$ . Medium formulation was determined by the gradient plate assay. The active component of spent medium was (1) of a molecular weight smaller than 500, (2) sensitive to lyophilization, (3) not extractable by various organic solvents of different pH's and (4) volatile in nature. The antifungal activity could be removed by either precipitating  $NH_4^+$  or entrapping gaseous  $NH_3$  from spent medium with tetraphenylboron. Results suggested that  $NH_3$  released from the deamination of asparagine by bacteria was responsible for the antifungal activity observed *in vitro*.

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PREDICTIVE BIOASSAY FOR A CLASS OF *PSEUDOMONAS FLUORESCENS* DEMONSTRATED TO CAUSE PLANT GROWTH PROMOTION. T.V. Suslow, P. Gill, N. McCarter-Zorner, D. Matsubara. Advanced Genetic Sciences, Inc., 6701 San Pablo Ave., Oakland, CA 94608.

A bioassay predictive of a group of *Pseudomonas fluorescens* capable of modifying plant development and reducing root infection by soil-borne fungi has been developed. Strains of *P. fluorescens* Biotype B, identifiable by this test, have plant growth promotion/biocontrol potential in greenhouse and field studies. A diagnostic dark green, non diffusible pigment is produced by the *P. fluorescens* when cells are grown in co-cultivation with *Escherichia coli*. Typically, viable cells of *E. coli* HB101 are spread on Luria's media at log 7.0 and allowed to grow for 2-4 hrs. Single colonies or dilutions from plant or soil isolation material are spotted or spread over the *E. coli* lawn. Of the hundreds of bacteria tested, only those similar taxonomically to strains E6 (Kloepper and Schroth, 1980; Yuen and Schroth, 1985) and NZ130 (Suslow, et al. unpublished) form the diagnostic dark green pigment after incubation and fluorescence.

Reciprocal competition between INA<sup>+</sup> wild-type and INA<sup>-</sup> deletion mutant strains of *Pseudomonas* on strawberry blossoms. Lindemann, J., Joe, L. and Moayeri, A. Advanced Genetic Sciences, Inc., Oakland, Ca. 94608.

Near isogenic lines of *Pseudomonas* strains differing in ice nucleation activity (site-directed deletion mutants) (Green, Corotto and Warren, 1985) and antibiotic resistance (spontaneous mutants) were spray inoculated onto blossoms of greenhouse grown strawberry plants. Inhibition of one bacterial strain by its near isogenic relative was dose dependant rather than strain dependant. INA<sup>-</sup> deletion mutants of *P. syringae* and *P. fluorescens* biotype A inoculated at 10<sup>7</sup> CFU/blossom inhibited growth of their INA<sup>+</sup> parental strains inoculated at 10<sup>4</sup> CFU/blossom. INA<sup>+</sup> parental strains inhibited INA<sup>-</sup> derivatives when inoculum doses were reversed. No inhibition occurred when two strains were inoculated simultaneously at equal doses (either 10<sup>7</sup> or 10<sup>8</sup> CFU/blossom).

## 655

ANALYSIS OF SIDEROPHORE-RELATED RESTRICTION FRAGMENT LENGTH POLYMORPHISM AMONG FLUORESCENT PSEUDOMONAS SPECIES. B.C. Hemming, E.C. Lawson and C.B. Jonsson. Biological Sciences, Monsanto Co., CR&DS, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Previous siderophore structural studies with *Pseudomonas* strains have revealed both similarity and heterogeneity of structure. The role of siderophores in the ecology of plant-associated pseudomonads is perhaps intermeshed with "strain specificity" exhibited in studies of plant growth, biological control and niche establishment. Siderophore-related DNA fragments produced from *P. syringae* pv. *syringae*, previously cloned and identified to at least 4 complementation groups (Loper et al., J. Gen. Micro. 130:1507-1515, 1984), have been subcloned and also used as probes in hybridization experiments against a collection of characterized strains. Genomic restriction enzyme analysis against probes of siderophore-related DNA indicates a sizeable genotypic diversity within this group. A spp. or perhaps a pathovar specific probe has been identified.

## 656

SUPPRESSION OF CUCUMBER DAMPING-OFF AND SURVIVAL OF PYTHIUM ULTIMUM IN CONTAINER MEDIA AMENDED WITH COMPOSTED HARDWOOD BARK. Chen, W., Hoitink, H. A. J., Kuter, G. A., and Schmitthenner, A. F. Dept. of Plant Pathology, OARDC-OSU, Wooster, OH 44691.

Suppression of *Pythium* damping-off in container media amended with composted hardwood bark (CHB) was determined with a cucumber seedling bioassay. Disease severity was higher (P=0.05) in media amended with CHB from the center (>50C) than from the edge (~25C) of compost piles. Suppressiveness was negated by heating (60 C, 5 days) or by addition of nutrients. In the absence of host plants, neither disease suppressive nor conducive media affected *Pythium* population development. However in planted (four replantings of 10 days each) disease suppressive, but not in conducive media, *Pythium* population development was suppressed. Data suggest that fungistatic factors and nutrient competition are at least partially responsible for the suppressive effect.

## 657

PREPARATION OF PROTOPLASTS FROM CONIDIA OF COLLETOTRICHUM GLOEOSPORIOIDES F. SP. AESCHYNOMENE. D. O. TeBeest and G. Weidemann, Dept. of Plant Pathology, 217 Plant Science, University of Arkansas, Fayetteville, AR 72701.

Protoplasts were obtained from conidia of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* by treatment of young conidia from broth cultures with a mixture of B-glucuronidase from *Helix pomatia* and chitinase from *Streptomyces griseus*. Conidia were incubated with 10mM ethylenediamine tetraacetic acid, 0.6M mannitol, 0.025M cysteine, and 1% 2-mercaptoethanol for 1 hour before enzyme treatment. Incubation of conidia with chitinase, B-glucuronidase, laminarinase, chitinase plus laminarinase or B-glucuronidase plus laminarinase was less effective than treatment with chitinase plus B-glucuronidase. Protoplasts were obtained within 2 hrs after treatment with chitinase plus B-glucuronidase and were stable in 0.6M mannitol buffered to pH 6.0 in 0.05M citrate-PO<sub>4</sub>.

HOST SPECIFICITY OF COLLETOTRICHUM PISI AND COLLETOTRICHUM GLOEOSPORIOIDES F. SP. AESCHYNOMENE. G. J. Weidemann, R. D. Cartwright and D. O. TeBeest, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Pathogenicity and virulence of *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, the mycoherbicide Collego, and two isolates of *Colletotrichum pisi* (*C. gloeosporioides*) were compared on *Aeschynomene virginica*, 28 varieties of *Pisum sativum* and several other genera within the Leguminosae. *Colletotrichum gloeosporioides* f. sp. *aeschynomene* could be differentiated from *C. pisi* on the basis of host specificity within the Leguminosae and on virulence within *P. sativum*. *Colletotrichum gloeosporioides* f. sp. *aeschynomene* was pathogenic to *A. virginica*, *P. sativum*, *Vicia faba* and *Lathyrus odoratus*. *Colletotrichum pisi* was pathogenic to *P. sativum*, *V. faba* and *L. odoratus*, but not *A. virginica*. *Colletotrichum pisi* was more virulent to *P. sativum* than was *C. gloeosporioides* f. sp. *aeschynomene*.

## 659

COMPARISON OF FUSARIUM OXYSPORUM F. SP. APII ISOLATES FOR VIRULENCE ON CELERY CULTIVARS. Elmer, W. H. and Lacy, M. L., Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48823.

Isolates of *Fusarium oxysporum* f. sp. *apii* Race 2 from Michigan, California and New York and one isolate of Race 1 from France were compared for virulence on 2 yellow and 9 green celery (*Apium graveolens* var *dulce*) cultivars that varied in resistance to Fusarium yellows in Michigan. Seedlings were transplanted into soil artificially infested with wheat straw colonized by each isolate. Virulence was assessed by rating vascular discoloration in the crown and by dry weights of the foliage after 6 weeks in the greenhouse. All Race 2 isolates caused disease on yellow and green cultivars, but varied in virulence. Race 1 caused disease on 'Golden Detroit' and on the green cultivar 'Bishop', but was avirulent on 'Golden Spartan' and all other green cultivars. This is the first report of loss of resistance to Race 1 in a green cultivar ('Bishop') selected from 'Tall Utah 52-70' which is highly resistant to Race 1.

## 661

HISTOPATHOLOGY OF RESISTANT AND SUSCEPTIBLE TOMATO CULTIVARS INOCULATED WITH VERTICILLIUM DAHLIAE (KLEB.) RACES 1 AND 2. J. Armen and P. B. Shoemaker, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Roots of 10-day-old tomato seedlings of *Verticillium* susceptible Walter (W) and resistant Flora-Dade (FD) were dipped into conidial suspensions (10<sup>6</sup>/ml) of *V. dahliae* races 1 (R1) and 2 (R2). The fungus was reisolated from surface disinfected root sections of each of the four treatments 48 hr after inoculation. *V. dahliae* was not isolated from stem or petiole sections of FD/R1, but the fungus was recovered from root, stem, and petiole tissue of the other three combinations up to 12 da after inoculation. Mycelial growth in the xylem vessels corresponded with recovery of the pathogen from infected tissues. The degree of fungal colonization was always greatest in W/R1. Conidia were usually found in association with the hyphae. Mistochemical tests for gel formation were negative. Tyloses were present in all cv/race

combinations and in uninoculated controls, but there was no association of tyloses with pathogen development.

#### 662

OCCURRENCE OF FRANKLINIELLA OCCIDENTALIS IN LOUISIANA: A POSSIBLE CAUSE FOR THE INCREASED INCIDENCE OF TOMATO SPOTTED WILT VIRUS. D.R. Greenough, L.L. Black, Dept. Plant Path. & Crop Physiol., R.N. Story, L.D. Newsom, Dept. Entom., La. Agric. Expt. Sta., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803; W.P. Bond, Dept. Bio. Sci., Southeastern La. Univ., Hammond, LA 70402.

*Frankliniella occidentalis*, the western flower thrips, was identified among thrips collected in Louisiana in 1984. This is the first record of its occurrence in the state. During the past 6 to 8 years the incidence of tomato spotted wilt virus (TSWV) has increased dramatically in tomato, pepper, and tobacco crops occasionally reaching 60% infection in commercial fields and 100% in home gardens. Two reported thrips vectors of TSWV, *F. fusca* and *Thrips tabaci*, have been known to be present in the state for many years. It is hypothesized that the expanded geographic range of *F. occidentalis*, an efficient vector in the western U.S., is responsible for the recent increase of TSWV in solanaceous crops in Louisiana. A statewide survey is being conducted of the thrips species associated with solanaceous crops to study their role in the epidemiology of TSWV.

#### 663

Patterns of susceptibility among six tomato genotypes to infection by *Xanthomonas campestris* pv. *vesicatoria*. R. C. McGuire and J. B. Jones. Gulf Coast Res. & Ed. Ctr.; Bradenton, FL.

Although several popular tomato cultivars have been developed with resistances to fungal pathogens, none have an adequate level of resistance to bacterial spot. This disease, caused by *X. campestris* pv. *vesicatoria* (XCV), can decimate a crop when spread by rains or overhead irrigation. Four tomato genotypes (Campbell 28, Florida 3316, Hawaii 7998, and Ohio 4013-3), with various degrees of resistance to bacterial spot or wilt, were compared with the susceptible cultivars Lyconorma and Walter in a field experiment. After spray inoculation, leaf populations of XCV remained low during periods of dry weather; however, within 2 weeks of a heavy rain those on susceptible lines had begun to rise sharply. Population levels of Hawaii 7998 and Campbell 28 remained low during this period. Although levels of XCV on Campbell 28 did eventually rise also, those on Hawaii 7998 had not risen significantly when plants of other cultivars had become heavily necrotic.

#### 664

SNAP BEAN BROWN SPOT BACTERIA ISOLATED FROM WILD CLOVERS. S. A. Abdelshife and M. J. Goode, Dept. of Plant Pathology, Univ. of Ark., Fayetteville, AR 72701.

Samples of wild clovers were taken from areas near and up to 5 miles from snap bean, *Phaseolus vulgaris*, fields in Northwest Arkansas. Isolates of *Pseudomonas syringae* pv. *syringae* were obtained from lesions on leaflets following surface sterilization. Pathogenicity studies were made by inoculating detached bean pods. Weakly to highly pathogenic isolates were demonstrated. The most pathogenic isolates were obtained from white sweetclover, *Melilotus alba*, and white clover, *Trifolium repens*. Lesser pathogenic isolates were obtained from yellow sweetclover, *M. officinalis*, and low hop clover, *I. procumbens*.

#### 665

USE OF DETACHED TRIFOLIOLATE LEAVES FOR SIMULTANEOUS INOCULATIONS OF BEAN WITH MULTIPLE PATHOGENS. D. J. Hagedorn, D. A. Inglis, E. Carlson and D. P. Maxwell, Department of Plant Pathology, University of Wisconsin-Madison 53706.

Detached *Phaseolus vulgaris* trifoliolate leaves in glass petri dishes were inoculated simultaneously with multiple pathogens under controlled growth chamber conditions. Repeatable results were obtained when one leaflet was inoculated with *Colletotrichum lindemuthianum* (Cl), one with *Isariopsis griseola* (Ig), and one with both *Uromyces phaseoli* (Up) and *Xanthomonas campestris* pv. *phaseoli* (Xcp) providing leaves were kept in a 24 C dew chamber for 36 hr after inoculation then in a 24 C growth chamber. Inoculum dosages were: Cl - 0.2 ml of  $10^5$  conidia/ml applied to adaxial midrib; Ig - 0.2 ml of  $10^4$  spores/ml to abaxial leaf surface; Up - 0.2 ml of  $10^4$  uredospores/ml to abaxial surface; and Xcp -  $10^7$  cells/ml to sliced margin of the same leaflet. Symptoms were similar--whether detached leaves or nondetached leaves of greenhouse control plants were inoculated.

#### 666

RELATIVE SUSCEPTIBILITY TO STREPTOMYCES SCABIES OF POTATO TUBERS PRODUCED FROM STEM CUTTINGS AND SEED PIECES. R. Loria and B. A. Kempter, Department of Plant Pathology, Cornell University, Long Island Horticultural Research Laboratory, Riverhead, NY 11901

Potato stem cuttings (SC) and seed pieces (SP) were inoculated with *Streptomyces scabies*, and disease symptoms on tubers produced from these SC and SP were compared. Symptoms of common scab on SC and SP tubers were similar under greenhouse conditions. Tubers produced from both SC and SP of the cultivar Chippewa were susceptible to nine of the eleven isolates of *S. scabies* tested. Lesion surface area and lesion type ratings were lower on Superior than on Chippewa tubers from both SC and SP in greenhouse trials. Relative disease severity on tubers of Chippewa, Superior and Katahdin produced from SC, inoculated, and grown in the greenhouse, was also similar to that observed on tubers produced from SP in naturally infested soil in the field. Since resistance of SP tubers to *S. scabies* appears to be expressed in tubers produced from SC, SC may be useful in screening for disease resistance and pathogen virulence.

#### 667

OVERWINTERING AND AEROBIOLOGY OF CERCOSPORA ASPARAGI IN NORTH CAROLINA. C. J. Cooperman, S. F. Jenkins, and C. W. Averre. Dept. of Plant Pathology, N. C. State Univ., Raleigh, NC 27695.

Survival of *Cercospora asparagi* was studied at two locations in NC. Naturally infected stems of asparagus were placed 1 m above, directly on, and 15 cm beneath the soil surface. Samples from these treatments, plus stems from intact plants, were collected monthly and examined for presence of conidia of *C. asparagi*. The fungus survived well from Dec to Jun in all above ground tissue. In buried tissue only trace amounts of the fungus could be found past Feb. Airborne conidia of *C. asparagi* were monitored at both locations. Conidia were first collected in Apr but few conidia were trapped from Apr through late Jul; the greatest concentration of airborne conidia occurred during the week of Aug 29 to Sep 5, and their incidence followed a diurnal pattern with greatest detection between 0900 and 1300 hr. Lesions first appeared Jun 19; rapid disease progression occurred during Aug and Sep and was associated with development of the asparagus canopy.

#### 668

EFFECT OF DEEP MOLDBOARD PLOWING, PREPLANT SOIL FUMIGATION, AND POSTPLANT FUNGICIDE APPLICATION ON CONTROL OF SOUTHERN BLIGHT OF TOMATO. S. F. Jenkins. Dept. of Plant Pathology, N. C. State Univ., Raleigh, NC 27695-7616.

Deep moldboard plowing of soil (Norfolk sandy loam) followed by application, with a single chisel six inches deep, of 0, 75, or 150 l/ha Vorlex in the row resulted in 14, 6, and 2 sclerotia of *Sclerotium rolfsii* per 400 g air dried soil, respectively. Disease incidence in the same treatments was 34, 18, and 8 %, respectively. Disking soil and applying the same rates of Vorlex resulted in 28, 13, and 5 sclerotia per 400 g soil and disease incidence of 43, 32, and 21 %, respectively. Metam sodium, applied with water to soil (Orangeburg sandy loam) to a depth of 25 cm at broadcast rates of 0, 90, 180, 360, and 720 l/ha resulted in 20, 7, 5, 1, and 2 sclerotia per 400 g soil, respectively. Corresponding disease incidence was 52, 51, 29, 24, and 20 %, respectively. Application of PCNB at 0, 0.9, and 1.8 kg/ha to plots receiving Vorlex or metam sodium treatments did not change (P<0.05) disease incidence.

#### 669

CONTROL OF COTTONY LEAK OF CUCUMBER WITH DIFFERENT FORMULATIONS OF METALAXYL APPLIED AT VARIOUS RATES AND TIMES. D. C. Thompson, Univ. of Maine Coop. Ext. Serv., Fort Kent, ME 04743, and S. F. Jenkins, Dept. of Plant Pathology, N. C. State Univ., Raleigh, NC 27695.

Cottony leak of cucumber caused by *Pythium* spp. is a common fruit rot during warm, humid weather. Metalaxyl 2E or 5G was applied in a 30 cm band to cucumber plants 1 to 3 times during the growing season at rates varying from 0.14 to 17.93 kg/ha at seeding, 2 to 4 leaf stage, plant tip over or first flower. Fruit in the field were exposed to oat grains colonized with *P. aphanidermatum* placed around the plants at first flower. Higher rates, multiple applications, or applications applied close to harvest controlled fruit rot best. The 5G formulation provided better control and less phytotoxicity to foliage than the 2E formulation. In a greenhouse study, a microencapsulated granule formulation (5 MEG) controlled cottony leak at lower

rates and produced less phytotoxicity than the 5G and 2E formulations.

#### 670

SOUTHERN ROOT KNOT (MELOIDOGYNE INCOGNITA) OF SWEET POTATO: RELATIONSHIPS BETWEEN RESISTANCE LEVELS, YIELD, MARKET QUALITY AND POST-HARVEST PATHOGEN DENSITIES. P. D. Dukes, Alfred Jones, USDA, ARS, U. S. Vegetable Laboratory, Charleston, SC 29407, and M. G. Hamilton, Clemson University, Edisto Experiment Station, Blackville, SC 29817.

Sweet potato genotypes were evaluated in greenhouse and field tests for reactions to root knot (*M. incognita*, race 3). The reactions were measured by gall, egg mass, and root necrosis indices, pathogen reproduction, yield and market quality; reactions ranged from extremely susceptible, e.g. B-18 and 'Georgia Jet', to highly resistant, e.g. 'Sumor' and PI 399163. In addition to reduced number of roots, root size and total yield, several market quality traits, i.e. cracking, malformation, and internal discoloration, were affected. Assays of post-harvest soil samples from plots with highly susceptible plants contained much higher densities of the parasite than did samples from plots with highly resistant plants (>1500/100 cc vs <100/100 cc).

#### 671

TEACHING PLANT PATHOLOGY IN DEVELOPING COUNTRIES. Graydon Kingsland, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, S.C. 29631, and Wayne Sitterly, S.C. Coastal Experiment Station, Charleston, S.C. 29407.

A recently-completed USAID project mandated an educational program in plant pathology for two high school level technicians in the Republic of Seychelles. The level of education and training provided was determined by (1) the prior education of the students, (2) the potential learning abilities of the students, (3) the physical facilities available, (4) the need (or role) that the students will satisfy in society with the education, (5) the time allotted for training and education, (6) the characteristics of the teachers. Implementation of the program involved daily lectures, discussions, instructions, and demonstrations in all aspects of the basic principles of plant pathology, as related to ongoing laboratory and field projects. The technicians assumed a high level of competency in essential phases of laboratory and field research. They were well-qualified to fulfill their roles as field and laboratory assistants at project termination.

#### 672

AREAGRAM--A STANDARD AREA DIAGRAM PROGRAM FOR THE APPLE COMPUTER. W. W. Shane, C. E. Thompson, and P. S. Teng, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

AREAGRAM, a BASIC program, was developed for the Apple II series microcomputers to produce reference diagrams for disease assessment. Outlines fashioned by the user to represent plant parts can be filled to any degree with spots of various shapes and sizes. These diagrams can be recalled and displayed on the microcomputer screen in a predetermined or random sequence to test the student's ability to estimate disease. The performance of the student can be stored on a disk or printed out. Diagrams can be saved as binary files for printout on paper for tests without the use of a microcomputer and for reference in disease assessment work in the field. The program provides a rapid and accurate means to "calibrate" disease assessors and investigate visual perception of disease.

#### 673

IN VITRO INHIBITION OF PASTURE LEGUME *TERAMNUS LABIALIS* BY TWO FAST GROWING *RHIZOBIUM* ISOLATES FROM *LEUCAENA*. Robert Webb, Virgin Islands Agricultural Experiment Station, St. Croix 00850

Severe chlorosis of foliage, and discoloration, deformation and cracking of roots were observed on *Teramnus* seedlings inoculated with isolates of *Rhizobium* recovered from nodules from *Leucaena leucocephala*. Seedlings were grown aseptically in 25 x 200 mm enclosed tubes on seedling agar without a source of nitrogen. Mild chlorosis and root discoloration were noted on uninoculated seedlings and on seedlings inoculated with infective but ineffective, promiscuous rhizobial strains and other *Leucaena* specific isolates. No symptoms were observed on plants inoculated with effective strains or those grown on media amended with nitrogen.

#### 674

IN VITRO EFFECTS OF HERBICIDES ON MYCELIAL GROWTH OF AG-1 AND AG-4 RHIZOCTONIA SOLANI ISOLATES FROM CANOLA/RAPSEED. P. R. Verma and D. L. Mackintosh, Agriculture Canada, Research Station, Saskatoon, Saskatchewan, Canada S7N 0W7.

The effects of 22 herbicides on the mycelial growth of an AG-1 (Iso.No.4) and an AG-4 (Iso.No.35) *Rhizoctonia solani* isolate from canola were determined using herbicide-amended agar at 25°C. Each herbicide was tested at 1, 2, 5, 10, 25, 50 and 100 PPM. Both isolates were highly pathogenic, but AG-1 was slower growing and more virulent. All rates of nicosulfuron, barban, di-butoxy methyl, alachlor and 2, 4-DB inhibited growth of both isolates, except 1 and 2.5 PPM of 2, 4-DB and alachlor which were not inhibitory to AG-1. Ten PPM and higher rates of glyphosate, sethoxydim, ethal fluralin, atrazine and fluzifop butyl alcohol significantly reduced growth of both isolates. Generally, AG-4 isolate was more sensitive and exhibited greater growth reduction. A few herbicides showed some stimulatory effect on growth. Effects of some pre-emergence herbicides on disease incidence were discussed.

#### 675

MONILIOD CELL PROPAGULES IN *RHIZOCTONIA SOLANI*. M. Arasaki, P. S. Yehata, and J. Y. Uchida. Dept of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Pathogenicity studies with *Rhizoctonia solani* Kühn have often been hampered by the absence of quantitatively reliable inoculum. In cell suspensions obtained by blending 15-20 day-old cultures of *R. solani*, approximately 30% of the units were moniliod cells and of these over 90% were 1-4 celled. The moniliod cells and hyphal cells in these suspensions are collectively referred to as moniliod cell propagules (MCP) in present studies. Cultures of *R. solani* AG-4 isolate 985, grown on 10% V-8 juice broth have yielded  $2.2 \times 10^6$  MCP/50 ml in 250 ml-flask cultures in 20 days. Approximately 98% of the MCP germinated on water agar. At an inoculum density of  $8 \times 10^5$  MCP/10-cm pot, approximately 87% of alfalfa cv 'Ranger' plants were killed. By using MCP, a more sensitive estimate of inoculum potential of *R. solani* can be obtained when compared to grain-sand cultures, agar cultures, mycelial mats or sclerotia.

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THE USE OF ELISA FOR EARLY QUANTIFICATION OF *GAEUMANNOMYCES GRAMINIS TRITICI* ASSOCIATED WITH WINTER WHEAT ROOTS. H.M. ElNashaar, L.W. Moore and R.A. George, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

ELISA-double antibody sandwich system was used to measure *Gaeumannomyces graminis tritici* (Ggt) associated with roots of winter wheat. The seedlings were grown at 15±2°C in sand inoculated with 1.0 mm particles of oat grains colonized by Ggt (2.5, 5 and 10 mg inoculum/gm sand). The pathogen was readily detected in a homogenized suspension ( $10^{-3}$  dilution) of 6 day old roots that were exposed to the lowest concentration of inoculum. The level of Ggt in the roots was proportional to the level of inoculum used ( $r=0.94$ ). When wheat seeds were coated with antagonistic bacterial strains prior to sowing, the level of Ggt detected in the roots decreased as much as 66%. The results indicate: (i) the amount of Ggt associated with wheat roots can be measured within 6 days from seeding, and (ii) using this system, potential antagonists to Ggt can be detected within 8-10 days from planting.

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DISTRIBUTION OF SCLEROTIA OF *SCLEROTIUM ROLFSEII* IN TWO APPLE NURSERY SOILS IN OKLAHOMA. S. P. Tomasiño and K. E. Conway, Dept. of Plant Pathology, Oklahoma State University, Stillwater, OK 74078

Soil samples were collected from two apple nursery plots in Oklahoma to determine sclerotial density and distribution of *S. rolfseii*. Ten core samples were taken to a depth of 8 cm in plots divided into identical quadrats. Cores were randomly removed from each quadrat and bulked together. Samples were air-dried, wet-sieved and allowed to dry again. Viable sclerotial counts were determined by adding a dark soil base and 2% methanol to stimulate sclerotial germination. Fifty four quadrats (2.75 x 2.75 m) were sampled at Tahlequah, OK and sclerotia were recovered in 78% of the samples with an average sclerotial density of one per 210 g of soil. Thirty quadrats (2.05 x 2.75 m) were sampled at Stillwater, OK and sclerotia were recovered from all quadrats with an average sclerotial density of one per 250 g of soil. The horizontal distribution of sclerotia most closely fits the Poisson distribution at both locations.



EVALUATION OF THE AMINOPEPTIDASE TECHNIQUE FOR IDENTIFICATION OF CYLINDROCLADIUM SPECIES. C. Stevens, Tuskegee Institute, AL, M. A. Palmer, North Central Forest Experiment Station, St. Paul, MN 55108, and A. Y. Tang, Tuskegee Institute, AL 36088.

The aminopeptidase substrate specificities of *Cylindrocladium clavatum*, *C. scoparium*, *C. floridanum* and *C. crotalariae* were compared. Fungal isolates were individually incubated with each of 19 amino acid  $\beta$ -naphthylamides (AAN). The amount of hydrolyzed AAN was then determined fluorometrically by measuring the enzymatically released  $\beta$ -naphthylamine. This resulted in an aminopeptidase profile for each isolate that was reproducible under standardized conditions. *C. crotalariae*, *C. clavatum* and *C. floridanum* had unique aminopeptidase profiles, however the profile of *C. floridanum* was indistinguishable from that of *C. scoparium*. This technique shows promise as a rapid method for identification of *Cylindrocladium* species.

FUSARIUM SPECIES ISOLATED FROM ROOTS, RHIZOSPHERES AND SOILS OF Baccharis SPECIES IN BRAZIL. Thor Kommedahl (Univ. of Minn.) and George A. Bean (Univ. of Maryland).

The shrubs *Baccharis coridifolia* and *B. megapotamica* occur in pastures in Brazil and have been reported to be toxic to livestock. Root, rhizosphere and soil samples were assayed for *Fusarium* spp. and other fungi to search for toxic species. The following *Fusarium* species were isolated and expressed as percentages of the total *Fusarium* colonies from roots, rhizospheres and soils, respectively: *F. oxysporum*, 46, 55, 82; *F. sporotrichioides*, 39, 35, 1; *F. acuminatum*, 6, 5, 1; *F. equiseti*, 5, 3, 1; *F. solani*, 3, 1, 17; *F. sambucinum* and *F. poae*, 1, 1, 1; *F. merismoides*; *F. semitectum* and *F. tricinctum*, 0, 1, 0; and *F. avenaceum*, 0, 0, 1. Species were identified using Nelson, Toussoun, and Marasas's key to *Fusarium* species (Penn. State Univ. Press, 1983). Most of these fungi were isolated from *B. coridifolia* but a few came from *B. megapotamica*. The mycotoxin production capabilities are currently being investigated.

POPULATION DYNAMICS OF FUNGAL GENERA ASSOCIATED WITH WINTER WHEAT ROOTS. W. W. Bockus, R. Hammel, M. G. Holtmeyer, and S. L. Wootke, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Field-grown winter wheat roots were sampled every two weeks for six weeks after seeding and then monthly for the remainder of the season from four replicate areas in each of two locations. Roots were washed, blended and then diluted plated onto 1/4 strength PDA. Root segments were also plated onto Pimaricin-Vancomycin Agar to monitor *Pythium* spp. Fungal colonies were identified to genus microscopically and populations quantified. The nine most common genera included representatives of: *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phoma*, *Rhizopus* and *Trichoderma*. These genera accounted for over 67% of the colonies formed on 1/4 PDA throughout the season. Unidentifiable fungi (no spores) accounted for 26.4% of the colonies. *Pythium* spp. produced an average of 0.38 cfu/cm of root throughout the season.

FUSARIUM SPECIES COLONIZING CEREALS GROWN IN ROTATION WITH POTATOES. Robert W. Stack, Neil C. Gudmestad and Marcia P. McMullen. Plant Pathol. Dept., No. Dak. St. Univ. Fargo 58105.

In North Dakota potatoes are commonly grown in rotation with spring wheats or barley. Cereals are generally considered to be non-hosts of potato pathogens, but little is known of their possible role in maintaining soil populations of fusaria pathogenic to potatoes. Cereal crowns and roots were collected from 32 fields in spring of 1984. Twenty-two of these fields had a rotation including potatoes while 10 had never been cropped to potatoes. We isolated from washed cereal root and crown pieces onto Komada medium. Of a total of 608 *Fusarium* isolates, 91% were of 5 species. Levels of *F. acuminatum* and *F. oxysporum* were similar in potato and non-potato soils. Levels of *F. culmorum* and *F. equiseti* were lower, and levels of *F. solani* higher where potatoes had been grown. Of the less frequently isolated species, *F. avenaceum* and *F. merismoides* occurred only in potato rotated fields while *F. graminearum*, *F. moniliforme* and *F. moniliforme* var *subglutinans* occurred equally in both potato and non-potato rotated fields.

EFFECT OF INOCULATION TIMING ON THE INTERACTION BETWEEN GLOMUS INTRARADICES AND FUSARIUM OXYSPORUM f.sp. RADICIS-LYCOPERSICI IN TOMATOES. Caron, M., J.A. Fortin and C. Richard. Agriculture Canada Experimental Farm, L'Assomption, Québec, Canada J0K 1G0. Département de Sciences Forestières, Université Laval, Québec, Canada G1K 7P6. Agriculture Canada Research Station, Sainte-Foy, Québec, Canada G1V 2J3.

In previous studies, we have shown that the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* can decrease root rot of tomatoes and *Fusarium oxysporum* f.sp. *radicis-lycopersici* development in the substrate. To determine if this effect varies with the order of inoculation of the two fungi, tomato plants were inoculated with *Glomus* 4 wk before, simultaneously, and 4 wk after inoculation with *Fusarium*. *G. intraradices* was effective in reducing root necrosis, plant mortality and the number of *Fusarium* propagules in the substrate (calcined montmorillonite clay) under all inoculation conditions tested. Root endomycorrhizal colonization was not affected by *Fusarium* or the order of inoculation.

RAPID IMMUNOCYTOCHEMICAL DETECTION OF A CLAVARIA SP. IN THE ROOTS OF RHODODENDRON. W. C. Mueller and L. Englander. Department of Plant Pathology-Entomology, University of Rhode Island, Kingston, RI 02881.

An antiserum was prepared in rabbits against the fruiting bodies of the *Clavaria* sp. found growing in the vicinity of rhododendrons in Rhode Island. The purified antiserum was used to detect *Clavaria* in the roots of rhododendron by a modification of the immunoenzymatic technique described by James S. Geric. Rhododendron roots were washed and processed at room temperature in wells of spot plates. The roots were sequentially treated with absolute ethanol, the anti-*Clavaria* serum, commercially prepared goat anti-rabbit IgG conjugated to alkaline phosphatase, enzyme substrate coupled to Fast Blue BB (a diazo salt), and NaClO. Strands of hyphae on the surface of the roots and knots of hyphae in the epidermal cells stained a brilliant blue. No reaction occurred in roots treated with normal serum or to an antiserum prepared against *Peizizella ericae*.

Formation and Regeneration of Protoplasts from the Ectomycorrhizal Basidiomycete *Laccaria bicolor*. Bradley R. Kropp and J.A. Fortin, Département des Sciences Forestières, Université Laval, Québec, Canada, G1K 7P4.

Protoplasts were obtained from *Laccaria bicolor* using Novozyme 234 (Novo Industrie, Bagsvaerd Denmark) a multi-enzyme preparation with primary activity towards glucan. All other enzyme preparations and combinations of enzyme preparations (i.e. Chitinase, Cytohelicase, Driselase, Cellulase TV, Cellulase Onozuka, Novozyme + Cellulase TV, Novozyme + Chitinase, Novozyme + Cytohelicase, Cellulase TV + Chitinase + Cytohelicase) were less effective. Protoplast yield was strongly affected by fungal culture age, incubation temperature, and the osmoticum used. The best yields were obtained by digesting 4-day old cultures at 30°C in .6M Mannitol. Protoplast regeneration begins after 2-3 days in osmotically stabilized MMN nutrient solution (Marx, Phytopathology 59: 153-163). Most regenerated colonies were monokaryotic but some were dikaryotic. The ability of cultures from regenerated protoplasts to form mycorrhizae was tested.

PRODUCTION OF A SPECIFIC ANTIBODY IN CHICKENS TO A MYCORRHIZAL FUNGUS. MacFall, J.S., Berbee, J.G., Cook, M.E., 1,2 Dept. of Plant Path., Univ. of Wisc.,-Madison 53706 3 Poultry Sci. Dept., Univ. of Wisc.,-Madison 53706

Mycelium of a new mycorrhizal fungus (*Hebeloma* sp.) of conifers was grown in liquid culture, washed by filtration, lyophilized, ground in liquid N<sub>2</sub>, and suspended in PBS. This material as well as the supernatant and resuspended pellet from centrifugation were injected separately into the breast muscle of White Leghorn chickens. Antibodies from egg yolks were tested by indirect ELISA with goat anti-chicken horse-radish peroxidase conjugate. There was a strong reaction to the injected fungus, but a weak cross reaction to *H. cylindrosporium* and *H. sarcophyllum*, and no reaction to the mycorrhizal fungi *Pisolithus tinctorius*, *Thelephora terrestris*, and *Laccaria laccata*. Only hyphal fragments of the injected fungus stained blue on nitrocellulose membrane immunoblots by egg antibodies, goat anti-chicken alkaline phosphatase conjugate and the substrate Naphthol-AS-phosphate.

CHLAMYDOSPORE PRODUCTION BY *GLOMUS DIMORPHICUM* IN THE ROOTS OF DIFFERENT HOSTS. S.M. Boyetchko and J.P. Tewari, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

*Glomus dimorphicum* Boyetchko and Tewari is mycorrhizal with barley in Alberta and produces many hyphae in the root system. The chlamydospore production in the roots is very rare. *Glomus* is an obligate symbiont and the presence of chlamydospores in the host roots is known to be important in the use of infected roots as inoculum. Pot culture experiments were therefore conducted to identify hosts in which the chlamydospore production in the roots may be appreciable. The roots of red clover and stringless green beans contained appreciable numbers of chlamydospores, in addition to the hyphae, whereas those of corn, alfalfa and onion have so far revealed only the hyphae. Therefore, red clover and stringless green bean can be used for raising the root-borne inoculum of *G. dimorphicum*.

EFFECT OF NITROGEN FERTILIZATION ON DISEASE DEVELOPMENT IN RICE D. E. Groth and D.M. Brandon, Rice Research Station, La. Agri. Exp. Stn., L.S.U. Agricultural Center, P. O. Box 1429, Crowley, LA 70527-1429

Severity ratings for various rice (*Oryza sativa* L.) diseases were collected from the N fertilization field experiments at the LSU Rice Research Station, Crowley, LA in 1984. Diseases were rated at plant maturity using 0-9 scales. Sheath blight (*Rhizoctonia solani*) and leaf smut (*Entyloma oryzae*) increased with increasing N levels while brown spot (*Bipolaris oryzae*) and narrow brown leaf spot (*Cercospora oryzae*) decreased with increasing N levels. Average change in disease severity was 2-3 disease units between 0 kg N/ha and 168 kg N/ha applied preplant. Evaluation of multiple disease resistance was most consistent at the 101 to 136 kg N/ha rates. Rates in this range will be used in future disease nurseries. Under low disease pressure in 1984, adjustment of N fertilization for maximum yields was more important than adjustment for disease control.

THE EFFECT OF BACTERIA ON UREDIA FORMATION BY *PUCCINIA RECONDITA* F. SP. *TRITICI* ON SPRING WHEAT. M. A. McVey and G. D. Statler, Department of Plant Pathology, North Dakota State University, Fargo, 58105.

The interaction of bacteria and urediniospores of *Puccinia recondita* f. sp. *tritici* on the phylloplane of wheat plants was investigated in a greenhouse study. The bacterial isolates used were *Erwinia carotovora* spp. *atroseptica*, *Pseudomonas syringae* pv. *phaseolicola* and *Bacillus megaterium* pv. *cerealis*. Primary leaves of 10-day-old seedlings of Thatcher, Olaf and CI8515 wheat were inoculated with 24-hour-old bacterial cultures. Bacteria were applied until runoff by an aerosol sprayer at densities of  $10^3$ ,  $10^5$ , and  $10^7$  colony forming units per ml. Urediniospores were applied with oil inoculators at 24 hr or 48 hr following inoculation with bacteria. Ten days after inoculation with urediniospores the number of uredia were counted on a 5 cm segment of the primary seedling leaf, 1 cm from the leaf tip. Preliminary results indicate *Pseudomonas* and *Bacillus* isolates reduced the number of uredia.

IMPROVEMENT OF POTATO PROFITABILITY THROUGH FIELD MAPPING OF *VERTICILLIUM DAHLIAE* INOCULUM POTENTIAL IN THE SOIL. Leah Tsror (Lahkim), Abraham Nachmias, James Krikun, Plant Disease Diagnostic Laboratory (PDDL), Gilat Exp. Station Negev 85-280, Israel.

Soil fumigation with Vapam (300-800 L/ha) is one of the main methods for the control of *Verticillium* wilt of potato. In the Negev region, soil treatment is based on nematode counts, and unfortunately, fields are fumigated whether infested with *V. dahliae* or not. During the last 3 years we have developed a method of mapping potato fields for *V. dahliae* infestation in the soil. Inoculum potential in soil samples (10-20/ha) was determined by a baiting technique using eggplant or flax as a susceptible host. An alternative technique was based on monitoring visual *Verticillium* symptoms on susceptible crops (usually cotton) grown prior to potato. Based on the information on nematode levels and infection rates of different seed lots, a better decision can be made on the necessity for expensive fumigation and the utilization of fields for seed tubers or consumption.

REACTION OF ARGENTINE RUST DIFFERENTIAL SUNFLOWER LINES TO FOUR AMERICAN RACES OF *PUCCINIA HELIANTHI*. S. Yang, USDA-ARS, P. O. Drawer 10, Bushland, TX 79012.

Four North American (NA) races (1, 2, 3, 4) of *Puccinia helianthi* Schw. (T. Gulya, USDA, Fargo, ND) were inoculated to 14 Argentine [7 Antonelli's (A) and 7 Luciano and Luciani's (LL)] rust differential sunflower (*Helianthus annuus* L.) lines. Antonelli's differential lines were provided by H. Shands, DeKalb-Pfizer Genetics, MN. NA race 1 attacked only Impira INTA NAL and Guayacan INTA NAL. NA race 2 also attacked Morden 307-1 (A and LL) and Pergamino (A); NA race 3 attacked Impira INTA Sel 11 (A) and F 164A (LL), and NA race 4 attacked Morden 307-1, F 164A, F164A x Morden 307-1 (LL), and P 94 (LL). These results indicate that NA race 1 is similar to Antonelli's race 311 and also Luciano and Luciani's race 1. NA races 2 and 3 are similar to Luciano and Luciani's races 2 and 3, respectively, but different from Antonelli's races 465, 745, 340, 767, 805, 827, and 823. NA race 4 is similar to Antonelli's race 793 but different from Luciano and Luciani's race 4.

MORPHOLOGICAL VARIATION OF TELIOSPORES IN *NEOVOSIA HORRIDA*. T.T. Matsumoto<sup>1</sup>, N.G. Whitney<sup>2</sup>, M.R. Bond<sup>3</sup>, and M.H. Foy<sup>3</sup>. <sup>1</sup>CDPA 1220 N St., Sacramento, CA 95814. <sup>2</sup>Texas A & M University, Beaumont, TX 77843. <sup>3</sup>USDA-ARS PDRL, Frederick, MD 21701. Morphological characters of teliospores such as exospore, spore size and color of *Neovossia horrida* (= *Tilletia barclayana*) were compared with isolates from California, Texas, and Arkansas. The hyaline sheath which surrounds the teliospores was experimentally removed with various chemical and enzymatic procedures. Direct observation using scanning electron microscopy and light microscopy were used to study this variation. Exospore characters were classified as spiny, intermediate and truncated. Teliospore size and color were determined by light microscopy in Shear's mounting medium. Considerable morphological variations were observed between isolates. These morphological characters and range in variation were used to compile specific criteria for the identification of this species which can be used by quarantine personnel. This information can prevent the confusion with *Neovossia indica* (= *Tilletia indica*) which is a quarantine pest of wheat. In 1984, two commercial wheat samples were found contaminated with teliospores of *Neovossia horrida* in California.

EVALUATION OF BARLEY CULTIVARS FOR KERNEL DISCOLORATION. M.R. Miles and R.D. Wilcoxson, Dept. of Plant Path., Univ. of Minn., St. Paul, MN 55108.

Eight cultivars differing in kernel discoloration severity were grown at Crookston, Morris, Rosemount and St. Paul, MN in 1982, 1983 and 1984. Kernels were visually evaluated for discoloration on a 1 to 5 scale. A score of 1 indicated little discoloration and a score of 5 severe discoloration. Chevron and CI9539 were the least severely discolored with mean scores of 1.2 and 1.4 whereas Karl was the most severely discolored with a mean score of 3.7. The commercial cultivars were intermediate. Robust,

Bumper and Morex had scores of 2.5 and 2.6, 2.6, respectively, and did not vary significantly from Glenn and Larker, scores of 2.8 and 2.9, respectively, but were significantly less severely discolored than Karl. Discoloration did not vary significantly with locations and years. However, the location by year interaction was significant, probably due to increased severity of discoloration at Morris in 1982, and at Rosemount and St. Paul in 1983.

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EVALUATION OF SOILS FOR SOYBEAN ROOT ROT POTENTIAL AND DISEASE SEVERITY. A.F.Olah and A.F.Schmitthenner. The Ohio State University. Plant Pathology Dept. OARDC. Wooster, OH 44691.

A soybean leaf-disc bioassay was used to evaluate *Phytophthora megasperma* f.sp. *glycinea* (Pmg) in 224 field soils collected in 1983. Pmg level (# of infected discs) was not correlated with high or low areas in fields, vacuum time required to partially dry slurried soils or moisture content of soils at bioassay. High levels of Pmg were found in 10%, moderate in 24%, low in 36% and none in 29% of soils studied. During 1984 *Phytophthora* damage was evaluated in 65 fields using metalaxyl (broadcast, 4 oz a.i./acre) with soybeans resistant or with high, moderate or low tolerance to Pmg. Significant ( $P=0.10$ ) stand increases (5 fields), yield increases (5 fields) and stand plus yield increases (2 fields) were obtained. In fields with high Pmg metalaxyl significantly increased stand in resistant varieties and yield of low-tolerant varieties. Disease levels were too low to adequately determine the relationship between Pmg level in soil and disease severity.

#### 696

REPRODUCTION AND SURVIVAL OF A MINNESOTA POPULATION OF *Heterodera glycines* RACE 5 FOLLOWING ONE TO THREE GROWING PERIODS ON NINE CROP AND TWO WEED SPECIES. M.E. Sortland, and D.H. MacDonald, Dept. of Plant Pathology, Univ. of Minn., St. Paul, MN 55108.

The effect of nine crop and two weed species on the reproduction and survival of a Minnesota population of *Heterodera glycines* race 5 was investigated using naturally infested soil in the greenhouse. Following one, two and three growing periods of approximately 40 days each, selected root systems were stained to determine infection. Mature female nematodes or cysts retaining eggs were extracted from soil. The capacity of *Heterodera glycines* race 5 to infect a susceptible crop following one, two or three growing periods of the test species was determined by a soybean bioassay. *Heterodera glycines* race 5 reproduced well on soybeans, adzuki beans and peas, but not on other crop and weed species. Such crops could be used in a crop rotation scheme to reduce this nematode population in the field, but the rotation must extend through two seasons and preferably beyond three.

#### 697

PATHOGENICITY OF BINUCLEATE *RHIZOCTONIA SOLANI*-LIKE ORGANISMS TO ALFALFA. P. S. Yahata, M. Aragaki and J. Y. Uchida. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

A high frequency of *Rhizoctonia solani* Kühn and *R. solani*-like organisms was associated with collar and root rots of legumes in Hawaii. Numerous binucleate *R. solani*-like isolates representing anastomosis groups CAG-2, -4, -7, three unidentified Hawaiian groups, and two independent isolates were made from alfalfa (*Medicago sativa* L.) and beans (*Phaseolus* spp.). Pathogenicity of representatives from each of the groups and two independent isolates were tested on alfalfa seedlings. Fifty alfalfa seeds were sown on each pot, maintained in the greenhouse, and assayed for disease development 13 days later. All binucleate *R. solani*-like isolates tested, were pathogenic to alfalfa. In general, *R. solani* AG-4 isolates from various legumes were more virulent to alfalfa than these binucleate, *R. solani*-like organisms.

#### 698

Effects of Water Potential on Anthracnose in Alfalfa. W.W. Miller and B.D. Thyr. The University of Nevada and USDA/ARS, Reno, Nevada.

*Colletotrichum trifolii* grows optimally in-vitro at

-1.0MPa. Alfalfa inoculated with *C. trifolii* was grown in soil columns under stress cycles of 0 to -0.033MPa, 0 to -0.6MPa, and 0 to -1.3MPa. Disease severity and plant mortality were significantly correlated to water treatment ( $r = .80$  and  $r = .87$ , respectively). Medium and high water stress treatments resulted in greater disease severity while plant mortality was greater at low and high water stresses. Decline in dry matter production due to inoculation was also significantly correlated to water treatment ( $r = .79$ ) with the greatest decreases occurring in low and high stress treatments. Mortality and reduced yield in low stress treatments may have been confounded by rhizosphere oxygen depletion.

#### 699

INCIDENCE OF AND TILLAGE METHODS ASSOCIATED WITH GRAY LEAF SPOT OF FIELD CORN IN MARYLAND. K. L. Smith, USDA-ARS-SNECL, Beltsville, MD 20705 and A. P. Grybauskas, Dept. of Botany, University of Maryland, College Park, MD 20742.

Gray leaf spot (GLS) of field corn, caused by *Cercospora zeae-maydis*, was found in 20 of 23 counties and 58 of 105 fields inspected in Maryland during Aug. and Sept. of 1984. This range far exceeds the prior known range of GLS in Maryland. The incidence of this disease was related to farmer-described tillage practices as follows: 35% in conventional tillage, 54% in minimum tillage, and 69% in no-till. GLS incidence was related to percent of soil surface covered with corn debris as follows: 31% for no surface corn debris, 73% for 1-25% surface corn debris, and 86% for greater than 25% surface corn debris. Counties with the highest incidence of GLS were those where the culture of continuous no-till corn predominated. In counties where little or no GLS was found one or more of the following practices of corn culture prevailed: conventional tillage, rotation of corn with soybeans and/or small grains, and silage production.

#### 700

ONE CAUSE OF MORTALITY OF SLASH PINES AFFECTED BY FUSIFORM RUST IN THE GREENHOUSE OR FIELD. C. H. Walkinshaw, Southern Forest Experiment Station, P. O. Box 2008, GMP, Gulfport, MS 39505.

Fusiform rust of slash pine (*Pinus elliotii* Engelm. var. *elliottii*) can kill more than 50% of trees with stem galls. Highest mortality is seen when stem infection occurs in the first, second, or third growing season. Resulting galls grow to approximately 25 cm in 5 years. Besides elongation, growth often ceases in the stem immediately below the gall, causing overgrowth galls. The tree dies one or two seasons later. This process is being studied in the field, greenhouse, and laboratory. The results are illustrated by field and greenhouse photographs and photomicrographs of fixed tissues. These illustrations and data on occurrence of overgrowth galls show that this girdling is a wound reaction. Also, this abnormal gall growth in the field and greenhouse appears similar to wounding by mechanical means, incompatibility of scion and stock, and infections by other organisms.

#### 701

DAMAGE TO RESIDUAL TREES FROM THINNING TIMBER STANDS USING BIOMASS HARVESTING TECHNOLOGY. W.D. Ostrofsky, R.S. Seymour, and R.C. Lemm. CFRU, College of Forest Resources, Univ. Maine, Orono 04469.

A mechanized whole-tree harvesting system, recently introduced to the northern New England region, was used for thinning two northern hardwood stands in western Maine. Damage levels were higher than those reported for similar stands thinned by conventional methods. Between 19% and 54% of the residual trees were injured, depending on stand type, skid trail spacing, and whether trails were designated prior to harvesting. Significantly more ( $P = 0.05$ ) trees were injured in a paper birch/yellow birch stand (49%) than in an oak/beech stand (32%), and more trees had root wounds (27%) in the birch stand, compared with the oak/beech stand (9%). Tree feller-bunchers, not operationally confined to skid trails, accounted for 66% of all injuries. If not carefully planned and executed, such harvesting may lower residual tree quality, and may result in serious and rapid forest stand decline.

#### 702

ERADICATION OF DECAY FUNGI IN DOUGLAS-FIR TIMBERS WITH FUNGICIDS.

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U.S.D.A. Forest Service, P. O. Box 5130, Madison, WI 53705

Fumigation with Vapam or chloropicrin of horizontal Douglas-fir timbers having interior decay prevented reinfection by decay fungi for 7 years. Mold fungi, however, were frequently isolated. Eleven additional fumigants plus vapam were tested on efficacy in killing cultures of *Poria placenta*, *P. carbonaria*, *P. xantha*, *Fibroporia vaillantii*, *Lentinus lepideus*, *Antrrodia serialis*, *Serpula incrassata*, and *Gloeophyllum trabeum* implanted in Douglas-fir timbers. Vapam proved the most effective with efficacy up to 16 months at 0.61 m from the point of application. Of the other fumigants tested, Busan 40, Nylone, and sodium bisulfite were next most effective. Fumigant toxicity was generally greatest during the first 4 months following treatment. Differences in fungal sensitivity to fumigants were observed.

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SYMPTOMOLOGY OF THE "TOP BLIGHT" DISEASES OF DOUGLAS-FIR BARE-ROOT SEEDLINGS IN NURSERIES IN THE PACIFIC NORTHWEST. P.B. Hamm, E.M. Hansen, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, and A.M. Kanaskie, State Department of Forestry, Salem, OR 97310.

Top Blight, an ill-defined syndrome, describes at least three distinct periods of mortality. In mid-summer, when seedlings are 3 mo old, a hypocotyl rot occurs at or just above the soil line. Seedling tops quickly turn brown while roots are not discolored. *Fusarium oxysporum* is isolated. From Sept. through Nov., cankers, centered on bark fissures, lateral or terminal shoots, or needle scars, appear between the midstem and seedling apex. *F. roseum* (Fr) and *Phoma eupyrena* (Pe) are recovered. A third type of symptom, Dec. through May, is associated with soil collars built up by irrigation and splashing rain. Seedlings are girdled at or above the cotyledon scar, with top symptoms sometimes not evident until new growth suddenly wilts during the Spring. *Er* and *Pe* are most frequently isolated from diseased seedlings.

704

SPREAD AND INTENSIFICATION OF WHITE PINE BLISTER RUST IN THE SOUTHERN SIERRA NEVADA. J. T. Kliejunas. USDA Forest Service, Forest Pest Management, San Francisco, CA 94111.

White pine blister rust (*Cronartium ribicola*) entered northern California in 1929 and advanced southward on sugar pine (*Pinus lambertiana*) to the central Sierra Nevada by 1944. Surveys in 1971-72 found that the rust had spread in the 1960's to isolated centers 150 miles further south. Incidence and impact surveys on the Sierra and Sequoia National Forests in 1982-83 found rust at 22 unreported locations and at the southern extent of sugar pine in the Sierra Nevada. Rust was adversely impacting the resource in local situations where significant spread and intensification had occurred since 1972. Canker incidence generally decreased from stream bottom to upper slope, but the disease was also found on ridgetops. Cankers in lower branches of smaller trees were most common. The southern Sierra Nevada climate is favorable for infection most years and local spread and intensification will continue.

705

WHITE PINE BLISTER RUST IN YOUNG SUGAR PINE PLANTATIONS IN THE MID-ELEVATION SIERRA NEVADA. Chen Mo-mei, F. Cobb and R. Heald, Depts. of Plant Pathology and Forestry, University of California, Berkeley, CA 94720.

Sugar pine seedlings were included in mixed-species plantations on Blodgett Research Forest (av. elevation 1330M) beginning in 1976. In 1983, the disease was detected on planted saplings, and a study was initiated in 1984. A total of 1277 saplings in 11 plantations and 476 scattered naturally-occurring saplings was examined; 16 percent of the planted saplings (1-53% of seedlings per individual plantation) and 12 percent of the natural saplings were infected. The years of origination of the infected internodes were determined: 60% of the infections were on 1981 tissue; 15% on 1982; 10% on 1980; 6% on 1979; and less than 1% on tissue of other years. Local weather data indicate that 1981 (with 11 days) and 1982 (with 6 days) were the only years since 1977 with more than one day of conditions favorable to infection. There was a strong correlation between infection and distance to *Ribes*. Both mature pycnia and aecia were detected during Fall, 1984.

706

TUBERCULARIA CANKER AND DIEBACK OF RUSSIAN OLIVE IN NORTH DAKOTA. H. W. Sengpiel and R. W. Stack, N.D. Highway Dept., Bismarck and Plant Pathol. Dept., ND State Univ. Fargo 58105

Russian olive (*Eleagnus angustifolia* L.) is widely planted throughout the northern great plains. Many trees show cankering of stems and dieback of tops. Affected Russian olive trees were examined at 80 locations throughout North Dakota including windbreaks, highway landscape plantings and urban sites. Trees showed a range of symptoms, from cankers to dieback to premature mortality. *Tubercularia ulmea* Cart. was consistently isolated from affected areas on symptomatic trees. Sporodochia of *T. ulmea* were frequently present on the bark surface of cankered stems. Perithecia of the teleomorph, *Nectria cinnabarina* (Fr) Fr. were also found occasionally. In the field, large cankers girdling main stems were frequently associated with remains of previously killed small branches. Cankering by *T. ulmea* was determined to be a main cause of dieback and death of Russian olive in North Dakota.

707

GREEN ASH STEM DECAY IN NORTH DAKOTA: RELATION OF CONKS AND STEM CANKERS TO INTERNAL DECAY. J. A. Walla, Plant Pathology Dept., North Dakota State Univ., Fargo, ND 58105.

In 1984, 50 live green ash in a shelterbelt planted in 1941 in southeast North Dakota were randomly selected. Tree height and diameter and presence of outward indicators of stem decay (fruiting bodies, stem cankers) were recorded. The trees were felled and stems greater than 8 cm diameter were cut into 1 m long sections. All sections were split lengthwise, through defects if present, to find small pockets of decay and extent of larger areas of decay. Isolations were made from discolored and decayed wood in each section. Volume of apparent decayed wood, discolored wood and all wood was calculated. More than 50% of the trees contained decayed wood as compared to 22% expected from outward indicators of decay. Decayed wood volume was less than 1% of total wood volume. At least four decay fungi were present, compared to two decay fungi expected from outward indicators. Green ash in additional shelterbelts will be similarly examined to determine the efficacy of estimating amount of stem decay based on outward indicators.

709

COMPARTMENTALIZATION OF *CERATOCYSTIS FAGACEARUM* IN OAK TISSUES. F. H. Jainter and S. W. Fraedrich, Department of Forestry, Clemson University, Clemson, SC 29631

Natural infections in South Carolina of the oak wilt disease, caused by *Ceratocystis fagacearum*, are few and relatively static. Infected red oaks are seldom quickly killed and may survive for several years or even recover. Trees exhibit noticeable vascular streaking in xylem of branches and stems during the early stages of infection. In nine turkey oak trees inoculated on April 30, 1980 with a S. C. isolate of *C. fagacearum* and harvested on August 16, 1982, *C. fagacearum* was not recovered even though the trees had partially wilted during the season of inoculation and the following season. There was a significant increase in tylose formation in xylem vessels during the year of inoculation and the following two years in crowns and upper and lower stems, and a similar, but with additional one-year delayed effect in roots. Tyloses were present

in fewer than 50 percent of the vessels in any given year, indicating a limited and not long-lasting physiological effect of infection on the host.

#### 710

Bacteria associated with flux from white oaks. M. L. Double and G. K. Bissonnette, WVU Plant Path. and Ag. Micro., P.O. Box 6057, Morgantown, WV 26506, and S. C. Haynes, Plant Pest Control Division, WV Department of Agriculture, Charleston, WV 25305

During the summer of 1984, copious amounts of an aromatic flux were observed oozing from bark crevices near the ground on numerous white oaks in southern WV. The source of the flux appeared to be discolored 1-to-2 year-old xylem tissue. Samples of flux from several trees yielded a bacterium and an actinomycete, tentatively identified as *Streptococcus* sp. and *Streptomyces* sp., respectively. A number of coryneform-like bacteria also were isolated. These bacteria are non-motile, Gram-positive to Gram-variable pleomorphic rods that produce acid from glucose, lack pigment, do not digest starch, gelatin or cellulose, are not sensitive to 2,3,5-triphenyl tetrazolium chloride, grow in 7% salt and produce bacteriocins. Unlike plant-associated corynebacteria, they are facultative anaerobes. The incidence and relationship of these organisms to the flux is being evaluated.

#### 711

GROWTH CULMINATION OF LOBLOLLY PINES GROWING ON SOILS OF DIFFERENT PREDICTED LITTLELEAF DISEASE RISK. S. W. Oak and F. H. Tainter, USDA Forest Service Southern Region, Forest Pest Management, Asheville, NC 28803, and Department of Forestry, Clemson University, Clemson, SC 29631.

Loblolly pine is recommended as an alternative to shortleaf pine in managed conifer forests affected by littleleaf disease on the southern Piedmont. However, growth stagnates in some loblolly pine stands before desired harvest age and this could be related to predicted littleleaf risk. Volume growth was measured and correlated with crown health class and predicted littleleaf risk for 100 trees in each of 17 loblolly pine stands. Volume growth rate culminated at age 28 on high and intermediate risk sites for trees with severe crown symptoms and at age 40 for trees with light crown symptoms. Volume growth rate had not culminated by age 50 for trees with healthy crowns, regardless of predicted littleleaf risk. Optimum harvest age can be recommended by predicting littleleaf disease risk class and determining incidence of trees with crown symptoms.

#### 712

PATHOGENICITY OF *FUSARIUM MONILIFORME* VAR. *SUBGLUTINANS* TO LOBLOLLY PINE CONES. Jane Barrows-Broadus, USDA Forest Service, Carlton St., Athens, GA 30602.

Sixteen-month-old cones of loblolly (*P. taeda* L.) pine were inoculated with *Fusarium moniliforme* var. *subglutinans* (FMS) after wounding with a dissecting needle in one of three locations: (1) apical scales (as), (2) central fertile scales (fs), and (3) base of the peduncle (bp). Inoculation treatments (I) consisted of  $10^7$  conidia/ml FMS in water, sprayed to runoff. Controls (C) were sprayed with sterile water. Ten weeks later, necrosis of tissues adjacent to a wound was observed in all cones except those in bp-I and bp-C treatments. Recovery of FMS from cone tissues was as follows: as-I 77%; as-C 24%; fs-I 61%; fs-C 6%; bp-I 9%; and bp-C 6%. Radiographs indicated that there was no effect of treatments on percentage of filled seed. When samples of filled seeds (20/treatment) were cultured, all germinated contamination-free except for seeds from treatment fs-I, in which 10% of the seeds were colonized by FMS. Although inoculation of mature cone scales resulted in their colonization by FMS, damage to seeds by FMS probably occurs in earlier stages of development.

#### 713

INFLUENCE OF AGE OF CULTURE AND 2 EXTRACTION METHODS ON YIELD OF ENZYMES FOR STARCH GEL ELECTROPHORESIS OF *FOMES ANNOSUS* ISOZYMES. William J. Orpington, USDA Forest Service, P.O. Box 245, Berkeley, CA 94701

*Fomes annosus* isolates were grown for 1, 2, and 3 weeks in potato-dextrose broth (Difco) at 24-25°C. Extracts of mycelial mats were obtained either by crushing for 30 sec. with a glass rod or by subjecting the mycelium to 30 sec. of agitation from the bit of a vibrating engraver. Starch gel electrophoresis was then performed on the extracts following the method of Conkle, et al. (1982, USDA Forest Service, General Technical Report, PSW-64). The 3-week-old cultures

extracted with a glass rod yielded the most readable zymograms. Of 27 enzyme systems assayed, 19 were detected in this study. Of those, glutamate-oxaloacetate transaminase, glucose-6-phosphate dehydrogenase, catalase, beta esterase, sorbitol dehydrogenase, uridine diphosphoglucose pyrophosphatase, malate dehydrogenase, shikimic dehydrogenase, and acid phosphatase yielded the most distinct bands.

#### 714

EFFECTS OF TEMPERATURE, SOIL WATER POTENTIAL AND INJURY ON DEVELOPMENT OF SYCAMORE ANTHRACNOSE. Vernon Ammon, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State, MS 39762.

Temperature and moisture stress were evaluated for effects on symptom development in sycamore seedlings inoculated with *Gnomonia platani*. Seedlings maintained at soil moisture potentials of 0.3, 3.0 and 10 bars did not develop disease symptoms under ambient greenhouse conditions, nor did those inoculated at 13° and 19° C at 100% relative humidity. Symptoms did develop, however, at both temperatures if leaves were injured prior to inoculation. Symptoms failed to develop on inoculated seedlings at combined temperature and moisture regimes of 13°, 19° and 24° C and 0.3, 3.0 and 10.0 bars, respectively.

#### 715

PERMEABILITY OF ASYMPTOMATIC, RESIN-SOAKED AND VERTICILLADIELLA PROCERA-BLACK-STAINED PINE SAPWOOD. W. Elliott Horner and S. A. Alexander, Dept. of Plant Pathology, Physiology and Weed Science, VPI & SU, Blacksburg, VA 24061.

Longitudinally oriented cores (5 mm diam.) were excised from pine sapwood with and without symptoms associated with white pine root disease caused by *Verticilladiella procera*. Permeability of these cores was compared by measuring the amount of water conducted in response to a 250 KPa pressure differential applied with a Scholander pressure bomb. Sapwood with neither macroscopic nor microscopic evidence of resin-soaking readily conducted water. Conduction through resin-soaked sapwood was significantly reduced ( $P=0.05$ ). Black-stained sapwood typically contained hyphae of *V. procera*, and either behaved as resin-soaked sapwood or allowed passage of the pressurizing gas rather than water. The zone of resin-soaked sapwood that typically developed between black-stained and clear sapwood areas had a greatly reduced permeability which may account for symptoms of wilt associated with this disease.

#### 716

SEED-BORNE FUNGAL PATHOGENS OF BITTERBRUSH (*PURSHIA TRIDENTATA*). David L. Nelson, USDA For. Serv., Intermtn. For. and Range Exp. Stn., Shrub Sci. Lab., Provo, UT 84601.

Bitterbrush seed from 18 locations in seven western states were tested for seed-borne fungi. Numerous fungi were isolated following seed stratification for 60 days at 1 C. A *Sclerotium* was isolated from 11 of the 18 sources, from 2 to 100 percent (avg. 28.6%) of seed. A *Diplodia*-like fungus was isolated from 17 of the sources from 2 to 90 percent (avg. 35.4%) of seed. Virtually all seed were destroyed, apparently during stratification, and did not germinate. Treatment of seed prior to stratification with NaClO, H<sub>2</sub>O<sub>2</sub>, and HgCl<sub>2</sub> reduced but did not eliminate these fungi. And, because seed embryos appeared healthy, the results suggest the fungi are borne in the seed coat. In pathogenicity tests on germinating *Purshia* seeds using 6 *Sclerotium* and 5 *Diplodia*-like isolates, 100 percent of the seedlings were invaded and killed (except for three isolates 46, 82, 92%). Both fungi appear to induce severe pre- and post-emergence damping-off.

#### 717

DIFFERENTIAL IRRIGATION EFFECTS ON BLAST DEVELOPMENT IN PURE AND MIXED STANDS OF UPLAND RICE. J.M. Bonman and B.A. Estrada, International Rice Research Institute, P.O. Box 933, Manila, Philippines.

Drought increases rice blast disease, caused by *Pericarpia oryzae*. In upland rice field plots, line source sprinkler irrigation was used to induce a gradient of water stress from 41 to 52 days after seeding (DAS). Rice plants in the center of each plot were inoculated with *P. oryzae* and uniform infection developed before differential irrigation began. The response of cultivars C22, UPLR13, and IR10011-16-3 sown in pure and mixed

stands was evaluated across the gradient. Only C22 was susceptible to leaf blast. At 63 DAS, the peak of disease development, it had 21% diseased leaf area (DLA) in the most stressed plot, but only 1% DLA in plots that received the most water. The response to blast across the gradient differed significantly between the cultivar mixture and the pure stand of C22. The line source sprinkler irrigation technique should be useful for studies of water stress/blast interactions in upland rice.

718

THE RELATIONSHIP OF SCLEROTINIA SCLEROTIORUM ASCOSPORE GERMINATION ON BEAN BLOSSOMS TO DISEASE DEVELOPMENT IN A BEAN CANOPY MICROCLIMATE. C.L. Campbell and J.R. Steadman, Dept. of Plant Path., University of Nebraska, Lincoln, NE 68583-0722.

When ascospores were placed on blossoms of *Phaseolus vulgaris* beans, temperatures above 27°C produced a rapid decline in germination after 6 hr incubation. The microclimate temperature with open bean canopies can exceed 27°C which then reduces disease initiation. Conformation of senescent blossoms may influence ascospore germination through availability of free moisture with nutrients leached from blossoms and held within blossom convolutions. This would have a significant effect on blossom colonization and initial infection and may explain disease escapes in the field.

719

REDUCED FITNESS IN THE FIELD OF LABORATORY STRAINS OF COCHLIOBOLUS HETEROSTROPHUS. C. J. R. Klittich and C. R. Bronson, Dept. of Plant Pathology, Seed and Weed Sciences, Iowa State University, Ames, IA 50011.

Race O strains of *Cochliobolus heterostrophus* (*Helminthosporium maydis*) adapted to laboratory culture were used to inoculate 0.3 ha of maize in 1983 and 1984. Leaf blight lesions were collected throughout the growing season and single-conidial isolates (1,198 in 1983 and 1,933 in 1984) were characterized. The percentage of isolates that were identified as lab strains by cycloheximide resistance and colony morphology decreased significantly compared with field isolates (cycloheximide sensitive, wild-type morphology) both years, dropping from 100% to 19% over 9 weeks in 1983 and from 98% to 17% over 8 weeks in 1984, indicating a rapid spread and ascendancy of naturally-occurring strains into and throughout the plot. The lab strain used in 1983 was not isolated from lesions collected in 1984, indicating that it did not overwinter at a detectable level. These data suggest that the overall fitness of the lab strains is lower than that of naturally-occurring strains in the field.

720

AUTORADIOGRAPHY OF CORN SILKS INOCULATED WITH RADIOLABELED ASPERGILLUS FLAVUS CONIDIA. D. H. Tucker, Jr., L. E. Trevathan, and T. F. Kellogg, Mississippi State University, Mississippi State, MS 39762.

Laboratory and glasshouse experiments were conducted to investigate the use of radioisotopes in studies of the mode of corn kernel infection by *Aspergillus flavus*. Inoculum was prepared by culturing *A. flavus* conidia on Czapek's solution agar amended with uniformly labeled  $C^{14}$  sucrose. Conidia were washed with sterile, distilled  $H_2O$  until the filtrate contained only background levels of radioactivity. Silks on ears of corn growing in a heated glasshouse were atomized with 0.7 ml of radiolabeled conidia suspended in sterile, distilled water ( $1 \times 10^7$  conidia/ml). Silks were examined at weekly intervals from inoculation until harvest for evidence of *A. flavus* colonization. Ten percent of the autoradiographs examined contained images of radioactive conidia with hyphae following the silk. The hyphae grew in two directions from the location of conidia on silks. No autoradiographs showed labeled hyphae reaching the kernels.

721

VIRULENCE, EVOLUTION, AND DISTRIBUTION OF PUCCINIA STRIIFORMIS IN WESTERN UNITED STATES. Roland F. Line and A. Qayoum, ARS-USDA and Washington State University, Pullman, WA 99164.

From 1960 to 1984, 32 races have been detected in western USA. Based on geographic barriers and prevailing winds, we divided western USA into six regions: eastern WA and OR and northern ID (R1), western MT (R2), southern ID and northern UT (R3), western OR (R4), northwestern WA (R5), and central CA (R6). R1 had the most races. The races in R2, R3, and R4 were the same as some races in R1. All races in R2 and R3 were first

detected in R1. Two of 23 races in R1 were first detected in R4. R5 had the second greatest number of races and many occurred only in R5. Of the few races in R6, the least virulent and aggressive was unique for R6. Inoculum movement within R1, R2, R3, and R4 appears to be common but not between R5 or R6 and those regions or between R5 and R6. New races were associated with introduction of resistant cultivars in R1, R2, R3, and R4 but not always in R5 and R6. Wild grasses probably contributed to race variability in R5 and R6.

722

RELATIONSHIP BETWEEN PECAN SCAB DISEASE PROGRESSION ON LEAVES VERSUS NUTS IN THREE PECAN CULTIVARS. F.W. Nutter, Jr., T.R. Gottwald, and P.F. Bertrand, Dept. Plant Pathology, Univ. of GA, Athens 30602; USDA-ARS, Orlando, FL 32803; and Coop. Ext. Serv., RDC, Tifton, GA 31793-0748.

Pecan leaf scab epidemics are difficult to quantify because severely infected leaves defoliate yet, flushes of new growth also occur throughout the season. We used area under the disease progress curve (AUC) based on eight severity assessments at approximately 20-day intervals to quantify the relationship between scab development on leaves versus scab development on nuts. Experiments were conducted over a 4-yr period and involved the cultivars Schley, Stewart, and Wichita. AUC values (leaves) for Stewart were significantly lower compared with Schley and Wichita. Although Schley and Wichita had similar AUC values for leaves, the regression coefficient relating AUC (leaves) to AUC (nuts) was three times higher for Wichita. This analysis indicates that there is a relationship between the leaf and nut phases of pecan scab epidemics and that cultivar resistance may affect one or both phases.

723

SURVEY OF PRUNING WOUND CANKERS CAUSED BY PHYTOPHTHORA SYRINGAE IN ALMOND ORCHARDS. M. A. Doster and R. M. Bostock, Department of Plant Pathology, University of California, Davis, CA 95616.

Two almond orchards were surveyed in 1984 for cankers caused by *Phytophthora syringae* that were located at pruning wounds. The heights and diameters for all pruning wounds were measured for 308 trees in one orchard and for 485 trees in the other. The percentage of pruning wounds with cankers was 10.5% and 23.4% in the two orchards. The percentage of pruning wounds with cankers increased linearly ( $r^2=0.97$ ) as the diameter of the pruning wound increased. Cankers were observed at all heights between 0.5 and 5 m, but in one orchard the percentage of infected pruning wounds increased with height of the wound, whereas in the other orchard disease incidence was approximately the same for pruning wounds at heights between 1.5 and 3.5 m. The occurrence of pruning wound cankers was not uniform nor random throughout the orchards. During summer, cankers in these orchards ceased expansion and *P. syringae* could no longer be isolated from them. The fungus was consistently isolated from fallen almond leaves on the orchard floor.

724

PRODUCTION OF PYRENOPHORA TRITICI-REPENTIS PSEUDOTHECIA ON STRAW FROM RESISTANT AND SUSCEPTIBLE WHEAT CULTIVARS. B. L. Norman, W. W. Bockus, and P. J. Raymond, Department of Plant Pathology, Kansas State Univ., Manhattan, KS 66506.

After harvest, straw was collected from Tam 105 (susceptible) and Red Chief (resistant) plots which had been infected with tan spot during the season. After various treatments, the straw was placed in 1 m square plots (1190 g/plot) and monitored for numbers of pseudothecia produced. Treatments included autoclaved and nonautoclaved straw and straw sprayed (3 times), or not sprayed with *Pyrenophora tritici-repentis* conidia. There were 24.6% fewer pseudothecia on Red Chief straw in the fall; however, by the following spring, when mature ascospores were ejected, numbers of pseudothecia on Red Chief and Tam 105 were virtually identical. Results indicated that conidia could not initiate colonization of autoclaved straw to produce pseudothecia. Similarly, spraying infested, nonautoclaved straw with conidia did not increase pseudothecia number.

725

EFFECT OF TILLAGE TREATMENTS ON INCIDENCE OF CITRUS BLIGHT. D. O. Chellemi, M. Cohen, R. R. Pelosi, D. V. Calvert, and R. M. Sonoda, University of Florida, IFAS, Agricultural Research and Education Center, Ft. Pierce, FL 33454.

Incidence of citrus blight on rough lemon rootstock was recorded for three different tillage treatments over a seven-year period. Tillage treatments were replicated three times in 1-ha blocks and consisted of either surface tillage (ST) to 15 cm with surface liming of 2.24 mt/ha dolomitic limestone, deep tillage (DT) to 105 cm with the same liming as ST, and deep tillage plus lime (DTL) with 56 mt/ha of agricultural and coarse grade dolomitic limestone mixed to a depth of 105 cm. Blight first appeared 8 years after transplanting in the ST and DTL treatments and progression was linear in relation to time ( $r^2 = .93$  and  $.94$ ) with the annual rate of increase being similar (4.2 and 6.2%). In the DT treatment blight did not appear until 10 years after transplanting and increased at a very slow annual rate (.44%) until the thirteenth year after transplanting when it began to increase at rates similar to the other treatments.

726

INFLUENCE OF ATMOSPHERIC HUMIDITY ON SPORULATION OF *SPHAEROTHECA PANNOSA* VAR. *ROSAE*. K. Hural and D. L. Coyler, Dept. of Botany and Plant Pathology, Oregon State University, and USDA-ARS, Corvallis, OR 97331

The influence of atmospheric humidity on sporulation of *Sphaerotheca pannosa* var. *rosae*, which causes rose powdery mildew, was studied on leaf disks of susceptible cultivar Samantha. Leaf disks were inoculated on the adaxial surface with conidia of *S. pannosa* race 2, and incubated at 21° C for 2 days. The number of germinated conidia per disk was determined before exposing disks to 20, 50, 80 and 95% relative humidity (RH). RH was maintained with glycerol solutions inside insulated chambers held at 21° C. After 5 days the disks were removed and the number of conidiophores per disk was determined with an epi-illuminating microscope. Disks were rinsed with a 0.1% aqueous solution of Tween 20 and the number of conidia produced per disk was determined with a hemacytometer. At 95% RH the amount of sporulation was significantly greater than that at 20 and 50% RH.

727

MEASURING SPORULATION OF INDIVIDUAL LESIONS OF POWDERY MILDEW DISEASE ON TOMATOES WITH A PORTABLE SPORE SAMPLER. J. C. Correll, V. J. Elliott, and D. J. Jacobson, Department of Plant Pathology, University of California, Berkeley, CA 94720.

A portable spore sampler was used to assess sporulation of individual lesions of powdery mildew disease of tomatoes, caused by the fungus *Leveillula taurica*. Infections are first visible as small light green lesions which expand and become bright yellow. Some lesions may become necrotic. Lesions were classified by the degree of chlorosis and/or necrosis. The lesion size was measured and sporulation quantified. In general, sporulation per mm sq. is greatest when lesions are light green and less than 150 mm sq. Sporulation dropped off considerably as lesion size increased. The portable spore sampler provides a rapid non-destructive method of quantifying sporulation of individual lesions in the field. These data will aid in making a lesion development model for this disease.

728

MODIFICATION OF EPIMUL TO PERMIT CHANGES IN DISEASE DEVELOPMENT RATES. I. S. Hoang, P. S. Teng, and A. P. Roelfs, Dept. of Plant Pathology, and Cereal Rust Lab., USDA, ARS, Univ. of Minnesota, St. Paul, MN 55108

EPIMUL, a spatial and temporal computer simulation model, was modified and tested with field data from a Minnesota experiment. Wheat cultivars resistant or susceptible to *Puccinia graminis* f. sp. *tritici* race 15-TNM, were grown in various ratios. EPIMUL has adjustable variables for daily multiplication factor (DMFR) and latent period (NLPD) but they are fixed for the duration of an epidemic. These variables were modified to fit the rapidly changing conditions of a continental climate. A good fit was obtained by changing DMFR three times and NLPD twice during a 41 day epidemic. The single initial focus of EPIMUL was modified for multiple initial foci that occur with exogenous inoculum. The original section of host-pathogen compatibility was replaced by changing the number of sites available for infection as the proportion of the resistant cultivar was changed in the mixture.

729

MORISITA'S INDEX: A MEASURE OF SPATIAL PATTERN OF PLANT DISEASE. W. Schuh and M. J. Jeger, Department of Plant

Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

Various techniques based on quadrat sampling have been used to identify dispersion in plant, animal, and microbial populations. Several of these are utilized to analyze spatial pattern in plant diseases. Morisita's (Res. Popul. Ecol. IV:1, 1962) index, calculated by a binary series of quadrat sizes, is a measure of dispersion that is largely independent of the number of quadrats and the number of individuals per quadrat. The index can be used to determine a) whether dispersion is random, uniform, or clumped b) the intraclump distribution; and c) clump size at several hierarchical levels. Applications of the index have been made in many disciplines; but, with the exception of nematodes, few with respect to plant disease. Use of the index is illustrated by incidence of downy mildew (*Peronosclerospora sorghi*) infection in sorghum.

730

A MODEL FOR TREATING PEANUT WITH FUNGICIDE SPRAYS FOR MAXIMIZING POD YIELDS. R.H. Littrell, F.W. Nutter, Jr. and F.M. Shokes, Departments of Plant Pathology, Univ. of GA, Coastal Plain Station, Tifton 31793; Univ. of GA, Athens 30602; and Univ. of FL, Agricultural Research & Education Center, Quincy 32351.

A stripped plot design was used to study the timing effect of initiating and terminating 14-day fungicide schedules on pod yield. Spray schedules (using chlorothalonil at the recommended dosage) were initiated 25, 50, or 75 days after planting (DAP) and constituted main plots while subplot treatments consisted of spray termination dates of 75, 100, and 125 DAP. A surface-response curve was developed to estimate pod yield based on the timing and number of sprays. Maximum yields were obtained when sprays were initiated at 25 DAP and terminated at 125 DAP. Extending the spray schedule to 125 DAP provided the highest return when sprays were initiated at 50 DAP. The earlier spray schedules were initiated, the smaller the return from increasing the number of sprays. Thus, early season control may allow sprays to be omitted late in the season whereas schedules initiated in mid-season require more sprays to obtain near-maximum yields.

731

A POTATO PLANT GROWTH MODEL FOR EXPLORING THE NATURE OF THE EARLY DYING SYNDROME. Apple, J.D. and Overton, W.S., Dept. of Botany and Plant Pathology, Statistics, Oregon State University, Corvallis, OR 97331.

A model of a single potato plant was constructed from a general theory of plant growth processes and parameterized with field data collected during the 1983 and 1984 growing seasons. The model is decomposed into seven subsystems that require daily input during a 150 - 200 day season characteristic of the Columbia Basin potato production area. Subsystems that are responsible for normal physiological processes in the plant include, transpiration, photosynthetic production, mother tuber metabolism, and allocation of carbohydrates. Each of these subsystems integrates daily input variables with current states of the plant to determine plant behavior. An additional subsystem modifying plant behavior, referred to as physiological adaptation, is responsible for integrating external biotic and abiotic as well as internally generated plant stress. A subsystem translates the current status of each of the plant organs (biomass) into capacities to perform work based on the age and stress on the plant. The model was used to simulate early dying, a common disease in the Columbia Basin, by comparing direct effects of vascular tissue blockage caused by *Verticillium dahliae* with indirect effects through the physiological adaptation subsystem.

732

EUTYPA DIEBACK OF GRAPEVINE IN WASHINGTON STATE: DISEASE INCIDENCE AND EFFECT ON YIELD. D. A. Johnson and J. D. Lunden, Irrigated Agriculture Research and Extension Center, Box 30, Prosser, WA 99350

Commercial vineyards of *Vitis labrusca* 'Concord' in southcentral Washington were surveyed during the springs of 1983 and 1984 (134 vineyards sampled) for the incidence of Eutypa dieback. The mean incidence of Eutypa dieback increased in vineyards from 0.3 to 1.6 to 7.8 to 15.7% as the diameter of vines increased from <38 to 38-44 to 45-51 to >51 mm, respectively. The highest disease incidence observed in a vineyard was 76%. Sprinkler irrigation did not affect disease incidence. When yields of healthy and diseased vines were compared, total yield, number of clusters, and weight per cluster were significantly reduced on diseased vines. Yield components generally decreased as disease severity increased from moderate to severe. The mean yield loss was 75% with a range of 62 to 95% on vines with severe symptoms, and 41% with a range of 19 to 65% on vines with moderate symptoms of Eutypa dieback.

WORLD PLANT PATHOGEN DATABASE. M. H. Royer and W. M. Dowler, USDA, ARS, Plant Disease Research Laboratory, Fort Detrick, Building 1301, Frederick, Maryland 21701.

Increased travel, trade, and movement of people and plant parts throughout the world have increased the risk of importing unwanted plant diseases to countries in which the diseases have not yet been recorded. Researchers at the Plant Disease Research Laboratory are establishing a computerized database of important diseases on major crops throughout the world to enhance our ability to assess the potential threats to agriculture in the United States. Biology, epidemiology, geography, host range, meteorology, yield loss, genus and species, symptomatology, common names, control, and major references are being contributed voluntarily by researchers around the world. The information will be made available to the contributors so that the information obtained from different researchers who work on the same disease from around the world may be compared.

## 734

IMPACT OF FOLLAR DISEASES ON WHEAT YIELD IN LOUISIANA. Louis Anzalone, Jr., Dept. Plant Path. and Crop Physiol., La. Agric. Exp. Stat., La. State Univ. Agric. Ctr., Baton Rouge, LA 70803.

Portions of a naturally occurring disease syndrome on winter wheat were reduced or eliminated with repeated application of Indar, a selective control for leaf rust (LR), caused by *Puccinia recondita* f. sp. *Tritici*, or Tilt, a broad spectrum fungicide for control of LR and glume blotch (GB), caused by *Septoria nodorum* Berk. The mean annual percent yield reductions during 1983, 1984, and 1985 for unsprayed control plots compared to treated plots of three cultivars were 47% and 48% in McNair 1003 (susceptible to LR and GB) for Indar and Tilt treated plots, respectively, 21% and 40% in Coker 68-15 (moderately susceptible to LR and GB) for Indar and Tilt treated plots, respectively, and 1% and 29% in Florida 301 (resistant to LR and susceptible to GB) for Indar and Tilt treated plots, respectively. Apparently, LR and GB can cause significant wheat yield losses in LA with the amount of grain loss depending on the disease susceptibility of the cultivars grown.

## 735

DEVELOPMENT OF A WHEAT GROWTH MODEL FOR USE IN MEASURING DISEASE LOSS. I. S. Hoang, P. S. Teng, and A. P. Roelfs. Dept. of Plant Pathology, and Cereal Rust Lab., USDA, ARS, Univ. of Minnesota, St. Paul, MN. 55108

For practical yield loss assessment, a simple yet robust crop model is necessary. Robertson's factorial yield weather model (FYWM) estimated the yearly-base yield through summation of the products of several regression equations between yield and weather variables over a 50 year period. FYWM was modified to estimate the daily-base yield of wheat (Y), i.e. dry matter of a whole plant or tiller, at time (t). Each daily Yt is a product of the individual regressions of the t-1 values for yield and precipitation; maximum temperature; minimum temperature; and solar radiation. To obtain a unique solution for those equations the iterative computer program package (SAS NLIN procedure with Marquardt Method) was applied. A reasonable graphical fit of the model was obtained in 1984 with the cultivars Alex and Baart using the FYWM derived in 1983, from the cultivar Chris.

## 736

EFFECT OF DISEASE AND PLANT COMPETITION ON CROP YIELD IN MONOCULTURES AND MIXTURES OF A RESISTANT AND SUSCEPTIBLE WHEAT CULTIVAR. Helen Miller Alexander and A. P. Roelfs, Dept. of Biology, Univ. of Louisville, Louisville, KY 40292 and Cereal Rust Lab., USDA, ARS, Univ. of Minnesota, St. Paul, MN 55108

Both disease and plant competition can affect yield in cultivar mixtures. To examine these factors, seed of two morphologically distinct cultivars, one resistant and one susceptible to race 15-TNM of *Puccinia graminis* f. sp. *tritici*, were mixed in the proportions 0, 60, 80, 90, 99, and 100% resistant plants and sown in replicate plots, 30 x 36 m. A portion of each plot was inoculated and the rest was treated with a systemic fungicide. The rate of disease development and final disease levels were inversely related to the proportion of resistant plants present. Total yield/area was lowest in susceptible monocultures and highest in resistant monocultures. Yield of the resistant cultivar was depressed in mixtures compared to its performance in monocultures. Disease decreased the average

head weight of the susceptible cultivar, with the effect diminished in plots with a high proportion of resistant plants.

## 737

FACTORS AFFECTING THE ASSESSMENT OF DISEASE INTENSITY IN SIMULATED PLANT STRUCTURES. G. A. Forbes and M. J. Jeger, Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

Disease intensity at measured levels (1,5,10,25,50, and 75% area diseased) of morphologically different, simulated (sketched) plant structures was estimated by 12 people. Estimation error was analysed according to three models: a) absolute error, b) relative error, and c) error based on a logarithmic scale. The effects of assessor, plant structure, intensity, and the interactions intensity x assessor and intensity x structure were highly significant. Order of assessment had no effect. Absolute error was 2.6% overall and ranged from 0.3 to 15.9% among plant structures, and from 1.4 to 16.2% among assessors. All intensities were overestimated, except 75%, which was underestimated by 5.3%. Eight of 12 assessors overestimated intensity. The greatest absolute errors occurred at 25 and 50% intensities. The intensity x structure interaction was not due to natural groupings of plant structures. Lesions occurring as blotches were more accurately estimated than as small spots.

## 739

THE EFFECTS OF *PUCCINIA POLYSORA* ON GROWTH AND YIELD OF CORN IN PENNSYLVANIA AND MARYLAND. R. N. Raid, S. P. Pennypacker, and J. S. Melching\*. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, and (\*USDA-AR Plant Disease Research Laboratory, P.O. Box 1209, Frederick, MD 21701.

Field research was conducted during the 1983 and 1984 growing seasons to determine the effects of *Puccinia polysora*, the causal agent of southern corn rust, on growth and yield of corn. The experiment was conducted at two locations, Rock Springs, PA, and Frederick, MD. Disease foci were created by transplanting inoculated plants into 0.8 ha plots prior to pustule formation. Plant growth measurements and disease assessments were recorded weekly at specified distances from the primary inoculum source. Yield losses near foci were as high as 41 and 19 percent at Frederick and Rock Springs, respectively. Results indicate that environmental conditions favorable for significant yield losses due to southern corn rust existed in both Maryland and Pennsylvania.

## 740

QUALITATIVE ALTERATIONS IN PROTEIN SYNTHESIS DURING DIFFERENTIATION OF NODAL PLATE-DERIVED CALLUS FROM SORGHUM BICOLOR (L.) MENCH. C. A. Hozniak AND J. E. Partridge, Dept. of Plant Path., Univ. of Nebraska, Lincoln, NE 68583-0722.

Calli were derived from excised coleoptilar nodal sections of three-day-old sorghum seedlings and cultured on Murashige and Skoog medium (1962) as modified by Bhaskaran et al (1983). 2-4-D and Picloram were supplied as growth regulators at concentrations of 0-14µM and 0-12µM, respectively. Through the manipulation of growth regulator concentrations, various stages of differentiation were sampled for protein content. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of total protein detected patterns of synthesis which are



unique to organogenic or embryogenic tissues. Tissues which have apparently lost regenerative capabilities produce an abundant 27kd protein which increases in quantity with tissue age. Analysis of callus tissues through 2D-PAGE may prove to be a significant tool in ascertaining regenerative capabilities and provide an understanding of events surrounding organogenesis and somatic embryogenesis.

containing soil collected from rhizosphere of healthy and diseased citrus field trees showing symptoms of "declinio". Part of soil was previously autoclaved. Ten months after the transference, plants growing in the soil from the rhizosphere of diseased trees showed an intense leaf chlorosis when compared with the controls. Histological studies performed in root samples showed the presence of amorphous-like plugs in the xylem vessels only in chlorotic plants as well as the blockage in water uptake. Analysis of the trunk tissue for zinc accumulation did not show any difference between control and chlorotic plants. Results exclude the possibility of the presence of living microorganisms in the soil. However, this does not exclude the possibility of the presence of toxic substances in the soil samples from the rhizosphere of diseased plants. This possibility is under study.

#### 745

DETECTION OF PHYTOALEXIN ELICITORS FROM *ASPERGILLUS FLAVUS* USING A PHASEOLUS VULGARIS-COTYLEDON ASSAY. T. E. Cleveland, USDA, ARS, SRRR, P.O. Box 19687, New Orleans, LA 70179.

Biologically active molecules (e.g. phytoalexins, elicitors) may limit growth and infestation of plants by *A. flavus*. A bean cotyledon assay was utilized to identify elicitor-active fractions in *A. flavus* cultures. Experiments using this assay demonstrated that both the fungus and dialyzed, autoclaved culture filtrates elicited accumulation of kievitone, phaseollin and phaseollinisoflavan. Kievitone inhibited growth of *Cladosporium* sp. and *A. flavus* at 100 and 500  $\mu\text{g ml}^{-1}$ , respectively. Polygalacturonase (PGase) has been reported (Lee and West, 1981. Plant Physiology 67:633) to be a phytoalexin elicitor. Two PGases were purified by PAGE from *A. flavus* culture filtrates. Elicitor activities and other characteristics of these enzymes will be reported. Purified elicitors will be tested for activity in plants susceptible to infestation by this fungus.

#### 742

STUDIES OF THE PRIMARY INFECTIONS OF COFFEE PLANTS BY *HEMILEIA VASTATRIX*. Elza M.F. Martins and Walkyria B.C. Moraes. Plant Path. Dep. E. I. Biológico, CP 7119, 01000 São Paulo, SP, Brazil

Development of *Hemileia vastatrix* in coffee leaves of resistant (R) and susceptible (S) cultivars (cv) was monitored with microscopy. For both cvs, the stage of germination and appressoria formation were similar. From 11 to 22h after inoculation (ai) a dense, granular seething mass was observed beneath the tip of some appressorium. Substomatal vesicles (SV) were observed 20h ai and at 36h, 70% of all appressoria formed over stomata (infection site) showed SV in both cvs. Haustorial mother cells (HMC) were visible 32h ai in both cvs. Scv showed, at 40h ai, 50% of the infection sites with 1-2 HMC, while Rcv only 20%. Colonies with more than 5 HMC were observed 40h ai only in Scv. Haustoria were visible in the susceptible cells of Scv, 46h ai, and 4h later in Rcv. However, in Rcv haustoria were smaller than in Scv. A cell wall thickening was observed in epidermic host cells, like yellow fluorescent beads, 20h earlier in Rcv than in Scv. Subsidiary cells around the infection site collapsed 26h ai in same proportion in both cvs.

#### 743

CARBOHYDRATES FROM *HEMILEIA VASTATRIX* AND THE ELICITATION OF GLYCEOLLIN IN SOYBEAN. Sylvia D. Guzzo and Walkyria B.C. Moraes. Plant Biochemistry Dept. I. Biológico, CP 7119, 01000 São Paulo SP, Brazil

Preparations of filtrates of water-washes of autoclaved (FEA) and nonautoclaved (FEV) uredospores of *H. vastatrix* that induce protection in coffee plants and other host-parasite systems are able to elicit the accumulation of glyceollin in soybean cotyledons. Samples are rich in carbohydrates, mainly arabinose, glucose, mannose and galactose. Fractionation with EtOH showed different abilities for the fractions in eliciting glyceollin. The highest activities were found for FEA in the 80% fraction and for FEV in 20% and 80% ones. Further purification did not change the ability of fractions in eliciting glyceollin on inducing protection in coffee plants, suggesting the non-specificity of the mechanisms involved in both events. Further treatment with periodate showed the involvement of sugar residues in the non-specific mechanisms. Further studies are being undertaken.

#### 744

CHLOROTIC SYMPTOMS IN LEAVES OF CITRUS PLANTS GROWING IN POTS WITH SOIL FROM ORCHARDS WITH "DECLINIO". M. Julia G. Beretta, Mônica Fogaça and Walkyria B.C. Moraes. Plant Biochemistry Dept. Instituto Biológico, CP 7119, 01000, São Paulo, SP, Brazil

Two years old healthy citrus plants were transferred to pots

#### 746

PEROXIDASE ISOZYMES ASSOCIATED WITH ROOTS OF *PHASEOLUS VULGARIS*. Frederick G. Albert and Anne J. Anderson, Department of Biology, Utah State University, Logan, UT 84322.

Characterization of peroxidase activities associated with bean roots has demonstrated an intense activity at the root surface. This root surface activity decreased with age of the plant whereas peroxidase in the rhizosphere increased. Native PAGE demonstrated differences in the isozymes present from a wash of intact sterile roots compared with an homogenate of sterile roots. Washes displayed 2 fast moving cathodic bands whereas homogenates possessed 8 additional forms. Additional bands were observed also in washes prepared for greenhouse-grown plants which had extensive colonization by saprophytic pseudomonads. Molecular sizing demonstrated the peroxidase in bean washes to coelute with an agglutinin which may be involved in root welfare. This agglutinin recognized cells of an aggressive bacterial root colonizer *P. putida*. Additional techniques which have separated the agglutinin from the peroxidase, indicated that these activities reside in different structures.

#### 747

INHIBITORY EFFECTS OF EXTRACELLULAR PRODUCTS FROM *COLLETOTRICHUM LINDEMUTHIANUM* ON TONOPLAST FUNCTION. H.M. Griffiths,<sup>1</sup> C.S. Tepper,<sup>1</sup> D.P. Briskin<sup>2</sup> and A.J. Anderson.<sup>1</sup> <sup>1</sup>Department of Biology and <sup>2</sup>USDA/ARS Utah State University, Logan, UT 84322.

Extracellular fungal components from the  $\alpha$  race of the bean pathogen, *Colletotrichum lindemuthianum* inhibited proton uptake and membrane charge formation in sealed vesicles prepared from bean hypocotyls. Inhibition of these ATP-dependent functions by nitrate but not by vanadate suggested the vesicles were enriched for tonoplast. Purification of fungal components by ion exchange and molecular sizing separated fractions active and inactive as inhibitors of tonoplast function. Several fractions, with different carbohydrate to protein ratios, inhibited both proton uptake and membrane charge formation. Similar inhibition was observed with vesicles from incompatible (Dark Red Kidney) and compatible (Great Northern) stems. These effects, of defined fungal products on tonoplast function, may play a role in plant cell necrosis that occurs upon challenge of bean by incompatible or compatible *C. lindemuthianum* races.

#### 748

EFFECT OF CELL WALL FRAGMENTS RELEASED BY PECTATE LYASE ON THE HYPERSENSITIVE RESPONSE IN TOBACCO TISSUE. C. J. Baker, USDA,

Pectate lyase was purified to electrophoretic homogeneity from periplasmic shock fluids of an *Escherichia coli* strain containing a cloned isozyme (pi 8.3) gene from *Erwinia chrysanthemi*. Pretreatment of tobacco leaf tissue with this enzyme (less than 0.1 umole reaction product produced/min/ml) prevented the hypersensitive response (HR) to *Pseudomonas syringae* pv. *tabaci* and also inhibited bacterial multiplication. To determine if enzyme reaction products were involved, purified tobacco cell walls were treated with similar levels of enzyme at pH 6.0. Heat stable factors were released which also prevented the HR when infiltrated 3-24 hr. prior to bacterial inoculation. Heat-inactivated enzyme or cell walls not treated with enzyme did not produce this effect.

749

THE EFFECT OF POSTHARVEST INFILTRATION OF CALCIUM, MAGNESIUM, OR STRONTIUM ON DECAY, FIRMNESS, RESPIRATION, AND ETHYLENE PRODUCTION IN APPLES. W. S. Corway, USDA, Agricultural Research Service, Horticultural Crops Quality Lab, Beltsville, MD 20705 and C. E. Sams, Dept. of Plant and Soil Science, Univ. of Tennessee, Knoxville, TN 37996.

'Delicious' and 'Golden Delicious' apples were treated at harvest with the chloride formulations of three cations, calcium (Ca), magnesium (Mg), and strontium (Sr), by pressure infiltration (68.95 kPa). After 5 mo storage at 0 C, the fruits were removed and wound inoculated with a conidial suspension of *Penicillium expansum*, and stored for 7 days at 20 C. Fruits were then rated for decay severity, ethylene production, respiration, firmness, injury, and analyzed for the content of the appropriate cation. It was found that Ca was the optimum cation for reducing decay, maintaining fruit firmness, suppressing ethylene production, and causing the least amount of injury. Cation treatments had little effect on respiration, and Mg caused the most injury to the fruit surface.

750

CYTOPATHOLOGY OF *XANTHOMONAS CAMPESTRIS* PV. *CITRI* FROM FLORIDA AND MEXICO. R. H. Lawson, M. D. Brannigan, E. L. Civerolo. USDA-ARS, FNC Lab and Fruit Lab, Beltsville, Md. 20705.

A Florida isolate (F1) of *Xanthomonas campestris* pv. *citri* produced water-soaked lesions with chlorotic halos at sites of wound inoculation on detached leaves of Mexican lime, *Citrus aurantifolia*. Epidermis surrounding the wound site of 1 and 2 wk infections was similar to healthy controls in scanning electron microscopy (SEM). Transmission electron microscopy (TEM) revealed bacteria in stomates and intercellular spaces in association with fibrillar material. Organisms were confined to water-soaked and adjacent chlorotic tissue. Bacteria were observed intracellularly as cell walls degraded. A Mexican isolate (14B) produced brown leaf pustules composed of hyperplastic host cells erupting through the epidermis with bacteria in stomates and intercellular spaces. The occurrence of intracellular bacteria and water-soaking in the absence of pustule formation distinguishes the cytopathology of the Florida and Mexican isolates.

752

ROLE OF ZINC IN TABTOXIN PRODUCTION BY PATHOVARS OF *PSEUDOMONAS SYRINGAE*. R. D. Durbin and T. F. Uchytel, USDA, ARS, Department

Tabtoxin-producing strains of *Pseudomonas syringae* were grown in Woolley's medium to mid-log phase (48 h), and tested for their ability to hydrolyze tabtoxin by the action of a Zn metalloprotease in the periplasmic gel. Analysis after 1 h incubation with 1  $\mu$ M Zn showed: 11 *tabaci*, 5 *coronafaciens* and 3 *tabaci* (*angulata*) strains required Zn for activity; 2 *tabaci* and 2 *coronafaciens* strains could not hydrolyze tabtoxin even with Zn; and 5 *tabaci* (*angulata*) strains did not require Zn for hydrolysis. Pathovar *tabaci* (ATCC 11528) in Woolley's medium with 1  $\mu$ M Zn grew identically to Zn-minus controls; only tabtoxinine- $\beta$ -lactam accumulated, whereas only tabtoxin accumulated in the controls. In inoculated plants only tabtoxinine- $\beta$ -lactam was found with strains whose peptidase were activated by Zn; however a mixture of tabtoxin and tabtoxinine- $\beta$ -lactam was found with strains unable to hydrolyze tabtoxin. The presence of both chemical forms of the toxin has implications for site-of-action studies since each could have a separate target.

753

EXTRACELLULAR PROTEINS ASSOCIATED WITH INDUCTION OF DIFFERENTIATION IN BEAN RUST UREDOSPORE GERMINATIONS. Epstein, L. L., Laccetti, L. L., Staples, R. C., Hoch, H. C., and Hoese, W. A. Boyce Thompson Institute, Ithaca, NY 14853 and NY State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

The bean rust fungus [*Uromyces appendiculatus* (Pers.) Unger] has a contact-sensitive mechanism to recognize the leaf stomata. Stomatal recognition is followed by mitotic nuclear division and the differentiation of the first infection structure, the appressorium. Nuclear division of germlings incubated on a normally inductive scratched surface was significantly reduced by either Pronase E or trypsin, but not by heat denatured enzymes, trypsin mixed with trypsin inhibitor or several other enzymes. Pronase E significantly reduced adhesion of the germlings to the substrate, but did not decrease rates of germination or germ tube elongation. These data suggest that proteins in the extracellular matrix may be involved in the thigmotropic response.

754

INFECTION OF OATS BY VIRULENT AND AVIRULENT ISOLATES OF *HELMINTHOSPORIUM VICTORIAE*. Penelope Hanchey, Dept. Plant Pathology and Weed Science, Colo. State Univ., Fort Collins, CO 80523.

Three oat cultivars susceptible to Victoria blight were inoculated with a virulent or avirulent isolate of *H. victoriae* and examined by epifluorescence or electron microscopy. After 24h, autofluorescent, epidermal wall appositions were twice as frequent beneath appressoria of the avirulent isolate compared with those of the virulent isolate. Apposition fluorescence increased after aniline blue staining. Small aniline blue-positive appositions were found in the mesophyll of plants infected with the virulent isolate. The earliest ultrastructural effect in susceptible interactions was a separation of the membrane from the wall and the appearance of dense material between the wall and membrane. This effect, which resembles that found with victorin, occurred prior to penetration. Less response was found with the avirulent isolate, but after 48h, hyphae were autofluorescent and the size and number of epidermal wall appositions exceeded those found with the virulent isolate.

755

RACE DISTRIBUTION OF *RHYNCHOSPORIUM SECALIS* ON BARLEY COMPOSITE CROSS POPULATIONS AND PURELINE CULTIVARS IN CALIFORNIA. E. A. Crandall, L. F. Jackson and R. K. Webster\*. Departments of Agronomy and Range Science and Plant Pathology\*, University of California, Davis, CA 95616.

Variability of the causal agent of barley scald, *Rhynchosporium secalis*, was evaluated for three years in three locations in California on five composite cross populations (CC 11 F26 and F48; CC V F24 and F33; CC XXI F17) and three pureline cultivars (Prato, CM 72, UC 566) of barley. Composite cross populations were advanced one generation per year and replanted at the location where they were produced. The frequencies of naturally occurring races were compared between populations grown under differential disease pressures provided by varied environmental conditions. These comparisons were made by classifying races as simple, intermediate or complex based on the disease reactions of fifteen differential cultivars. Differences were found between pureline cultivars and composite populations and between populations advanced at different locations.

HISTOPATHOLOGY OF WOUNDED ALMOND BARK IN RELATION TO INFECTION BY *CERATOCYSTIS FIMBRIATA*. G. E. Middleton and R. M. Bostock. Department of Plant Pathology, University of California, Davis, CA 95616

*Ceratocystis fimbriata* Ellis & Halst. is a wound invading fungus which causes perennial cankers in stone fruit trees damaged by harvesting equipment. Bark wounds made in 'Non Pareil' almond trees became resistant to infection by spores of *C. fimbriata* within 10-14 days after injury. Frequency and size of cankers declined significantly as the time interval between wounding and inoculation increased. The development of complete resistance was correlated by histochemical analyses to deposition of lignin-like and suberin-like material exterior to the zone of meristematic activity. However, partial resistance was observed 2 to 4 days after injury and prior to detection of lignin or suberin, which suggests that additional factor(s) contribute to wound resistance. These studies indicate that protective treatments must be applied to fresh wounds and remain effective for 10 to 14 days after wounding.

## 757

DIFFERENTIAL RECOGNITION OF COLLETOTRICHUM LINDEMUTHIANUM ELICITORS IN COMPATIBLE AND INCOMPATIBLE BEAN CULTIVARS. Craig S. Tepper and Anne J. Anderson, Dept. of Biology, Utah State University, Logan, UT 84322.

Cell wall  $\beta$  glucans from *Colletotrichum lindemuthianum* are potent elicitors, yet they cannot explain race-cultivar specificity. However, certain extracellular components from the  $\alpha$  race may demonstrate cultivar-specific elicitor activity. Purification of  $\alpha$  race extracellular components, by a variety of chromatographic techniques, has revealed distinct types of elicitor structures. These elicitors at concentrations less than  $10^{-6}$  M protein and carbohydrate trigger the production of low molecular weight phenolics in the incompatible Dark Red Kidney. One class of elicitor which does not adsorb to DEAE Sephadex or CM Sephadex has been characterized as a galactoglucomannan. This galactoglucomannan displays high levels of elicitor activity when assayed for the production of low molecular weight phenolics on the incompatible Dark Red bean cultivar but no elicitor activity on the Great Northern bean cultivar.

## 758

A PROFILE OF PHYTOALEXIN EXPRESSION IN THE COTTON LEAF. Hampden J. Zeringue, Jr., USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Boulevard, P.O. Box 19687, New Orleans, Louisiana 70179.

A ten-day time-course study of the production of five autofluorescent compounds, (lacinilene C, lacinilene C 7-methyl ether, scopoletin, 2-hydroxy-7-methoxycadalene, and 2,7-dihydroxycadalene) in the cotton leaf induced by cell-free mycelial extracts of *Aspergillus flavus* showed that the lacinilenes and the cadalenes accumulate in a cyclic fashion. The initial increase at two days is followed by greater increases at six days after treatment. It will also be demonstrated that these five autofluorescent compounds are either localized in the 6 mm diameter treated area or in a 3 mm area immediately surrounding the 6 mm treated area of the leaf.

## 760

COMPARATIVE IMMUNOLOGICAL STUDY OF CUCUMBER, MUSKMELON AND

WATERMELON PEROXIDASE ISOZYMES ASSOCIATED WITH INDUCED RESISTANCE. J. A. Smith and R. Hammerschmidt. Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824-1312.

Induced resistance in cucurbits is accompanied by a marked increase in intercellular peroxidase (PO) isozymes. The PO isozymes are low molecular weight (ca. 20-30 kd) glycoproteins. Antibodies raised against cucumber PO isozymes were tested for their cross reactivity with intercellular PO isozymes induced in muskmelon and watermelon. An ELISA assay was used to quantitate cross reactivity of anti-cucumber peroxidase with muskmelon and watermelon PO isozymes. ELISA absorbance values with muskmelon and watermelon PO were, respectively, 46% and 33% of cucumber values. Likewise, muskmelon and watermelon PO produced precipitation patterns of partial identity with cucumber when tested in the Duchterlony double diffusion assay. The use of antibodies for *in vivo* quantitation and ultrastructural localization of PO will also be discussed.

## 761

QUANTITATION AND LOCALIZATION OF EXTENSIN BY IMMUNOCHEMICAL METHODS. T. A. Conrad, R. Hammerschmidt and D.T.A. Lampert. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

The role of extensin, a cell wall protein, in induced resistance, is being investigated by immunochemical methods. After production of an antibody against the glycosylated and deglycosylated forms of extensin precursor, a competitive indirect enzyme immunoassay was developed. After adsorbing purified precursor on microtiter plates, antibody and sample are incubated therein. Bound antibody is determined by subsequent incubation with an enzyme labeled secondary antibody followed by chromogenic substrate. The assay can detect purified precursor at 100 pg/ml and is being used for quantitation of soluble extensin in cucumber seedlings following resistance induction by heat shock. Fluorescent labeled antibody is also being used to localize extensin in these seedlings.

## 762

SURVEY OF VIRULENCE IN PERONOSCLEROSPORA SORGHII ISOLATES FROM INDIA, ETHIOPIA, NIGERIA, TEXAS (USA), HONDURAS, BRAZIL, AND ARGENTINA. M. N. Pawar, R. A. Frederiksen, TX. Agric. Exp. Sta., College Station, TX 77843; L. K. Mughogho, ICRISAT, Patancheru (P.O.), A. P. 502, India, and M. R. Borde, PDRL, Frederick, MD 21701.

Sixteen worldwide isolates of *Peronosclerospora sorghii* were tested for virulence on 75 sorghum cultivars. These cultivars were previously identified as resistant to three pathotypes from Texas and to some regional populations of *P. sorghii* in a multilocal screening program of ICRISAT for sorghum downy mildew resistance. All isolates were identified as distinct pathotypes on the basis of their differential sporulation on the host cultivars. The host-pathogen interactions were consistent with a gene-for-gene hypothesis. Isolates from India, Nigeria, and Ethiopia attacked a greater number of sorghum cultivars which remained resistant to other isolates. Sorghum cultivars QL-3 India, IS1032, IS2473, IS3546, IS14332, IS14387, IS22227, IS22228, IS22229, IS22230 were identified as resistant to all isolates tested.

## 763

CALCIUM OXALATE CRYSTALS IN THE CULTURES OF *MYCENA CITRICOLOR*, CAUSAL AGENT OF THE AMERICAN LEAF SPOT OF COFFEE. D.V. Rao, J.P. Tewari, C. DuFresne, and W.A. Ayer, Depts. of Plant Science and Chemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5.

Light and scanning electron microscopy revealed numerous prismatic crystals along the hyphae of *Mycena citricolor* growing in potato-dextrose agar and potato-dextrose broth. Pure samples of these crystals were isolated. They were shown to be composed of calcium oxalate based on their shape, solubility, SEM X-ray energy dispersive microanalysis, carbon-13 nuclear magnetic resonance spectroscopy and fast atom bombardment mass spectrometry. The oxalic acid yields were in the range of 0.5-1 g/L of broth as determined by permanganate titration. Sequestering of calcium as oxalate suggests that oxalic acid may be playing a key role in the pathogenesis by *M. citricolor*.

## 763a

UNIQUE STRAIN OF *BOTRYOSPHERA DOTHIDEA* INVOLVED IN PEACH TREE

FUNGAL GUMMOSIS. P. L. Pusey, C. C. Reilly and W. R. Okie, USDA-ARS, S.E. Fruit & Tree Nut Res. Lab., Byron, GA 31008.

Wound inoculations of peach trees (*Prunus persica*) involved *Botryosphaeria dothidea* isolates from peach inside (PI) and outside (PO) the reported peach fungal gummosis area and from non-peach hosts inside (NI) and outside (NO) the gummosis area. All isolates induced gumming; however, observations for longer than one year revealed that 12 of 13 PI isolates continued to induce gumming at a high level, whereas gum exudation had ceased or was not different ( $P=0.05$ ) from the uninoculated check for 10 NI isolates (except three from *Prunus* spp.), 5 NO isolates and 1 PO isolate. The PI isolates and some NI isolates increased tree death and caused lenticel-associated symptoms. Peach isolates of *B. obtusa* and *B. rhodina*, included for comparison, caused the same general response of peach as did *B. dothidea* isolates from non-peach hosts. Results indicate that *B. dothidea* isolates from peach trees with typical gummosis symptoms represent a new pathogenic strain of the fungus.

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CHLORATE RESISTANCE IN *MACROPHOMINA PHASEOLINA* OBTAINED FROM CORN, SOIL, AND SOYBEAN. C. A. S. Pearson, J. F. Leslie, and F. W. Schwenk, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Chlorate ( $\text{ClO}_3^-$ ) is a nitrate analog. *Macrophomina* isolated from soybean field soil, or from corn or soybean tissue, was grown on defined medium with and without 120  $\mu\text{M}$   $\text{KClO}_3$ . Growth was scored after 7 days at 30 C. Corn isolates were chlorate resistant and grew normally, producing numerous dark sclerotia on medium containing  $\text{KClO}_3$ . Isolates from soil and soybean tissue were chlorate sensitive. Sensitive isolates were divided into two classes based on growth on the chlorate medium. One class of sensitive isolates grew sparsely with a feather-like sclerotial pattern while radial growth of the other class was almost completely restricted. *Macrophomina* isolates from corn and soybean appear to differ in their ability to utilize certain nitrogenous compounds. Such differences might reflect metabolic capabilities which could lead to host specialization within this genus.

765

PURIFICATION OF A TOXIN FROM CULTURE OF *STEMPHYLIUM BOTRYOSUM* PATHOGENIC ON ALFALFA. Dana Kelly Heiny and David G. Gilchrist, Department of Plant Pathology, University of California, Davis, California 95616.

Isolates of the cool-temperature biotype of *Stemphylium botryosum* Wallr., causal agent of Stemphylium leafspot of alfalfa, produce a phytotoxic molecule when cultured in a defined liquid medium. Culture filtrates purified by gel filtration and/or chromatofocusing cause necrosis resembling Stemphylium leafspot when injected into alfalfa leaflets. The molecular weight of the toxic component estimated by gel filtration is approximately 19,500. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of the purified fractions with toxic activity revealed two major protein bands ( $M_r=19,500$ ; 26,900). The biological significance of these proteins in this disease is being investigated via attempts to produce monospecific antibodies for quantification and localization of toxin in culture and infected tissue.

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EFFECT OF TRIAZINE HERBICIDES ON APOTHECIAL DEVELOPMENT IN *SCLEROTINIA SCLEROTIUM*. W.L. Casale and L.P. Hart, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The specific effects of several herbicides on the development and morphology of apothecia of *S. sclerotiorum* were examined. The percentage of sclerotia germinating carpo-genically in soil containing 4  $\mu\text{g}$  to 10 mg atrazine/g or in 10-50  $\mu\text{M}$  atrazine solution was the same as that in controls without atrazine, but stipes were distorted, and only infertile, abnormally-shaped apothecial discs were produced. Numerous stipes grew from the aborted hymenia of immature apothecia soaked in 50  $\mu\text{M}$  atrazine for 30 min. Metribuzin at 500  $\mu\text{g}/\text{g}$  soil completely prevented carpo-genic germination, but normal apothecia developed at lower concentrations. These herbicides did not affect mycelial growth rate on water agar containing 50  $\mu\text{g}/\text{ml}$ .

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EFFECT OF GLUCOSE ON THE ACCUMULATION OF AMMONIUM IN CULTURES OF

*BIPOLARIS MAYDIS* RACE T. T. W. Bigschoff and M. O. Garraway, Dept. Plant Path., The Ohio State Univ., Columbus, OH 43210.

To examine the effects of glucose on the accumulation of ammonium in cultures of *Bipolaris maydis* race T, we incubated the fungus in a mineral salts liquid medium with 2 g/l glucose and 4 g/l L-asparagine (L-asn) for 48 hr at 28 C on a reciprocal shaker (100/min) and transferred the mycelium to fresh media containing 2 or 5 g/l glucose with or without L-asn. Twenty-four hrs after transfer ammonium levels were 13.1 and 0.6  $\mu\text{moles}/\text{ml}$  with L-asn and 2 or 5 g/l glucose while no ammonium was detected in cultures lacking L-asn. Forty-eight hrs after transfer ammonium levels increased and were 23.0 and 13.1  $\mu\text{moles}/\text{ml}$  in cultures with L-asn and 2 or 5 g/l glucose while no ammonium was detected in cultures lacking L-asn. Glucose was no longer detected either in cultures with L-asn and 2 g/l glucose 24 hrs after transfer or in cultures with L-asn and 5 g/l glucose 48 hrs after transfer. The data indicate that ammonium production on media containing L-asn may be related to the depletion of glucose from the culture medium by *B. maydis* race T.

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UTILIZATION OF PECTIC SUBSTANCES BY *CYTOSPORA CINCTA* FROM PEACH. Elke Endert-Kirkpatrick and David F. Ritchie, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Five strains of *Cytospora cincta* ranging from low to high virulence were tested for production of tissue-macerating enzymes in broth culture. Oxalic acid, pectinolytic enzymes, and cellulolytic enzymes were quantified in cell-free culture filtrates by permanganate titration, carrot disc maceration, and cellulose agar clarification, respectively. All strains produced equivalent amounts of oxalic acid and pectinolytic enzymes, whereas none of the strains produced cellulolytic enzymes. Pectinolytic enzymes were not detected when pectic substances were omitted from the culture medium. Growth of two highly virulent strains on 12 carbon sources within a carbon-free synthetic medium indicated that maximum mycelial growth and pycnidial formation occurred with citrus pectin and tannic acid. Sodium polypectate and polygalacturonic acid supported less growth than citrus pectin but were better carbon sources than sugars or polysaccharides.

769

OOSPOROGENESIS OF *PHYTOPHTHORA CAPSICI*. J. Y. Uehida and M. Aragaki, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Oospore formation by *Phytophthora capsici* Leonian was studied with 30 isolates obtained from solanaceous and non-solanaceous hosts. Of the 30 isolates, 4 belonged to the A1 compatibility type, 7 were A2 and 19 were infertile or A0. Oospore production varied among the 11 fertile isolates from abundant to very few. Seven of the fertile isolates produced a few oospores in unpaired (solo) cultures. An interaction between light and temperature on oosporeogenesis was observed. Inhibition of oospore formation by light was found to be temperature dependent. At 16 C, effect of light through the spectrum 290 to 750 nm was minimal, but at 24 C there was pronounced inhibition in the blue zone (440-470 nm). Oospore formation was inhibited at 31 C, independent of light treatments.

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GENETICS OF *USTILAGO HORDEI*: GENE CONTROL OF FUNGICIDE RESISTANCE. Carol E. Henry, Bethsheba Bullock, Vonda M. Gaines, Felix Onyiah, Department of Biological Sciences, Chicago State University, Chicago, Illinois 60628.

Spontaneous, UV and chemically induced mutants of *Ustilago hordei* (Pers.) Lagerh. were characterized for fungicide resistance by plating sporidia at high density ( $10^4$ ) using the gradient plate technique of Szybalski and Bryson in combination with Vogel's complete medium and the fungicides chloroneb and thiabendazole. The maximum non inhibitory concentrations of the mutants were determined. These mutants were then used to determine gene control of fungicide resistance by the teliospore test. Various wild type mutant sporidial matings when inoculated into barley seeds (*Hordeum vulgare* L.) resulted in heterozygous diploid teliospores in smutted host inflorescences. Teliospore analysis indicated that fungicide resistance is polygenic and controlled by dominant, semi-dominant and recessive genes.

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FUNCTIONAL ANALYSIS OF THE ADXIN-INDEPENDENCE GENES, SEQUENCE

ANALYSIS OF THE CYTOKININ-INDEPENDENCE GENE OF AGROBACTERIUM PLASMID pTiAg63, AND THE RELATIONSHIP OF THESE LOCI TO HOST RANGE. R.R. Carlson, W.G. Buchholz, R.E. Hurlbert, H.I. Malkawi, and M.F. Thomashow. Dept. of Bacteriology, Washington State University, Pullman, WA 99164-4340.

The host range of A. tumefaciens Ag63 is very narrow and is a function of its Ti plasmid, pTiAg63. Insertion of the pTiA6 cytokinin-independence gene into the pTiAg63 T<sub>B</sub>-DNA results in a Ti plasmid which encodes a host range expanded relative to pTiAg63. This suggested that the pTiAg63 cytokinin-independence gene was not functional in all hosts. DNA sequence analysis revealed a deletion in this gene. This accounts for part, but not all, of the host range limitations of pTiAg63 because addition of the cytokinin locus did not fully expand the host range. We reasoned that the pTiAg63 auxin-independence genes might also limit host range. This was not the case since substitution of them for the corresponding genes of pTiA6 did not affect the host range phenotype of pTiA6.

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A CUBIC MODEL FOR PARASITE:HOST:ENVIRONMENT SPECIFICITY STUDIES  
L. E. Browder and T. M. Shehab Eldin, USDA-ARS, Plant Pathology, KSU, Manhattan, KS 66506

Previously used concepts of modeling parasite:host specificity can be expanded to also account for the effect of variation in environment. A three-dimensional, cubic model that accounts for binary variation in these factors in relation to one set of corresponding gene pairs is suggested. This model expresses the premise that a definitive parasite genotype interacting with a definitive host genotype in a definitive environment results in a definitive aegricorpus phenotype. Within this premise, certain phenotype-data configurations logically indicate differences in parasite genotype, host genotype, or specificity of aegricorpus genotypes to environment. This logic can be used to hypothesize genetic relationships of parasites and hosts by sequentially analyzing subsets of phenotype data from 2 parasites, 2 hosts, and 2 environments within datasets from large experiments. Considering specificity of aegricorpus genotype to environment also may provide improved control strategies.

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USE OF DRY BEAN CANOPY ARCHITECTURE COMPONENTS TO PREDICT WHITE MOLD DISEASE POTENTIAL. C.L. Campbell, J.R. Steadman, and D.P. Coyna. Dept. of Plant Path., University of Nebraska, Lincoln, NE 68583-0722.

Eighteen Phaseolus vulgaris cultivars were compared using a simple rating system developed to quantify and define four major canopy components: vining, growth habit, tunnel formation between rows, and canopy density. There was a positive correlation between canopy architectural ratings and severity of white mold disease caused by Bclartotinia sclerotiorum. Prediction of white mold disease potential using architectural ratings is more difficult for cultivars which exhibit high susceptibility or resistance to the pathogen.

777

A COMPARISON OF TECHNIQUES FOR SCREENING SOFT WHITE SPRING WHEAT FOR BLACK POINT RESISTANCE. K. L. Connor and Thomas, J. B., Agriculture Canada Research Station, Lethbridge, Alberta, Canada T1J 4B1.

Field tests of soft white spring wheat lines have identified several sources of black point resistance of which line SWS15 appeared to be the most resistant. Comparisons of inoculation techniques using an isolate of Alternaria alternata demonstrated that needle inoculation or vacuum infiltration provided the most reliable means of screening for black point resistance. Inoculation treatments such as dips or sprays gave low, inconsistent results, which did not allow the identification of resistant lines. Inoculation prior to anthesis tended to produce lower levels of black point discoloration than inoculations at anthesis or the mid-dough stage. Black point severity was not influenced by spore concentrations between  $10^4$  to  $10^5$  spores/mL, but at higher concentrations ( $5 \times 10^5$  spores/mL) black point severity was reduced.

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ASSESSMENT OF RESISTANCE TO PEACH TREE FUNGAL GUMMOSIS. K. O. Britton, F.F. Hendrix, Dept. of Plant Pathology, Univ. of Ga., Athens, GA 30602. P.L. Pusey, C.C. Reilly, W.R. Okie, U.S.D.A. Fruit and Tree Laboratory, Byron, GA 31008 and J.W. Daniell, Dept. of Horticulture, Univ. of Ga. Agric. Expt. Sta., Experiment, GA 30212.

Two field tests assessed resistance of peach cultivars to fungal gummosis caused by Botryosphaeria spp. Both naturally-occurring and wound-inoculated trees were rated (0-4 scale) for gum exudation. Inoculated twigs were removed and plated in serial sections on acidified potato dextrose agar to measure fungal colonization rates, and the extent of vascular discoloration. Cultivars 'Summergold' and 'Winblo' ranked high on gum exudation but were colonized at low rates compared to other cultivars. Vascular discoloration was more extensive in cultivars prone to gum. Results suggest that neither gum ratings nor fungal reisolation rates accurately assess the complex host-parasite interaction in peach tree gummosis individually.

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ACTIVE RESISTANCE OF CABBAGE TO *XANTHOMONAS CAMPESTRIS* PV. *CAROTAE* AND PROTECTION AGAINST BLACK ROT AGENT, PV. *CAMPESTRIS*, BY CO-INOCULATION. Douglas R. Cook and David J. Robeson, ARCO PCRI, 6560 Trinity Ct., Dublin, CA 94568-2685

When the compatible pv. *campestris* and incompatible pv. *carotae* were inoculated into host tissues, initial growth rate of each pathovar was similar. However after 2-3 days, growth of pv. *carotae* ceased and the viable population remained constant. Cessation of growth was accompanied by the development of localized necrosis. In contrast, growth of the compatible pv. *campestris* continued logarithmically, necrosis was not apparent after 2-3 days, but instead spreading lesions developed after 7 days. Co-inoculation of compatible pv. *campestris* and incompatible pv. *carotae*, resulted in an incompatible reaction. Active resistance was also induced by injection of heat-killed bacteria of the incompatible pv. *carotae*, which afforded localized protection against challenge with the living compatible pathogen. The results are consistent with the specific elicitor-specific receptor hypothesis of biochemical recognition.

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INDUCED RESISTANCE TO *PERONOSPORA TABACINA* IN SUCKERS AND ROOT-ED SHOOT APICES OF TOBACCO. Yigal Cohen and Sara Peer, Bar-Ilan University, Israel 52100.

Tobacco plants (cvs. Ky 16 and Burley 21) were protected against blue mold incited by *P. tabacina*, by injecting conidial suspension of the pathogen to the lower part of the stem or by pouring conidial suspension onto the soil surface around the stem base. Suckers developed on protected plants were mostly free of stem necrosis and exhibited full susceptibility to a foliage challenge with the pathogen. Plants developed from rooted stem apices taken from protected plants were also susceptible to foliage infection with *P. tabacina*. We hypothesized that foliage resistance in stem-infected tobacco plants results from disturbed source-sink relationships.

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RESPONSE OF BEAN CALLI TO FILTRATE FROM *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* AND COMPARISON TO WHOLE PLANT DISEASE REACTION. C. L. Hartman, G. A. Secor, J. R. Venette, and D. A. Albaugh, Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Callus cultures of nine bean (*Phaseolus vulgaris* L.) cultivars were tested for response to a toxic filtrate from liquid cultures of *P. syringae* pv. *phaseolicola*. Callus reaction to the filtrate ranged from totally necrotic with no growth to no necrosis and normal growth. Plants from each of the nine bean cultivars were inoculated in the greenhouse with a bacterial suspension of the pathogen. Charlevoix (red kidney) had the most severe symptoms in the greenhouse, and the calli were severely affected (necrosis, no growth) by the filtrate. Emerson (great northern) was resistant to the pathogen in the greenhouse, and the calli developed no necrosis on medium containing filtrate. Results suggest that a callus screening system could identify bean cultivars resistant to halo blight.

783

COMPARATIVE RESPONSE OF POTATO LEAVES, TUBER TISSUE AND STEM CALLUS TO CULTURE FILTRATES OF *VERTICILLIUM* SPP. C. L. Hartman and G. A. Secor. Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Determination of disease response of existing and new cultivars is often difficult to achieve. Challenge of various tissues *in vitro* with culture filtrates offers a potential system for determination of disease response. Excised potato leaves, tuber pieces and stem callus were tested for response to filtrates of *Verticillium dahliae* (Vd) and *Verticillium albo-atrum* (Vaa). Leaves were either sprayed with broth cultures or

allowed to imbibe culture filtrates of Vaa and Vd. Stem callus cultures were grown on callus growth medium containing culture extract. Leaf and callus tissues, but not tuber tissue, reacted to cultures or filtrates. Disease response among cultivars was similar regardless of inoculum, filtrate or tissue used. The response of cultivars to Vaa and Vd could potentially be determined with this system.

784

THE ULTRASTRUCTURE OF LEAF EPICUTICULAR WAX IN CANOLA. K.L. Conn and J.P. Tewari, Dept. of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5.

The epicuticular wax in canola is known to confer resistance against *Alternaria brassicae* by providing a water-repellent surface and by forming a barrier restricting the diffusion of leaf exudates. The conformation of the wax is crucial in both these aspects. Therefore, an ultrastructural study of the epicuticular wax on the leaves of *Brassica napus* and *B. campestris* by SEM was undertaken. Freeze-drying and air-drying methods of leaf preparation were compared. The material prepared by the first method revealed considerable disturbance in the wax layer, whereas no disturbance was evident when the second method was used. Using the second method, at least three crystal types were observed. These included plate-like crystals, filamentous, sometimes branched crystals, and rods, present singly or forming blocks. The rods and filamentous crystals projected out from the leaf surface.

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USE OF DRY INOCULUM FOR FIELD TESTING REACTION OF *PISUM SATIVUM* L. TO *FUSARIUM OXYSPORUM* F. SP. *PISI* RACE 1. J. B. Mullen and D. J. Hagedorn, Department of Plant Pathology, University of Wisconsin-Madison 53706.

A method for field inoculation of pea (*Pisum sativum* L.) seed with *Fusarium oxysporum* f. sp. *pisi* race 1 was studied for screening wilt resistance. Inoculum was produced by growing mycelium and microconidia of the pathogen for two weeks on pea seed sterilized by two autoclavings, drying this mixture, and then grinding into a powder. Five treatment levels (0, 2 x 10<sup>6</sup>, 4 x 10<sup>6</sup>, 9 x 10<sup>6</sup>, and 2 x 10<sup>7</sup> cfu/inch of planted row) of the powdered inoculum was mechanically planted with seed of two resistant (Charo, AG38SF) and two susceptible (Mini, Abador) pea cultivars in twenty foot rows using four replicates in a completely randomized design. The highest treatment level consistently wilted, then killed all plants of the susceptible cultivars. Stand and yields of the resistant cultivars were unaffected by treatment.

786

A LEVANSUCRASE INHIBITOR FROM SUGARBEET FUNCTIONS AS AN ANTIBIOTIC. W. M. Bugbee, U.S. Department of Agriculture, ARS and Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

An extract from sugarbeet roots exhibited inhibitory activity against a levansucrase (LS) that had been purified from the culture filtrate of a sugarbeet strain of *Bacillus circulans*. The inhibitor was heat stable, had a molecular weight of ca. 560, and contained two sugar and three amino residues. Growth of *Corynebacterium sepedonicum*, *C. nebraskense*, and *B. circulans* was inhibited if the culture broth contained 2% purified inhibitor. LS inhibitory activity in plant extract depended on the source of the extract. LS inhibitory activity was diminished if sugarbeet roots were frozen or submerged under water. The inhibitor was not effective against invertase. Tomatoes were partially protected against stunting if inoculated with a mixture of *C. sepedonicum* and the inhibitor. This primary report of a naturally occurring inhibitor of LS helps to explain the sugarbeet's resistance against invading microflora.

787

AN INHIBITOR OF COWPEA MOSAIC VIRUS (CPMV) POLYPROTEIN PROCESSING FROM ARLINGTON COWPEAS. Christopher B. Glascock, Fernando Ponz, and George Bruening, Department of Plant Pathology, University of California, Davis, CA 95616.

We have investigated a possible mechanism by which cowpea (*Vigna unguiculata*) line Arlington resists infection by CPMV. We designate this line as "operationally immune" to CPMV because several days after inoculation sensitive assays detect no infectivity or

virions. Protoplasts of Arlington leaves are highly resistant to CPMV. A polyprotein translated from CPMV RNA is cleaved by a CPMV proteinase that is essential for CPMV replication. Previous work from this laboratory has implicated a proteinase inhibitor from Arlington protoplasts as the mediator of resistance to CPMV and has shown that extracts of Arlington protoplasts but not of protoplasts from susceptible cowpeas inhibited a crude proteinase preparation. We show here that CPMV RNA 1 translated *in vitro* gives rise to a proteinase activity that also is specifically inhibited by extracts of Arlington protoplasts. Further progress in the purification of the inhibitor of the *in vitro* produced proteinase will be presented.

#### 778

INHERITANCE OF REACTION TYPE 4 AGAINST DOWNY MILDEW IN CUCUMIS MELO. Y. Cohen<sup>1</sup>, C. E. Thomas<sup>2</sup>, S. Cohen<sup>1</sup>, and H. Eyal<sup>1</sup>.  
<sup>1</sup>Dept. Life Sciences, Bar-Ilan University, Ramat-Gan, Israel; <sup>2</sup>USDA, ARS, U. S. Vegetable Laboratory, Charleston, SC 29407.

Reaction type (RT) 4 indicates a high level of resistance against downy mildew incited by *Pseudoperonospora cubensis* in *Cucumis melo* germplasm. This RT was stabilized in an inbred line of *C. melo* PI 124111 which was used as the resistant parent in crosses with the susceptible cultivars 'Homed' and 'Ananas-Yokneam'. Artificial inoculations were used to evaluate the parental, F<sub>1</sub>, F<sub>2</sub>, and BC<sub>1</sub> generations for reaction type against the pathogen. F<sub>1</sub> plants had intermediate levels of resistance. F<sub>2</sub> populations segregated in a ratio of 6:9:1 (RT's 1:2 and 3:4). BC<sub>1</sub> to the resistant parent segregated in a ratio of 3:1 (RT's 2 and 3:4). These data indicate that two incompletely dominant genes condition inheritance of RT 4 from the resistant parent. Some data and observations also indicate possible involvement of cytoplasmic factors. Research supported by BARD.

#### 789

MONILIFORMIN PRODUCED BY CULTURES OF FUSARIUM SUBGLUTINANS ISOLATED FROM SWINE FEED. Ronald F. Vossender, Northern Regional Research Center, ARS, USDA, Peoria, IL 61604

Feed samples from Iowa suspected of causing vomiting and enlarged vulva, as well as mortalities of swine, were examined for toxicogenic fungi and mycotoxins. *Fusarium moniliforme* and *F. subglutinans* accounted for 43 percent and 18.5 percent, respectively, of the total count of  $4.75 \times 10^5$  propagules of filamentous fungi per gram of swine feed, but representatives of various *Penicillium* spp. and *Aspergillus* spp. were also found. Isolates of *Fusaria* were grown on cracked corn for 6 weeks at 25° C. *F. subglutinans* isolates (NRRL 13241, 13242-13245) elaborated 100-400 µg/g moniliformin. This toxin was not detected in the corn fermented with *F. moniliforme* or the swine feed. Other mycotoxins such as trichothecenes, zearalenone, and fusaric acid were not detected in the corn fermentations. Since information is meager concerning the presence of *F. subglutinans* in feed, these results suggest a need for additional studies into the incidence and distribution of this fungus on corn produced in the U.S.

#### 790

AFLATOXIN ESTIMATION IN CORN BY DETERMINATION OF BRIGHT GREENISH-YELLOW FLUORESCENCE IN AQUEOUS EXTRACTS. Elvind B. Lillehoj, Thomas J. Jacks, and Oscar H. Calvert, USDA, SRRC, 1100 Robert E. Lee Blvd., New Orleans, LA 70124 and Dept. of Plant Pathol., Univ. of Missouri, Columbia, MO 65211.

A procedure was developed for extraction of bright greenish-yellow (BGY) fluorescence from ground corn samples and subsequent determination of fluorescence emission in a fluorometer. The technique was designed to be inexpensive, rapid and utilizable at remote locations. A simple, *in vitro* method for production of the BGY-fluorescing material from kojic acid was characterized; the procedure provided a reference for standardization of the fluorescence. Water was identified as an effective solvent for extraction of the BGY-fluorescence from corn and stabilizer of fluorescence during subsequent extract storage. A correlation coefficient of  $r = 0.77$  was determined for BGY-fluorescence vs total aflatoxin levels (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) and  $r = 0.88$  for the two variables in samples containing only aflatoxin B<sub>1</sub> and B<sub>2</sub>.

#### 791

INHIBITION OF DAMAGING STORAGE FUNGI BY ASPERGILLUS GLAUCUS IN GRAIN. D. B. Sauer, USDA, ARS, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502.

In laboratory storage tests, corn and wheat inoculated with three members of the *Aspergillus glaucus* group sustained less damage and total fungal growth than grain inoculated with a mixture of five other species: *A. candidus*, *A. flavus*, *A. niger*, *A. ochraceus*, and *Penicillium citrinum*. Inoculation with the five species plus *A. glaucus* was less damaging than inoculation with just the five species. In several tests at 20 and 25C and 85 to 90% relative humidity, total fungal growth as indicated by ergosterol content, averaged three times higher in grain inoculated with the five species than in grain inoculated with *A. glaucus*. Grain inoculated with both groups and noninoculated grain averaged almost twice as high as the *A. glaucus*-inoculated grain. Moisture content also increased in some of the non *A. glaucus* treatments, particularly when *A. candidus* was a dominant part of the mycoflora.

#### 792

EVIDENCE FOR A "PATHOTOXIN" ROLE OF TRICHOPECENE METABOLITES PRODUCED BY A STRAIN OF MYROTHECIUM RORIDUM PATHOGENIC TO MUSKMELON (*CUCUMIS MELO* L.)

Joseph O. Kuti, Timothy J Ng and George A. Bean, Departments of Horticulture and Botany, University of Maryland, College Park, Maryland 20742.

A pathogenic strain of *Myrothecium roridum* Tode ex. Fr (ATCC #52485) isolated from an infected muskmelon fruit produced 4 trichoverroids (trichodermedienol A and B, roridin 1-2, and 16 hydroxyroridin 1-2) and the macrocyclic trichothecene roridin E in liquid culture. These compounds produced phytotoxic reactions on detached leaf tissues of muskmelon. Addition of roridin A or E to spores inoculated on leaf tissue accelerated growth of the fungus and stimulated rate and amount of sporulation. The addition of roridin A or E to the inoculum also caused some muskmelon genotypes normally resistant to the pathogen to become susceptible.

#### 793

PRESENCE OF A UNIQUE PLASMID COMMON TO SEVERAL ISOLATES OF CORYNEBACTERIUM SEPEDONICUM. B.D. Mogen\*, A.E. Oleson†, and G.A. Secor\*. Depts. of Plant Pathology\* and Biochemistry†, North Dakota State Univ., Fargo, ND 58105.

Several isolates of *C. sepedonicum* were examined for the presence of plasmids using an alkaline-lysis, CsCl purification procedure. Most isolates screened were plasmid positive and, regardless of geographic origin, contained a single plasmid which appeared to be identical from all isolates based on restriction enzyme analysis. The plasmid, designated pCsl, was calculated to have a molecular size of 42 kb, based on the sum of BamHI, SmaI, or SstI restriction fragments. However, the native plasmid migrates at a rate expected for a ccc molecule approximately three times this size in two different electrophoretic systems. Sensitivity of pCsl to 13 different restriction enzymes was tested. Eleven enzymes yielded restriction patterns of varying complexity, whereas two others (EcoRI and HindIII) failed to cleave pCsl. Preliminary evidence suggests that either the plasmid is devoid of these sites or analogous restriction-modification systems exist in *C. sepedonicum*.

#### 794

EFFECT OF WIND-GENERATED SAND ABRASION ON INFECTION OF CORN (*ZEA MAYS* L.) BY *CORYNEBACTERIUM MICHIGANENSE* SSP. NEBRASKENSE. T.R. Rochford, A.K. Vidaver, C.D. Gardner, and D.L. Arambust, Univ. of Nebraska, Lincoln, NE 68583 and USDA Wind Erosion Lab., Kansas State Univ., Manhattan, KS 66506.

Wind tunnel experiments were conducted to test the hypothesis that wind or sand damage facilitates infection of corn by *C. n. ssp. nebraskense* (Can). Seedlings received (1) sand abrasion followed by spray inoculation, (2) wind only, followed by spray inoculation, (3) abrasion with sand containing inoculum, and (4) control treatments. Inoculum concentration was  $2 \times 10^6$  CFU/ml. In treatments 1 and 2, 5 ml inoculum/3 plants were applied; in treatment 3, 1 ml inoculum/115 g sand. Seedlings were exposed to sand (31 g/cm-width/min) for 10 minutes. Disease symptoms were detected in plants of treatments 1 and 2 after 14 days. Can was isolated from plants in treatments 1, 2, and 3. In a repeat experiment, although clear disease symptoms were not detected after 14 days, Can was isolated only from plants in treatments 1, 2, and 3. Thus, wind or sand damage provides avenues for infection of corn by Can.

#### 795

EXTRACELLULAR POLYSACCHARIDE MUTANTS OF *CORYNEBACTERIUM*

Mutants of *Corynebacterium michiganense* pv *insidiosum* (Cmi) with an impaired extracellular polysaccharide (EPS)-producing ability were obtained by UV irradiation to assess the necessity of EPS to the *in planta* survival of the organism. Liquid cultures were used to compare mutant EPS production to that of wild type. Wild type EPS consists of three different-sized components with known sugar compositions. The mutants obtained by UV were of four types. Most had reduced EPS production. One type lacked the largest component, one lacked the mid-sized component, and one had only the smallest component. The sugar composition of some EPS components from mutants was different from that of wild type. Plant inoculations with mutant strains showed that the mutants were able to grow within host tissues despite the changes in their EPS composition.

#### 796

SUGARBEET: A NATURAL HOST FOR *CORYNEBACTERIUM SEPEDONICUM*. W. M. Bugbee, M. C. Gudmestad, G. A. Secor, and P. Nolte. U.S. Department of Agriculture, ARS, and Department of Plant Pathology, North Dakota State University, Fargo, ND 58105

The potato ring rot bacterium, *Corynebacterium sepedonicum* (Csep) was isolated from symptomless field-grown sugarbeet roots that were collected in eastern North Dakota. Endophytic bacteria were collected by centrifugation from homogenates of 60-90 day old field-grown roots. The pellets were assayed for Csep by immunofluorescent microscopy using monoclonal antibodies prepared against polysaccharide cell wall components (courtesy S. de Boer) of Csep. Pellets containing Csep were bioassayed by inoculation of eggplants. Approximately 30% of the roots harvested were positive for Csep using immunofluorescent microscopy. Eggplants developed symptoms and Csep was reisolated following inoculation with a positive sugarbeet homogenate pellet. Csep also was isolated from beet roots that had grown in pasteurized soil amended with tubers with ring rot symptoms.

#### 797

DOSE-RESPONSE RELATIONSHIPS OF *CLAVIBACTER XYLI* SUBSP. *XYLI* AND SUGARCANE CLONES DIFFERING IN TOLERANCE TO RATOON STUNTING DISEASE. N. A. Harrison and M. J. Davis, Univ. of Florida, Research and Education Center, 3205 College Avenue, Ft. Lauderdale, FL 33314.

Single bud cuttings from healthy stalks of eight sugarcane clones varying in tolerance to ratoon stunting disease were inoculated with 10-fold or 1000-fold serial dilutions of the F-1 isolate of *C. x. xyli*. Inoculum levels ranged from  $10^4$  -  $10^7$  cfu/ml. Sap from mature stalks of plants grown from the cuttings was examined for *C. x. xyli* using phase-contrast microscopy and/or a fluorescent-antibody staining procedure. Single stalks from 30 plants/clone/inoculum dose were sampled. Probit analyses of quantal responses of sugarcane clones to *C. x. xyli* gave  $ED_{50}$  values (dosage required to produce detectable levels of the bacterium in sap from 50% of the population) from  $7.38 \times 10^2$  cfu for clone CP 53-1 to  $1.8 \times 10^{12}$  cfu/ml for H 60-6909.  $ED_{50}$  values were inversely correlated with yield-loss estimates for the clones.

#### 798

EVALUATION OF PEA CULTIVARS FOR USE IN THE BIOASSAY FOR THE PATHOGENICITY OF *CORYNEBACTERIUM FASCIENS*. S. H. Kim and W. A. Woodward. Pennsylvania Dept of Agr. Harrisburg, PA 17110-9408.

Thirty-four garden pea (*Pisum sativum* L.) cultivars were evaluated in test tubes to rapidly confirm the pathogenicity of *Corynebacterium fasciens*. Pea seeds, surface sterilized with 0.5% NaOCl, were germinated on water agar (2-3 days). The germinated peas were soaked for 2 hrs in a mixture of 3, 5, and 7 day old bacteria cultures of 4 separate isolates. The bacteria were grown in an agitated liquid medium composed of 7g  $K_2HPO_4$ , 3g  $KH_2PO_4$ , 0.1g  $MgSO_4 \cdot 7H_2O$ , 10g sucrose, 2g L-asparagine and 0.01g thiamine HCl per liter. The inoculated peas were rinsed with sterile water and transferred to individual test tubes containing Hoagland's solution with 0.5% agar, and incubated with a 12 hr light/dark cycle at 19C until symptoms developed. The rapid, reliable diagnostic symptom for *C. fasciens* was consistent multishoot development from the base of the main stem. Alaska and Novella cultivars expressed 100% multishoot development within 7 days. Bolero, Mars, Oregon Sugar Pod, Snowbird and Wando showed the symptom in 14 days. Other cultivars were inconsistent in symptom expression.

#### 799

THE EFFECT OF *ERWINIA CAROTOVORA* PV *CAROTOVORA* ON GROWTH AND SURVIVAL OF PROTOPLAST DERIVED POTATO CALLI. R. J. Taylor and G. A. Secor. Dept. Plant Path., No. Dak. St. Univ., Fargo, 58105.

Protoplast derived potato calli (cv Crystal) were exposed either directly or indirectly to *Erwinia carotovora* pv *carotovora* (strain Ecc 71). For direct exposure, calli were inoculated with 0.5 ul of bacterial suspensions at  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  or  $10^9$  cfu/ml. For indirect exposure calli were grown on an agar bilayer medium. The lower layer consisted of 10 ml of callus induction medium supplemented with pectin (2 g/l) and contained bacteria at  $10^6$ ,  $10^5$ ,  $10^4$ ,  $10^3$  or  $10^2$  cfu/ml. The upper 10 ml layer was bacteria free. In all inoculated treatments the average callus diameter increased for 24 hours then declined. Over 90% of the calli were killed within 5 days, however, some calli survived as long as 14 days. Calli grown on the bilayer containing  $10^6$ ,  $10^5$  and  $10^4$  cfu/ml also decreased in size and most were killed within 9 days. Those exposed to  $10^3$  and  $10^2$  cfu/ml grew slightly. Approximately 20% and 7% respectively of these calli survived after 27 days.

#### 800

ALFALFA SPROUT ROT CAUSED BY *ERWINIA CHRYSANTHEMI*. Luellen Pierce and A. H. McCain, Dept. of Plant Pathology, University of California, Berkeley, CA 94720.

Alfalfa sprouts grown for consumption periodically become diseased during sprouting. Infected sprouts collapse into a yellowish, stinking mass. *Erwinia chrysanthemi* was isolated and shown to cause the disease. Temperature greatly affects disease severity. Little or no disease occurs at 15 or 18 C, but severe rot occurs at temperatures over 28 C. Disease severity is related to increasing concentrations of the pathogen, with disease occurring when seeds are soaked in a bacterial suspension of at least  $10^2$  cfu/ml. Soaking inoculated seeds for 2 hours in 0.12%, 0.25% and 0.5% sodium hypochlorite or calcium hypochlorite eliminated the disease. *E. chrysanthemi* does not survive longer than 1-2 weeks on inoculated seeds or in infected alfalfa sprouts. *E. chrysanthemi* also causes a rot of mung beans.

#### 801

RELATION OF CALCIUM CONTENT IN POTATO TUBER PERIDERM TO BACTERIAL SOFT ROT SUSCEPTIBILITY, INTERNAL BROWN SPOT AND SUBAPICAL NECROSIS OF SPROUTS. K. C. Tzeng, A. Kelman, K. E. Simmons and K. A. Kelling, Department of Plant Pathology and Department of Soil Science, University of Wisconsin-Madison, Madison, WI 53706.

Russet Burbank potato tubers from a factorial experiment involving fertilization with calcium, magnesium, and potassium contained various levels of periderm calcium. These tubers were assayed in the mist chamber for soft rot susceptibility shortly after harvest. Additional samples were examined for internal brown spot and subapical necrosis at harvest or after storage. Soft rot susceptibility of tubers inoculated with *Erwinia carotovora* pv *atroseptica* decreased as periderm calcium content increased. Incidence of internal brown spot of tubers and subapical necrosis of sprouts was inversely correlated with the periderm calcium content. Subapical necrosis of sprouts may serve as an indicator of low calcium content of tubers.

#### 802

SURVIVAL OF *ERWINIA AMYLOVORA* ON NON-HOST FLOWERS OF SWEET CHERRY. Sherman V. Thomson, Department of Biology, Utah State University, Logan, Utah 84322

Epiphytic colonization by *Erwinia amylovora* of flowers of fire blight hosts such as pear and apple occurs on the stigmatic surfaces of pistils. Multiplication occurs on apparently healthy stigmas, and populations frequently reach  $10^6$  to  $10^7$  cells per flower. However, there generally is no symptom development on these colonized host flowers. *Erwinia amylovora* survived on flowers of non-host sweet cherry flowers for up to 17 days in the field, but populations did not exceed the original inoculum levels. No symptoms were observed. Survival was only slightly better on the pistils when compared to survival on other flower parts. These data suggest that the stigmas of host flowers are conducive for growth of *E. amylovora* whereas non-host cherry flowers allow survival but do not permit significant multiplication.



A REVISED "CUP PLATE" ASSAY FOR DETERMINING THE PECTOLYTIC ACTIVITY OF CULTURE FILTRATES. Raymond J. Taylor and Gary A. Secor. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

The agar "cup plate" diffusion procedure for quantitatively determining pectolytic activity has been modified for increased sensitivity. The revised assay is run in 100 X 15 mm petri plates. Each plate contains 20 ml of 1% agarose (Type II), ammonium oxylate (0.5%), and sodium azide (0.2%) in phosphate buffer (0.2M, pH 5.3) with polygalacturonic acid (0.01%) as the substrate. Samples (35  $\mu$ l) are pipetted into wells punched in the agarose with a #1 cork borer. From 1 to 5 samples can be assayed per plate. After incubation at 37°C for 17 hours, the gel is developed with 10 ml of 0.05% ruthenium red for 30 minutes and the diameter of the clear zone of activity is measured microscopically. Pectinase equivalents as low as  $2.3 \times 10^{-4}$  units/ml can be detected. The modified assay requires less sample and reduces the problem of gel dehydration associated with the standard assay.

NUTRITION OF THE PLANT PARASITIC PROTOZOAN, *PHYTOMONAS DAVIDI*, IN SERUM-FREE AND CHEMICALLY DEFINED MEDIA. Meghnad Konai<sup>1</sup>, Robert E. Davis<sup>1</sup>, and Ing-Ming Lee<sup>2</sup>. <sup>1</sup>Plant Virology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705 and <sup>2</sup>Department of Botany, University of Maryland, College Park, MD 20742.

*Phytomonas davidi* (Trypanosomatidae), a flagellated protozoan found in latex vessels of plants in the Ruphorbiaceae, was cultured in a new chemically defined medium as well as in undefined media in which serum was replaced by a mixture of bovine serum albumin, lipids, hemin chloride, and Tween 80. Titer of the protozoan reached over  $10^8$ /ml from starting titers of  $10^2$  to  $10^3$ /ml. Keto acids and nucleosides and bases promoted growth. Growth was inhibited by cysteine in some media. Hemin was required for growth. Chemically defined and serum-free media present new opportunities for research on nutritional requirements of this agent, and their use offers a new approach toward eventual cultivation of protozoans associated with heartrot of coconut, coffee phloem necrosis, and Marchitez disease (fatal wilt) of oil palm.

IDENTIFICATION OF A *PSEUDOMONAS SYRINGAE* ICE GENE TRANSLATIONAL PRODUCT AND RECONSTITUTION OF ICE NUCLEATION ACTIVITY *IN VITRO*. A. G. Govindarajan and S. E. Lindow, Department of Plant Pathology, University of California, Berkeley, CA 94720.

*Escherichia coli* minicell strain P67856 and strain HB101 were transformed with the plasmid vectors pBR322 or pBR325 carrying 9 and 4.2 kb *ice* inserts from *Pseudomonas syringae* pv. *syringae* strain Cit7 respectively. Isolated minicells were pulse labeled *in vivo* with <sup>35</sup>S-methionine; a protein with a molecular weight of 145 + 3 Kd was detected only in *ice*<sup>+</sup> strains by 1 and 2 dimensional SDS-PAGE followed by autoradiography. A very minor protein having a mass of 158 + 8 Kd was also detected by Coomassie brilliant blue staining of SDS-PAGE separation of proteins from membranes isolated from *ice*<sup>+</sup> but not *ice*<sup>-</sup> *E. coli* HB101 cells. No protein corresponding to that observed in *in vivo* experiments was detected in coupled *in vitro* transcription-translation reactions. However, products of *in vitro* protein synthesis exhibited ice nucleation activity when reconstituted with phospholipids. An average of 267 ice nuclei active at -9°C per microgram of added plasmid DNA were detected.

A PRELIMINARY BIOLOGICAL CHARACTERIZATION OF THE BEET LEAFHOPPER TRANSMITTED VIRESCENCE AGENT (BLTVA) FROM CALIFORNIA. D.A. Sullivan, G.N. Oldfield, and D.J. Gumpf\*. USDA-ARS and Department of Plant Pathology\*, University of California, Riverside, CA 92521.

A single insect transmitted line of BLTVA, an agent previously reported in California in dual infections with *Spiroplasma citri*, was associated with mycoplasma-like bodies in the phloem of infected periwinkle, *Catharanthus roseus*. Transmission tests with *Circulifer tenellus* have demonstrated that the transmission biology is typical of leafhopper borne mollicutes. Species of Apocynaceae, Asteraceae, Brassicaceae, Ranunculaceae and Tropaeolaceae were identified as experimental hosts. In China aster, *Callistephus chinensis*, symptoms are very similar to those caused by the aster yellows agent.

PLAQUE ASSAY OF SPIROPLASMAVIRUS ON SENSITIVE INDICATOR LAWNS IN SERUM-FREE MEDIUM. R. E. Davis and I.-M. Lee. Plant Virology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705 and Department of Botany, University of Maryland, College Park, MD 20742.

Spiroplasmaviruses in partially purified preparations, in spiroplasma culture filtrates, and in extracts from plants and insects infected by *Spiroplasma citri* or corn stunt spiroplasma, induced plaques in lawns of a sensitive indicator strain of *S. citri* growing in agar overlays of a medium (LD57) lacking serum. Plaque size varied inversely with seeding density of indicator strain. Thus, plaques with diameters of from <0.2 to >5 mm were observed when a given cloned virus was assayed at different seeding levels of lawn. Plaques could be completely obscured when lawns were seeded with high concentrations of indicator strain. A similar relationship was found between plaque size and lawn density in a medium (LD8A) containing serum. Plaque efficiencies were similar in LD57 and LD8A. This is the first report of plaque assay in serum-free medium of a virus infecting a member of the class Mollicutes.

STRAINS OF CORN STUNT SPIROPLASMA DEFECTIVE IN HELICITY AND MOTILITY. I.-M. Lee and R. E. Davis. Department of Botany, University of Maryland, College Park, MD 20742, Plant Virology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

Medium LD59 was used for primary isolation of corn stunt spiroplasma (CSS) from diseased plants of corn from Mississippi. Strains triply cloned from one primary culture exhibited varying degrees of defects in helicity and motility. Some were nearly non-helical. Clones with less helicity were less motile, but one clone (strain I15) of normal helicity exhibited little or no motility. Strains with little helicity and/or motility developed tiny "fried-egg" colonies ( $\leq 0.2$  mm diam) on LD59 agar, while strains with greater helicity or motility developed small somewhat diffuse colonies. Strains with normal helicity and motility developed large uniformly diffuse colonies (up to 5 mm). All new strains were serologically indistinguishable from known CSS strains, but PAGE analysis of membrane proteins revealed minor differences. This is the first report of CSS strains defective in helicity and/or motility.

PRIMARY ISOLATION OF *SPIROPLASMA CITRI* AND CORN STUNT SPIROPLASMA IN SERUM-FREE MEDIUM. R. E. Davis and I.-M. Lee. Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705 and Department of Botany, University of Maryland, College Park, MD 20742.

Primary isolation of *Spiroplasma citri* and corn stunt spiroplasma (CSS) from diseased hosts was accomplished in a culture medium (LD57) lacking serum. Medium LD57 was similar to medium LD8A except that serum in LD8A was replaced by a serum substitute consisting of bovine serum albumin, cholesterol, fatty acids, phospholipids, and Tween 80. Primary isolations of *S. citri* from insects and plants were achieved with equal efficiencies in media LD57 and LD8A, and were obtained in broth and agar versions of both media. For isolations of corn stunt spiroplasma, the serum-free LD57 proved superior to LD8A. Cell titers reached within 7 days in LD57 were 10- to 100-fold higher than those in LD8A, and some strains developed colonies only on agar versions of LD57. Serum-free media offer a new approach toward eventual isolation of yet uncultured plant mycoplasma-like organisms (MLO) and other lipid-requiring agents.

SINGLE-STRANDED DNA VIRUS FROM A STRAIN OF HONEYBEE SPIROPLASMA: CHARACTERIZATION AND TRANSFECTION. S. McCammon and R. E. Davis. Plant Virology Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

A new spiroplasmavirus isolated from honeybee spiroplasma strain TS2 was propagated and plaque assayed in *Spiroplasma citri* strain M200H. The virus is a naked rod, 150-180 x 7-12 nm, with one flat and one rounded end, and it contains single-stranded circular DNA as shown by electron microscopy, melting profile, and enzyme analysis. Transfection of M200H with viral DNA occurred after the DNA was treated with RNase and protease while none occurred after S<sub>1</sub> nuclease or DNase treatment. A double-stranded replicative form (RF) of the viral DNA was isolated from infected cells. RF had a molecular mass of about 4.3 Md as determined by agarose gel

electrophoresis and had sites sensitive to restriction enzymes *HinfI*, *MspI*, *AluI*, and *HhaI* and one region sensitive to *S<sub>1</sub>* nuclease. Transfection with this RF was also achieved. Study of the virus has permitted development of methods for efficient introduction of foreign DNA into spiroplasmas.

#### 812

SUPPRESSION OF LEAF SCALD SYMPTOMS IN PLUM BY OXYTETRACYCLINE INJECTION. C. J. Chang, C. Yonce, and D. Gardner. Dept. of Plant Path., Univ. of Ga., Experiment, GA 30212, USDA SE Fruit & Tree Nut Lab., Byron, GA 31008, and Gardner Green Consulting, Savannah, GA 31419.

Six 7 yr old plum trees (*Prunus salicina* L.) showing leaf scald symptoms over the past 3 years were injected with oxytetracycline (OTC) (J.J. Mauget Co.) and 3 were injected with carrier as controls. Each tree was injected with two Mauget capsules each containing 0.16 g OTC in 4 ml solution in April, 1984. The presence and absence of scald symptoms on all branches were recorded in August. Two control trees developed scald symptoms on all branches. The other control tree developed early defoliation and more red leaves without apparent scald symptoms. Two treated trees were partially burned due to toxicity. On the remaining living branches, one tree showed no apparent symptoms, while the other developed scald symptoms in 1/4 (25%) branches. The other four treated trees developed scald symptoms on: 5/15 (33.3%), 1/13 (7.7%), 5/13 (38.5%), and 0/9 (0%) of the branches.

#### 813

REMISSION OF PHYLLODY SYMPTOMS IN ASTER YELLOWS AGENT INFECTED PERIWINKLES BY KINETIN SPRAY. C. J. Chang and R. C. Donaldson. Dept. of Plant Path., Univ. of Ga., Experiment, GA 30212.

Periwinkles infected with aster yellows mycoplasma-like organisms (AYMLO) were sprayed weekly with kinetin (2000 ppm in 0.05 N NaOH). The first spray started seven months after grafting when severe witches' broom and phyllody symptoms were fully developed. About four weeks after the first spray, the treated periwinkles began to show remission of the phyllody symptom by producing green and pink "semi-phyllody" flowers. More semi-phyllody flowers were observed as sprays continued. Four to five weeks later, instead of producing semi-phyllody flowers, the treated periwinkles produced normal color yet smaller size flowers, whereas the control periwinkles retained the witches' broom symptoms with no flower production. Under the electron microscope MLO particles were observed in the sieve tubes of both treated and control leaf petioles. Apparently, the MLO infection reduces the kinetin concentration *in vivo* resulting in phyllody symptoms.

#### 814

BACTERIAL STRAIN IDENTIFICATION BY COMPARATIVE ANALYSIS OF CHROMOSOMAL DNA RESTRICTION PATTERNS. D. Drahos, J. Brackin, G. Barry. Monsanto Co., Department of Biological Sciences, 700 Chesterfield Village Parkway, St. Louis, MO. 63198.

A rapid method has been developed to comparatively identify most gram negative and some gram positive bacteria by the analysis of chromosomal restriction patterns following gel electrophoresis. For this procedure DNA from a known bacterial

broth culture is prepared by a rapid (1-2 hour) extraction procedure, cut with a particular restriction endonuclease (usually *EcoRI*), and separated by agarose gel electrophoresis. The DNA banding pattern, visualized with ethidium bromide staining, is used as a standard to determine whether an unknown strain has a banding pattern identical to the known reference previously (or concurrently) analyzed. More importantly, a rapid differentiation between strains to the pathovar, and even subpathovar, level can often be made. For example, three distinct strains of *Erwinia carotovora* pv. *carotovora* were readily distinguished. This method can also bypass problems in identification caused by differential colony morphology.

#### 815

CLONING AND MOLECULAR ANALYSIS OF VIRULENCE-RELATED SEQUENCES OF *PSEUDOMONAS SYRINGAE* PVS. *SYRINGAE* AND *PHASEOLICOLA* ON BEAN. G. Bertoni, F. Niepold, D. Mills, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331-2902.

*Tn5* mutagenesis experiments using wild type *P. syringae* pvs. *syringae* (Pss) and *phaseolicola* (Psp) have produced eight mutants with reduced virulence on their host plant *Phaseolus vulgaris* (bean). The *Tn5*-containing *EcoRI* fragments from six of these mutants have been cloned, mapped, and shown to represent different sequences. Site-directed mutagenesis of cosmid sequences known to complement one Pss mutant has more precisely defined the region within an 8.5 kb segment which confers pathogenicity. Pathogenicity determinants are encoded within a 2.7 kb region at one end of this segment and also from another region 4.6 kb downstream. Although this mutated sequence showed homology with sequences in two other Pss strains and one Psp strain, the mutated sequence from a second mutant showed homology only to one Pss strain.

#### 816

PSEUDOMONAD PHYTOTOXIN, SYRINGOMYCIN STIMULATES THE PLASMA MEMBRANE ATPASE OF *RHODOTORULA PILIMANAE*. Lei Zhang and Jon Y. Takemoto. Departments of Biology and Chemistry/Biochemistry, Utah State University, Logan, UT 84322

The mode of action of the phytotoxin, syringomycin (SR), produced by *Pseudomonas syringae* pv. *syringae* was investigated using the fungus, *Rhodotorula pilimanae*, as a target organism. Fungal growth in liquid culture was inhibited by SR at levels below 1 µg per ml. At these levels, SR caused increases in the rates of cell uptake of tetraphenylphosphonium ion and dimethylloxazolinedione indicating effects on the charge and pH differences across the plasma membrane (PM). The PM was extracted and resolved on sucrose density gradients. SR at 2-5 µg per ml stimulated the vanadate-sensitive ATPase of the PM but not the mitochondrial ATPase. We propose that SR causes the electrogenic cellular extrusion of protons which is linked to the stimulation of the PM ATPase.

#### 817

SCANNING ELECTRON MICROSCOPY OBSERVATION OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* AND *P. SYRINGAE* PV. *TOMATO* ON TOMATO AND EPIPHYTIC WEED HOSTS. Rosa Mariano and S. M. McCarter, Department of Plant Pathology, University of Georgia, Athens 30602.

Scanning electron microscopy (SEM) was used to follow development of *Pseudomonas syringae* pv. *syringae* (PSS) and *P. syringae* pv. *tomato* (PST) on susceptible (PM 6203) and resistant (Ontario 7710) tomato plants and on suspected epiphytic hosts (*Anoda cristata* for PSS and *Datura stramonium* for PST). Young plants were misted to runoff with  $10^9$  c.f.u./ml, held in a lighted chamber at 25 C, and watered by subirrigation. Foliage from each plant was sampled daily for 6 days for SEM observation. Numbers of PSS and PST cells observed remained high throughout the 6-day period and were similar on the two cultivars. High populations of PSS and PST were also present on *A. cristata* and *D. stramonium*, respectively, throughout the observation period. On all hosts bacterial cells were clustered at epidermal cell junctions, along veins, around the base of trichomes, and occasionally within stomates. Fibrilla-entrapped bacterial cells were observed in all bacterium-host combinations.

#### 818

PATHOGENICITY OF *XANTHOMONAS CAMPESTRIS* PV. *PRUNI* ON DETACHED PEACH LEAVES. Randhawa, P.S. and Civerolo, E.L. USDA Fruit Laboratory, Beltsville Agricultural Research Center - West, Beltsville, MD 20705.

Young peach leaves were detached and surface sterilized with 70% ethanol. Inoculum ( $1 \times 10^6$  colony forming units (cfu/ml) of *Xanthomonas campestris* pv. *pruni* (Xcp) was infiltrated with a needleless syringe at several sites (approximately 70 cfu/site) on the adaxial side. The leaves were incubated on 0.5% water agar for 2 weeks at 25°C under 16h photo-period. Defined symptoms developed and the number of lesions produced at each site was directly proportional to the amount of inoculum infiltrated. Response of detached and attached leaves was similar. Symptoms specific for Xcp were not observed with 51 saprophytic bacterial strains tested at inoculum concentrations as high as  $10^8$  cfu/ml. At inoculum concentration of  $10^6$  cfu/ml, the detached leaf response distinguished the previously reported differential field resistance reactions of 21 of 22 peach cultivars.

819

ABERRANT SYMPTOMS ON CABBAGE CAUSED BY STRAINS OF *XANTHOMONAS CAMPESTRIS*. G. Y. K. Yuen and A. M. Alvarez, University of Hawaii, Honolulu, HI 96822.

*Xanthomonas campestris* (Xc) strains isolated from necrotic lesions on field-grown cabbage produced symptoms differing from typical black rot upon spray inoculation onto cabbage. Standard strains of Xc pv. *campestris* induced V-shaped chlorotic lesions with light brown necrosis and distinctive vein-blackening. In contrast, one group of field strains caused a leaf blight in which tissues rapidly collapsed starting at hydathodes resulting in a papery necrosis. The necrosis progressed inwardly in a circular, rather than a V-shaped, front. No vein-blackening was evident. A second group produced small, round, dark-grey necrotic lesions on the leaf lamina and at hydathodes. The latter set of symptoms were similar to those caused by strains of Xc classified as pv. *armoraciae*. The possibility that these represent different virulence types of Xc pv. *campestris* will be discussed.

820

DICHONDRA RUST IN CALIFORNIA. Robert D. Raabe, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Although previously reported from California, rust (*Puccinia dichondrae*) recently has become widespread on dichondra (*Dichondra micrantha*) in northern California. Teliospores are produced in pustules mainly on the lower surfaces of the leaves but occasionally on the upper surfaces or the petioles. Teliospores are released at maturity and are capable of germination as soon as they are in water. Germination is better at 18°C than at other temperatures, followed by 20°C and 24°C. Petioles of infected leaves are stimulated to grow to lift the infected leaves above the surrounding mat of leaves. The average length of the petioles of 50 infected leaves was 52.6 mm whereas the average length of petioles of 50 non-infected leaves in the same location was 22.9 mm.

821

ALTERNARIA LEAF SPOT OF ANIGOZANTHOS SPP. Robert D. Raabe, Department of Plant Pathology, University of California, Berkeley, CA 94720.

Plants of the genus *Anigozanthos* are native to Australia, where the common name is kangaroo paw. Recently the plants, particularly *A. manglesii* and hybrids of various species, have been grown commercially in northern and southern California as a source of cut flowers. A leaf spot, found on plantings was determined to result from infection by a species of *Alternaria*. Leaf spots are purple-black, are irregular in shape and vary usually from 1/2 to 1 cm across. Inoculations using conidia produced in culture have been successful on *A. manglesii*, *A. flavidus*, and *A. viridis*. The conidia of the fungus usually are borne singly on host tissues but occasionally are found in short chains of 2 or 3. The conidia vary from 68 to 132  $\mu$  in length by 14 to 36  $\mu$  in width. Because of the spore characteristics, the fungus falls between the sections *Brevicatenatae* and *Noncatenatae*, and because of this and the fact that this is a new host for a species of *Alternaria*, the fungus is tentatively named *Alternaria anigozanthi* n. sp.

822

PHYTOPHTHORA DIEBACK OF AZALEA AND SPATIAL PATTERN OF DISEASED PLANTS IN NORTH CAROLINA NURSERIES. D. M. Benson, Department of Plant Pathology, N. C. State University, Raleigh, NC 27695-7616

Phytophthora dieback of azalea (*Rhododendron obtusum*) was observed for the first time in nurseries in North Carolina during the summer of 1984. Azaleas in containers exposed to full sun as well as plants in the shade were infected. The disease first developed as water-soaked areas on leaves or necrotic lesions on stems of 1-yr-old Hershey Red and Hino Crimson. Eventually, entire shoots were killed. Phytophthora dieback was most severe on Hershey Red in all three nurseries surveyed in 1984. Isolations from leaf and stem tissue yielded *Phytophthora parasitica*, *P. cinnamomi*, and an unidentified, homothallic, *Phytophthora* sp. Development of Phytophthora dieback was associated with above normal rainfall in July 1984. Spatial pattern of diseased plants in quadrats was aggregated in two nurseries and random in a third based on indices of dispersion and fit to probability distributions. The aggregated pattern suggests that infection from secondary inoculum may be important in the disease.

823

PHYTOPHTHORA BLIGHT OF GOLDEN-FRUITED PALM (*CHRYSALIDOCARPUS LUTEASCENS*). N. M. Nagata and M. Aragaki, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

This is the first report of a seedling blight of *Chrysalidocarpus lutescens* (Bory) Wendl. (golden-fruited palm) caused by *Phytophthora nicotianae* B. de Haan sensu Erselius & De Vallavieille (= *P. parasitica* Dast.). The disease was confined to single outbreaks on the islands of Oahu and Kauai. Necrotic brown flecks and angular to irregularly shaped leaf spots, which expanded within a week to large lesions or covered entire leaflets, were characteristic of natural infections as well as artificial inoculations. Sporangia of P490, a single zoospore A1 isolate were  $48.1 \pm 4.6 \times 37.4 \pm 3.8$   $\mu$ m; chlamydozoospores were  $39.1 \pm 11.4$   $\mu$ m; oogonia were  $28.1 \pm 1.9$   $\mu$ m; length to diameter measurements of antheridia were  $10.0 \pm 1.3 \times 13.2 \pm 1.5$   $\mu$ m; and oospores were  $21.7 \pm 1.5$   $\mu$ m. Metalaxyl and fosetyl-Al, at 80 and 9500 ppm a.i. respectively, were effective in controlling this disease on golden-fruited palm by spray or drench applications.

824

FOLIAR BLIGHT AND DAMPING-OFF OF BRASSAIA ACTINOPHYLLA CAUSED BY COLLETOTRICHUM GLOEOSPORIOIDES. M. Aragaki, K. Y. Fitz, and J. Y. Uchida, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

*Colletotrichum gloeosporioides* Penz. was associated with blighted young leaves on large potted Brassaisia (*Brassaisia actinophylla* Endl.) plants and seedling damping-off. The foliar phase is characterized by black, necrotic young terminal leaves, deformed expanding leaves, and small leaf spots. Initial infection is limited to young tissue such as expanding leaves or seedlings. Both diseases were reproduced by isolates of *C. gloeosporioides* obtained from tissue with either disease symptom. Pathogenic isolates were also recovered from suspect seed lots, indicating the seed-borne nature of this pathogen. Isolates of *C. gloeosporioides* obtained from and pathogenic to Brassaisia produced only flecks on 3% of inoculated papaya fruit. Isolates of *C. gloeosporioides* isolated from papaya produced lesions on 30% of the inoculated papaya.

825

INFECTION OF THORNLESS BLACKBERRY BY BOTRYOSPHERA DOTHIDEA. J. L. Maas, Fruit Laboratory, USDA, Agricultural Research Service, Beltsville, MD 20705.

*B. dothidea* causes a serious cane canker disease of thornless blackberry in the eastern U.S. Since most cankers are first evident at nodes, isolations were made of lateral buds removed from canes in the spring and fall. In the spring, *B. dothidea* was isolated from 9% of surface sterilized buds with outer scales intact but from 0.3% of buds with outer scales and outermost emerging leaves removed. Spring isolations from scars of subtending leaf petioles resulted in 6.2% recovery. In fall, isolations from intact, buds and subtending scars resulted in 6 and 9% recovery, respectively. Isolations of persistently retained petioles in winter (December) resulted in 20-100% recovery of *B. dothidea*, depending on cultivar and degree of leaf senescence. Retention of petioles in February varied with cultivar, from 10-44%. Data suggest that *B. dothidea* aggressively colonizes senescing leaf petioles and, once established saprophytically, can invade healthy stem tissue.

826

HERITABILITY OF RESISTANCE TO THE SUGARCANE RUST

**PATHOGEN, PUCCINIA MELANOCEPHALA.** J. C. Comstock, K. K. Wu and S. A. Ferreira, Dept. of Genetics & Pathology, Hawaiian Sugar Planters' Association, P. O. Box 1057, Aiea, Hawaii 96701.

The heritability of sugarcane rust resistance was studied in two tests. The first test in 1983 involved 12 crosses with 13 parents; the second in 1984 involved 51 crosses with 27 parental clones. Rust grades were obtained visually when plants were 4-5 months old using a 1 to 9 scale based on leaf tissue area infected. Heritability values of 0.84 and 0.73 from the 1983 and 1984 tests, respectively, were determined by the regression of the progeny mean rust grade on the mid-point rust reaction of their parents. The high heritability values indicate that rust resistance can be easily incorporated in our variety development program. Resistant progeny were obtained in all crosses, although higher frequencies were obtained when one or both parents were resistant. In the 1984 study, 15, 43, and 79 percent resistant progeny were obtained using moderately susceptible, moderately susceptible x resistant, and resistant parents, respectively.

#### 827

**NUCLEAR CYCLE DURING TELIOSPORE GERMINATION IN TILLETIA CARIES, T. FOETIDA AND T. CONTROVERSA.** B.J. COATES AND J.A. HOFFMANN  
USDA-ARS LOGAN, UT 84322.

Germinating teliospores were stained with acetic orcein and examined with light microscopy. Nuclei in teliospores stained only after a period of incubation. Teliospores contained a single nucleus which underwent meiosis and then 0-2 synchronous mitotic divisions. As the spore produced a promycelium and then primary sporidia, the nuclei migrated from the spore into the primary sporidia. At this stage the number of nuclei is usually less than the number of primary sporidia. The nuclei divided synchronously in the primary sporidia, after which one of each pair of daughter nuclei migrated to the tip of the promycelium and then into any remaining anucleate primary sporidia. Supernumerary nuclei in the promycelium or in the primary sporidia remained contracted and autolysed. Mature primary sporidia usually contained a single interphase nucleus. When *T. caries* and *T. foetida* were germinated at 5C as compared to 15C, the nuclei in most teliospores underwent one less synchronous division and the number of primary sporidia was significantly reduced.

#### 828

**FUSARIUM SOLANI-INDUCED BRANCH AND TRUNK CANCERS ON CITRUS WEAKENED BY COLD WEATHER IN FLORIDA.** S. Nemeč, U.S. Department of Agriculture, ARS, 2120 Camden Road, Orlando, FL 32803.

Trunk and branch cankers formed in the spring and summer 1984 in many central Florida citrus groves that survived the freeze during Christmas 1983. *Fusarium solani* was consistently isolated from active lesions of cankered bark and phloem. *Phytophthora* was not isolated from cankered tissue. Visually healthy tree trunks injured or inoculated with *F. solani* developed the same kind of cankers, although those induced by injury were the smallest. Inoculations beginning in early December 1983 through mid-1984 resulted in cankers with vertical diameters of usually 10-90 cm 4 weeks after inoculation; canker size decreased to several centimeter diameter in late summer inoculations. Earliest cankers appeared as a water-soaked area under the bark around the point of inoculation, later sporodochia formed on the bark surface. Older cankers became dry and cracked but produced no gum. In the field, *F. solani* probably entered the tree through mechanical injuries or breaks in the bark caused by the freeze.

#### 829

**THE PERFECT STATE OF RHIZOCTONIA ORYZAE-SATIVAE: CAUSAL ORGANISM OF AGGREGATE SHEATH SPOT OF RICE.** P. S. Cunnell and R. K. Webster. Department of Plant Pathology, University of California, Davis, CA 95616.

The perfect state of *Rhizoctonia oryzae-sativae* (Saw.) Mordue was found near the water line on leaf sheaths of rice plants infected with aggregate sheath spot. This is the first report of the teleomorph of *R. oryzae-sativae* from nature. Fructifications are whitish, effuse, tenuous, occur in small inconspicuous patches and consist of a loosely organized layer of hyphae and basidia. The basidia are sphaero- to pyrropedunculate, 15-20.5 (-32) x 11.5-16.5 µm and are produced singly or in groups in a raceme-like arrangement. Each basidia bears 2, stout, cornute sterigmata, 12.5-23 x 4-5 µm. Basidiospores are globose, hyaline, smooth, repetitive, 9-16 x 6.5-15.5 µm. The perfect state of *R. oryzae-sativae* belongs in the genus *Ceratobasidium* and is a new species of *Ceratobasidium*, differing from all previously described species in that it has regularly 2 sterigmata and globose to subglobose spores.

#### 830 Withdrawn

#### 831

**SELECTIVE ISOLATION OF COLLETOTRICHUM SPP. FROM ALFALFA.** M. Y. Abdalla and L. H. Rhodes, Dept. of Plant Pathology, The Ohio State Univ., Columbus, OH 43210.

In an effort to develop a medium for selective isolation of *Colletotrichum trifolii* from alfalfa, several fungitoxicants were tested for in-vitro effects on saprophytic fungi frequently associated with diseased alfalfa tissue. In 25% lima bean agar (LBA) radial growth of *Fusarium roseum*, *Rhizopus stolonifer* or *Alternaria* sp. was completely suppressed for 6 days by 10 ppm benomyl, 25 ppm PCNB, or 25 ppm iprodione, respectively. LBA containing above fungitoxicants in combination plus 100 ppm streptomycin, 100 ppm chloramphenicol and 50 ppm tetracycline allowed consistent isolations of *C. destructivum*, *C. truncatum* and *C. gloeosporioides* from diseased and symptomless alfalfa stems with minimal growth of other microorganisms. *C. trifolii* was infrequently isolated, even from characteristic anthracnose lesions. Difficulties associated with isolation of *C. trifolii* from diseased alfalfa tissue are discussed.

#### 832

**CONTROL OF LETTUCE DROP (SCLEROTINIA SCLEROTIUM) Y.** Ben-Yehphet S. Bitton and A. Greenberger, Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, Israel.

The effects on lettuce drop of metham-sodium (MES) disinfection and a varying number of benomyl applications (2,3 and 6) in addition to the combined treatments, were studied at two sites naturally infested with *Sclerotinia sclerotiorum*. At each site half the field received MES by overhead irrigation whereas the control half received only an equivalent volume of water. Metham-sodium killed 85% of the *S. sclerotiorum* sclerotia in the top 10 cm of soil and of the remaining viable sclerotia, only 30% produced apothecia. Five-14 times fewer ascospores were deposited on the crop in MES-treated sites than in non-treated ones. Lettuce drop affected 30% of the plants in non-sprayed control plots; MES application alone, reduced the drop to 4%. Benomyl sprays alone also significantly reduced apothecia numbers and disease development and consequently increased yield although not to the extent observed with the MES treatment alone. Combined treatments produced the best disease control, but no significant yield increase compared to MES alone.

#### 833

**RICE BLAST CONTROL BY SEED TREATMENT WITH PYROQUILON**  
R.J. Williams, CIBA-GEIGY Ltd, Basle, Switzerland, K. Marjudin & R. Guyer, CIBA-GEIGY Ltd, P.O. Box 15/Rby, Jakarta, Indonesia.

In many trials in several countries in Asia and Latin America, treatment of rice seed with pyroquilon (CGA 49104), as a dry or slurry treatment, at 4-6 g a.i. per kg seed, has provided a high level of control of leaf blast for 40-60 days after sowing, even under heavy disease pressure in upland rice blast nurseries. Such a duration of control protects the rice crop during the time it is most vulnerable to devastating epidemics of leaf blast and, thus, seed treatment with pyroquilon is of great potential value to blast management programs. Seed treatment is a simple, efficient and relatively inexpensive way to protect the rice crop from leaf blast, even for small-scale rice farmers, as no specialised application equipment is needed, the treatment can be easily and rapidly carried out, and each seedling is protected from the start of its development. Such treatment integrated with use of resistant varieties could lead to durable leaf blast control.

#### 834

**CONTROL OF STRIPE RUST AND LEAF RUST OF WHEAT WITH TRIDIMENOL SEED TREATMENTS AND TRIADIMEFON FOLIAR SPRAYS.** R. B. Scott and R. F. Line, USDA-ARS, Pullman, WA 99164

Baytan (tridimenol) seed treatments used alone and in combination with foliar applications of Bayleton (triadimefon) were evaluated for control of naturally occurring stripe and leaf rust on wheat cultivars in Washington in 1981-1983. Baytan controlled rust but usually not beyond the jointing stage of plant growth; consequently, it usually did not improve yield. When the seed treatment was supplemented with a foliar spray at jointing to heading stages, the rusts were controlled, and yields were usually higher than when only one spray was applied. The yields were usually not as high as

when both early and late sprays were used. Baytan often delayed plant emergence and sometimes reduced plant stand and yield, especially when rusts were not severe. In conclusion, Baytan has potential for control of the rusts when used in conjunction with a foliar fungicide but its negative effect on plant establishment and yield must be considered.

### 835

STUDIES OF IN VITRO EFFICACY AND PHYTOTOXICITY OF STEROL INHIBITING FUNGICIDES ON ORGANISMS KNOWN TO CAUSE COMMON ROOT ROT OF WHEAT. Mathieson, J.T. and W.D. Worrall, 1984. Texas A&M Agricultural Experiment Station, Vernon, TX 76384.

Eight sterol inhibiting fungicides were evaluated for efficacy against Bipolaris sorokiniana and Fusarium spp. and for phytotoxicity to wheat (Triticum aestivum L.). Applied as seed treatments at the rate of 0.1 g a.i./kg the fungicides bitertanol, imazalil and triadimenol showed no phytotoxic effects, remaining fungicides exhibited varying levels of phytotoxicity. The ED 50 was determined by measuring the radial growth. ED 50 in vitro of B. sorokiniana ranged 10-100 µg/ml for triadimefon, 1-10 µg/ml for triadimenol, Bay HWG 1608, bitertanol, and imazalil, and 0.1-1.0 µg/ml for propiconazole, ectaconazole, and flutriafen. ED 50 in vitro of the Fusarium spp. ranged from 1-10 µg/ml for imazalil, triadimefon, and flutriafen to 10-100 ng/ml for ectaconazole, bitertanol, triadimenol, and Bay HWG 1608, and 1-10 ng/ml for propiconazole.

### 836

STEROL-INHIBITING FUNGICIDES FOR CONTROL OF SOUTHWESTERN RUST, Puccinia cacabata Presley, on Cotton. Deborah J. Young, L. M. Sullivan (University of Arizona Cooperative Extension Service, 450 S. Haskell, Willcox, AZ 85643), and Curtis E. Engle (Mobay Chemical Corporation, 53-205 Avenida Carranza, LaQuienta CA 92253).

The fungicides triadimefon (Bayleton), bitertanol (Baycor), and Bay HWG 1608 were screened for effectiveness in controlling southwestern rust on cotton (Gossypium hirsutum L.). Curative treatments of Baycor and Bayleton were applied at two rates (2 and 4oz active ingredient/acre) at first observed rust (7/18/84) and at two-wk intervals through 8/14/84. Baycor applications (4oz active ingredient/acre) significantly increased seed cotton yields as compared to other curative treatments and control. Preventative treatments of Baycor, Bayleton, and Bay HWG 1608 (same rates as above) were applied 6/5/84 and at three-wk intervals through 8/8/84. Bay HWG 1608 (4oz active ingredient/acre) significantly increased yield as compared to other preventative and control treatments.

### 837

EFFICACY OF FOSETYL-AL IN CONTROLLING METALAXYL-SENSITIVE AND-RESISTANT ISOLATES OF PHYTOPHTHORA INFESTANS. Yair Samoucha and Yigal Cohen, Bar-Ilan University, Israel 52100.

Twenty four isolates of P. infestans, collected in blighted potato fields in Israel during 1983-1985, were tested for sensitivity to metalaxyl and fosetyl-Al in potted potato (cv. Alpha) plants in growth chambers and in plants growing in plastic houses. While metalaxyl-sensitive isolates were fully controlled in plants sprayed with 1000 µg a.i./ml fosetyl-Al, metalaxyl-resistant isolates were unaffected in plants similarly sprayed with fosetyl-Al. Two interpretations of these data are suggested: (i) cross resistance occurs between the two fungicides, or (ii) the local fungal population is heterogeneous composed of Israeli isolates sensitive to both fungicides and European (imported) isolates resistant to metalaxyl and insensitive to fosetyl-Al.

### 838

EFFECTS OF FLOODING, CULTIVAR, AND CHEMICAL SEED TREATMENT ON ALFALFA STAND ESTABLISHMENT AND DAMPING OFF CAUSED BY PHYTOPHTHORA MEGASPERMA F. SP. MEDICAGINIS. L. H. Rhodes, D. K. Myers, and P. R. Henderlong, The Ohio State Univ., Columbus 43210.

Intermittent flooding of field plots for 2 wk after alfalfa seedling emergence reduced plant count (PC) and stand density rating (SDR) 10 wk after seeding in comparison to non-flooded plots. No difference (P=0.05) in PC between cultivars "Vernal" and "WL-312" (susceptible and resistant to Phytophthora megasperma f. sp. medicaginis (Pmm) respectively) were observed; however, "Vernal" had significantly higher SDR. Pre-plant seed treatment with

metalaxyl (up to 0.3 g a.i./kg seed) or pyroxyfur (up to 1.4 g a.i./kg seed) did not affect PC or SDR. In a greenhouse study, post-emergence damping off (PEDO) at 2 wk was higher, and PC and plant height (PH) at 4 wk were lower in field soil (FS) or autoclaved, Pmm-inoculated soil (Pmm-IS) in comparison to autoclaved soil. PEDO, PC, or PH did not differ between "Vernal" and "WL-312". PEDO decreased and PC and PH increased with increasing rates of metalaxyl (up to 0.3 g a.i./kg seed) in Pmm-IS or FS and with increasing rates of pyroxyfur (up to 1.4 g a.i./kg seed) in Pmm-IS.

### 839

SEPARATION AND ANALYSIS OF PENTAKETIDE MELANIN METABOLITES BY REVERSE PHASE HPLC. M. H. Wheeler and G. A. Greenblatt. National Cotton Pathology Research Laboratory, USDA, ARS and Dept. of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77841

Four intermediates and ten related secondary products involved in the production of fungal melanin in Verticillium dahliae, Pyricularia oryzae and a number of other imperfect and ascomycetous fungi were separated using reverse-phase HPLC. The metabolites were first adsorbed to reverse-phase Sep-Pak cartridges and then eluted with acidified acetonitrile. They were then separated on a C-18 column using a linear gradient of acidified acetonitrile. All but two of the compounds were separated with resolution factors of one or more. These two compounds could be separated isocratically. Examples are given where gradient and isocratic systems are used to analyze melanin metabolites from cultures and enzymic systems. The techniques should have wide applicability in future studies.

### 841

CONTROL OF FOLIAR DISEASES WITH FILM-FORMING ANTITRANSPIRANTS. O. Ziv and R. A. Frederiksen. Dept. of Field Crops, ARD, The Volcani Center, Bet-Dagan 50200 Israel, and Dept. of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

Several film-forming antitranspirants were evaluated as protectants from foliar pathogens on sorghum (Sorghum bicolor), wheat (Triticum aestivum), barley (Hordeum sativum), hydrangea (Hydrangea macrophylla) and crapemyrtle (Lagerstroemia caerulea). The antitranspirants 'Vapor Guard' and 'NU-Film' 17 (Miller Chemical and Fertilizer Co.), 'Wilt-Pruf' (Nursery Speciality), and 'Folicote' (Crystal Soap and Chemical Co. Inc.) reduced powdery mildew and rust in varying amounts, and effectively controlled some other foliar diseases. A 2-3% antitranspirant emulsion was sufficient to suppress the pathogen's development without causing visible phytotoxic effects or inhibiting plant growth. The antitranspirants controlled diseases as effectively as several commercial fungicides such as Benomyl, Bayleton, and Tilt.

### 842

OCCURRENCE AND CONTROL OF PLANT PARASITIC NEMATODES IN CREEPING BENTGRASS GOLF GREENS IN KANSAS. T. Todd and N. Tisserat, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Bentgrass greens from two Kansas golf courses with a history of summer thinning problems were sampled for plant parasitic nematodes. Maximum sample populations per 100 cm<sup>3</sup> soil for

parasitic nematode genera were 704 for Criconebella, 1232 for Helicotylenchus, 296 for Tylenchorhynchus, and 104 for Hoplolaimus. Application of ethoprop, isophenphos, and fenamiphos to a bentgrass green infested with Helicotylenchus and Criconebella spp. significantly reduced ( $p=0.1$ ) Helicotylenchus populations after 1 mo. After 3 mo, ethoprop and fenamiphos continued to suppress populations of Helicotylenchus. Criconebella populations were not significantly affected by any treatment. Fenamiphos-treated bentgrass plugs removed from plots and placed in the greenhouse for 122 days had significantly ( $P=0.05$ ) higher accumulations of grass clippings than non-treated plugs.

#### 843

GERMINATION OF Tilletia indica, T. controversa, AND T. caries TELIOSPORES AFTER INGESTION BY VARIOUS ANIMALS. J.L. Smilanick, M. Dupler, B. Coates, and J.A. Hoffmann. USDA-ARS, Logan UT 84322

Tilletia indica (TIM), T. controversa (TCK), or T. caries (TCT) teliospores were fed to Leghorn chickens and grasshoppers (Melanoplus sanguinipes), and TCT teliospores were placed in the rumen of a rumen-fistulated Holstein cow. Teliospores were recovered from feces and incubated on water agar under optimal germination conditions. All the feces contained viable teliospores, however, viability was reduced compared to non-ingested controls. Ingestion by grasshoppers reduced teliospore germination of TIM, TCK, and TCT to 70.0, 88.5, and 89.2% of controls, respectively; only TIM reduction was significant. Ingestion by chickens reduced TIM, TCK, and TCT teliospore germination significantly to 46.2, 71.7, and 8.3% of controls, respectively. Passage through a cow significantly reduced TCT germination to 36.1% of controls. TIM and TCK are subject to international quarantines. Movement of feces-derived fertilizers or of animals may contribute to dispersal of these pathogens.

#### 844

REDUCTION OF BEAN SEED EMERGENCE BY VACUUM INFILTRATION WITH BACTERIAL PATHOGENS. J. R. Venette. Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58105.

Bean seeds heavily contaminated with bacterial pathogens often fail to germinate or emerge. This study determined the level of Pseudomonas pathogen contamination that reduced bean emergence. Pregerminated Phaseolus vulgaris pinto and kidney bean seeds were vacuum infiltrated with four dilutions of Pseudomonas syringae pvs. phaseolicola and syringae, and were planted in sterile vermiculite in the greenhouse. Seeds vacuum infiltrated with water or untreated beans served as controls. Emergence was evaluated seven days after infiltration. Pv. syringae significantly reduced emergence of kidney ( $LD_{50}=5.6 \times 10^3$ ) and pinto beans ( $LD_{50}=1.5 \times 10^4$ ) when infiltrated with  $3 \times 10^6$  or  $3 \times 10^4$  cfu/ml. Emergence of beans treated with concentrations of 300 and 3 cfu/ml were not significantly different from controls. Only infiltration with  $3 \times 10^6$  cfu/ml of pvs. phaseolicola significantly reduced emergence of pinto beans ( $LD_{50}=2.2 \times 10^6$ ), but infiltration with  $3 \times 10^6$  or  $3 \times 10^4$  cfu/ml significantly reduced emergence of kidney beans ( $LD_{50}=9.1 \times 10^5$ ).

#### 845

EVALUATION OF SEED TREATMENTS FOR ERADICATION OF XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS FROM WHEAT SEED. R. L. Forster,

Univ. Idaho Res. & Ext. Ctr., Kimberly, ID 83341 and H. W. Schaad, Dept. Pl., Soil & Ent. Sci., Univ. Idaho, Moscow, ID 83843.

Several seed treatments were evaluated for eradicating Xanthomonas campestris p.v. translucens (Xct) from wheat seeds. Naturally infected seeds were treated with methoxyethylmercury acetate, methoxyethylmercury chloride, phenylmercury acetate, cupric hydroxide, calcium hypochlorite, sodium hypochlorite, calcium propionate, and acidified cupric acetate (ACA). After treating, seeds were assayed for Xct by 1) plating seeds and washings onto XTS agar and 2) sowing treated and untreated seeds in plots in Kimberly, ID. Xct was detected by laboratory assays in all treatments except ACA. In 1983 black chaff developed from all treatments except ACA. In 1984, black chaff developed in all treatments but was significantly less in ACA which was similar to check plots planted with seed having only a trace level of the pathogen.

#### 846

MICROFLORA ASSOCIATED WITH COMMON WHEAT VARIETIES IN TEXAS. Mathieson, J.T. and T.M. Mathieson. Research associate Texas Agricultural Experiment Station, Vernon, Texas and independent plant pathologist, Vernon, Texas 76384.

Seed harvested from eight hard red winter wheat varieties grown in research tests in five locations in Texas in 1984 were analyzed to determine fungal infestations. Varieties examined were Chisholm, Hawk, Mic, Payne, Sandy, Scout 66, Sturdy, and TAM-105. Seeds were obtained from plots located at Bushland, Chillicothe, Dallas, Overton, and Temple. One hundred fifty seeds of each variety were surface sterilized and placed onto PDA amended with streptomycin sulfate (200 µg/ml). Plates were incubated at 23C for 5-7 days and the total number and types of fungal colonies determined. Seeds were also scored for discoloration. Significant differences existed between locations but not varieties with reference to total colonies and seed discoloration. Common fungi present and frequency range were Alternaria spp. (23-80%), Bipolaris spp. (1-10%), and Fusarium spp. (0.4-1.5%).

#### 847

A new seedling disease of Cucumis melo incited by Fusarium equiseti in the south western San Joaquin Valley of California. G.C. Adams and R.G. Grogan; Dept of Botany and Plant Pathology, MSU, E. Lansing, MI 48824 and Dept of Plant Pathology, U of CA, Davis, CA 95616.

If soil temperatures remain cool, 15-18°C, following sowing, growth of seedlings is greatly reduced and hypocotyls exhibit dry sunken lesions. Symptoms are reproducible in seedlings grown at 15°C root temperature (27°C air temperature) in chloropicrin fumigated soils reinfested with F. equiseti (10,000 macroconidia/g air dried soil). After 14 days growth, seedlings in the infested soil were smaller by 40% in fresh weight than seedlings in fumigated soil. Characteristic lesions were present on hypocotyls and Koch's postulates were satisfied. Propagules of F. equiseti in field soils were 100 to 500 per gram air-dried soil. Disease symptoms and F. equiseti were not found in surveys of central and northern melon growing areas of the valley.