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ABSTRACTS

VERTICICLADIELLA PROCERA AND HETEROBASIDION ANNOSUM IN LOBLOLLY PINE PLANTATIONS. S.A. Alexander and W.E. Horner, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

In 3 thinned loblolly pine plantations located on annosus root rot low hazard soil types in the Holly Springs National Forest in Mississippi 117 trees were excavated from 9, 0.08 ha plots. Ten symptomatic roots were collected from each plot and removed to the laboratory for isolation. The roots were rated by symptom e.g. resinous and/or black stained. Verticicladiella procera and/or Heterobasidion annosum were isolated from 74% of the 90 roots sampled. H. annosum was present in 49% and V. procera in 46% of the roots. From 82 resinous roots, H. annosum was isolated from 51% and V. procera from 45%. From 20 black stained roots, V. procera was isolated from 65% and H. annosum was isolated from 10%. These findings suggest the possibility that V. procera may be contributing to the disease situation in these plantations. This is the first report of V. procera associated with H. annosum in loblolly pine plantations.

OBTAINING NUCLEASE-FREE HIGH MOLECULAR WEIGHT DNA FROM ERWINIA CAROTOVORA SUBSP. CAROTOVORA (EC14). C. Allen, V.K. Stromberg, and G.H. Lacy, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Isolation of high molecular weight (HMW) DNA from EC14, incitant of bacterial soft rot, is complicated by the presence of tenacious endonucleases complexed with bacterial DNA. pBR322 DNA was completely degraded in 60 min at 32C in the presence of EC-14 DNA isolated by a standard Marmur procedure. These endonucleases are resistant to extraction by phenol:chloroform and CsCl-ethidium bromide density gradient centrifugation, are not inactivated by 15 min at 100C, but are inactivated by 15 min at 121C. EDTA at 1.0mM but not 0.2mM inhibits acitivty. Nuclease-free HMW DNA was isolated from EC14 by lysing 1 g of cells in 16 ml saline-EDTA with 5 mg/ml lysozyme, 1% SDS, and 0.1 mg/ml proteinase K. Upon clearing, the lysate was extracted 3 times with phenol:chloroform, once with chloroform:isopentanol, and dialyzed. This procedure yielded mg quantities of DNA fragments > 100kb long, useful in hybridization studies, % G+C determination, and in construction of phage or cosmid genomic libraries.

MANAGEMENT OF LEAF RUST AND POWDERY MILDEW OF WHEAT UNDER MAXIMUM YIELD PRODUCTION. D.E. Babineau and E.L. Stromberg, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Foliar fungicides were evaluated for control of leaf rust and powdery mildew and their effect on yield on three soft red winter wheat cultivars: Blueboy, Coker 747, and Tyler. Plots (3.5 x 6 m) with rows 10 cm apart received 67 kg/ha N at Feekes' stages 3 and 6 and ethephon at stage 10. Disease indices were determined at stage 10.5. Leaf rust was severe on non-treated Tyler and moderate on non-treated Coker 747 and Blueboy while powdery mildew was most severe on non-treated Blueboy, moderate on non-treated Coker 747 and not detected on Tyler. Propiconazole was most effective in controlling leaf rust on Coker 747 and Tyler while benomyl + mancozeb was best on Blueboy. Triadimefon and propiconazole afforded equal levels of powdery mildew control on Blueboy and Coker 747. Yields of all cultivars tended to be greater as a result of fungicide applications although increases were not statistically significant (P=0.05) for Coker 747 and Tyler. Ethephon prevented lodging of Blueboy and Tyler while Coker 747 lodged slightly.

MORPHOLOGICAL COMPARISON OF PERONOSCLEROSPORA SACCHARI FROM TAIWAN WITH P. PHILIPPINENSIS FROM THE PHILIPPINES. M. R. Bonde, N. B. Duck, and G. L. Peterson. Plant Disease Research Lab., USDA-ARS, Bldg. 1301, Ft. Detrick, Frederick, MD 21701.

Maize seedlings and sugarcane cuttings were inoculated with conidia of P. sacchari (T. Miyake) C. G. Shaw (two isolates from Taiwan) and P. philippinensis (Weston) C. G. Shaw (two isolates from the Philippines), incubated in growth chambers 28 days (maize) or 35 days (sugarcane) at 26C day-21C night temperatures, and compared for symptoms. The plants then were moved into dew chambers (20C) to induce sporulation, and mature conidia were collected as they dropped onto water agar containing 1% copper sulphate to prevent germination. No significant differences in size or shape of conidia or conidiophores were noted when comparing the two species. Because P. sacchari and P. philippinensis could not be differentiated by morphological comparisons of their asexual stages, by symptoms, or by results of previous host range studies, they may be the same species.

CHARACTERIZATION OF SCLEROTINIA MINOR ISOLATES WITH TOLERANCE TO DICLORAN, IPRODIONE AND VINCLOZOLIN. T. B. Brenneman, P. M. Phipps, and R. J. Stipes, Dept. of Plant Path., Physiol., & Weed Sci., VPI & SU, Blacksburg, VA 24061.

Nine isolates of Sclerotinia minor with tolerance to dicloran (D), iprodione (I) or vinclozolin (V) were collected during in vitro ED_{50} assessments. They exhibited cross tolerance to all three fungicides and to pentachloronitrobenzene (PCNB). In field microplots infested with sclerotia from two tolerant isolates, both were less pathogenic to Florigiant peanut than the sensitive isolate from which they originated. Three spray applications of V (0.84 kg/ha) were effective in control of both sensitive and tolerant strains. Similar treatments with I (1.12 kg/ha) and D (2.8 kg/ha) provided only partial control. Disease incidence at harvest was suppressed 13, 20 and 84% as compared to untreated plots by D, I and V, respectively. Additional microplots were infested with equal numbers of sclerotia from three pairs of sensitive and tolerant isolates to compare competitive pathogenicity. Fifteen, 17 and 32% of isolates recovered from tissue at harvest were tolerant.

COMPARISON OF TILLAGE AND IRRIGATION COMBINATIONS ON STALK ROT OF FIELD CORN. K. J. Byrnes and R. B. Carroll, Plant Science Department, University of Delaware, Newark, DE 19717-1303.

Field corn (Zea mays) was grown in 1983 with conventional or no-tillage and was subjected to four irrigation treatments: (1) season long, (2) July 15 to end of season, (3) July 29 to end of season, and (4) no irrigation. Each treatment was replicated three times in a completely randomized design. Two hybrids, Pioneer 3358 and B73 x MO17, and two planting dates, May 1 and May 26, were included for each treatment. Stalk rot was rated by splitting the stalks and visually determining symptoms of infection. Stalk strength was measured in the field with a hand-held force gauge. For the early planting the interaction of no-tillage and irrigation significantly (P=.05) affected stalk rot on Pioneer 3358. For the late planting no-tillage significantly reduced stalk rot and increased stalk strength for Pioneer 3358, and irrigation provided the same effects for both hybrids.

GROWTH RESPONSE OF YELLOW POPLAR SEEDLINGS TO SIMULATED ACIDIC PRECIPITATION, OZONE, AND SULFUR DIOXIDE. A.H. Chappelka, B.I. Chevone and T.E. Burk, Dept. Plant Path., Physiol. & Weed Sci., and Dept. For., VPI&SU, Blacksburg, VA 24061.

Half-sib, 9 wk-old, yellow poplar, were exposed to O_3 and/or SO_2 (controls, 0.10 ppm O_3, 0.08 ppm SO_2 or 0.10 ppm O_3 + 0.08 SO_2, 4h/d, 5d wk) with simulated rain (pH 3.0, 4.3 or 5.6 1h/d, 2d/wk at 0.75 cm/h) for 6 wks, with rain applied either just before or after fumigation. Across all rain treatments, O_3 + SO_2 significantly decreased cumulative height growth (after 6 wks), and stem, leaf and total dry weights as compared to all other fumigation treatments. For these variables, there were no significant differences among rain treatments across fumigation treatments. A significant gaseous pollutant x rain interaction occurred for root dry weight. When pH 3.0 was compared to pH 5.6, dry weight decreased in the control and O_3 treatments and increased in the SO_2 and O_3 + SO_2 treatments. Fumigation of wet leaves as compared to dry leaves significantly reduced (> 15%) shoot growth, and stem and root dry weights across all other treatments.

CHANGES IN SOYBEAN METABOLITIES IN RESPONSE TO O_3 AND SO_2 FUMIGATION. B.I. Chevone[1], J.L. Hess[2], E. Pierson[2] and L.D. Moore[1], Dept. Plant Path., Physiol. & Weed Sci.[1], & Dept. Biochem. & Nutr.[2], VPI&SU, Blacksburg, VA 24061.

Soybeans (Glycine max) cv. 'Dare' (O_3 sensitive) and 'Williams' (O_3 tolerant), 45-d-old, were exposed simultaneously to 0.20 ppm O_3 and 0.70 ppm SO_2 or to filtered air for 2 h at 28 ± 2°C, 65 ± 10% R.H. and 380 ± 20 $\mu E\ m^{-2}s^{-1}$ PAR. Leaves were homogenized immediately after treatment and leaf concentrations of ascorbate, glutathione and H_2O_2, and glutathione reductase activity were measured. By the homovanillic acid assay, H_2O_2 concentrations were estimated to double in 'Dare' exposed to O_3 plus SO_2 compared to plants exposed to filtered air. A similar change in H_2O_2 levels was not found in the tolerant cultivar. The glutathione reductase activity was three-fold greater in 'Williams' compared to 'Dare' after fumigation. Ascorbate and total glutathione concentrations were not altered in fumigated plants.

ACIDIC PRECIPITATION AND OZONE EFFECTS ON GROWTH OF LOBLOLLY AND SHORTLEAF PINE SEEDLINGS. B.I. Chevone[1], Y.S. Yang[2] and G.S. Reddick[1], Dept. Plant Path., Physiol. & Weed Sci.,[1] VPI&SU, Blacksburg, VA 24061 and Dept. of Plant Path.,[2] Penn St. Univ., University Park, PA 16802.

Loblolly and shortleaf pine seedlings, 3 wks old, were exposed for 11 wks to all combinations of ozone (O_3) (0.00 or 0.08 ppm, 4h/d, 2d/wk) and simulated rain (pH 3.0, 4.3 or 5.6, 1h/d, 2d/wk at 0.75 cm/h). Seedlings were grown in Creedmoor or Altavista soils. After 11 wks, shoot and root dry weight and root length differed significantly between species and soils across pollutant treatments. Root length, at pH 3.0 compared to pH 5.6, was 27 and 20% less in loblolly pine on Altavista and Creedmoor soils, respectively. Root length was reduced 29% and root dry weight 28% at pH 3.0 compared to pH 5.6 in shortleaf pine on Altavista soil. Exposure to O_3 reduced shoot dry weight 20% in loblolly pine on Creedmoor soil and 17% in shortleaf pine on Altavista soil. Ozone also reduced root length 20% in shortleaf pine on Altavista soil across all pH treatments.

COMPARISONS OF THREE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) METHODS FOR THE DETECTION OF WATERMELON MOSAIC VIRUS (WMV), STRAINS 1 & 2. Robert F. Davis, Department of Plant Pathology, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, NJ 08903.

Three ELISA methods were tested for sensitivity and specificity using WMV-1 and WMV-2. All reactants were tested at various dilutions. The ELISA methods were 1) "standard double sandwich" (DS), 2) "double sandwich indirect" (DSI) using Fab and protein-A-enzyme conjugate, and 3) "direct-indirect" (DI) using direct adsorption of virus as the coating step and anti-rabbit enzyme conjugate (complete descriptions will be presented). Antibodies to WMV-2 were used for all tests. Specificity of reaction was highest with DS & DSI. The DI method reacted equally strong with both WMV-1 & WMV-2 except at high dilutions of sap. Sensitivity was greatest with DI, medium with DS, and lowest with DSI. Considerations of sensitivity, specificity, time and convenience will determine the most appropriate method. New Jersey Agricultural Experiment Station No. K-11191-2-84.

CURRENT STATUS OF VIRUSES INFECTING CUCURBITS AND MAIZE IN NEW JERSEY. Robert F. Davis, Department of Plant Pathology, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903.

Surveys were conducted to identify the viruses infecting cucurbit crops. Samples were collected from commercial fields and University test plots. Crops sampled included mainly Cucurbita pepo (summer squash and zucchini). Samples were assayed by double-sandwich indirect enzyme-linked immunosorbent assay (ELISA) for watermelon mosaic virus-strains 1 & 2 (WMV-1 & WMV-2), cucumber mosaic virus (CMV), squash mosaic virus (SqMV), and zucchini yellow mosaic virus (ZYMV). The percentages of infection for 122 plants tested were: WMV-2 (61%); CMV (15%); WMV-1 (4%); SqMV (2%); and ZYMV (0%). During 1982, maize dwarf mosaic virus (MDMV) occurred in near epidemic proportions in New Jersey. A survey indicated that MDMV was present in all major sweet corn growing areas. The incidence reached 100 percent in some fields. The major strain detected was the non-johnsongrass infecting strain (MDMV-B). New Jersey Agricultural Experiment Station No. K 11191-1-84 and K-11230-1-84.

VARIABILITY OF HOST REACTION OBSERVED IN RESPONSE TO PHAKOPSORA PACHYRHIZI SYDOW, CAUSAL AGENT OF SOYBEAN RUST. W. M. Dowler and J. L. Rytter. Plant Disease Research Laboratory, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701.

Several legumes commonly found in the southeastern and gulf coastal areas of the U.S. were evaluated for their reaction to the soybean rust causal organism, Phakopsora pachyrhizi. There were marked differences in symptom development on various hosts. Lesions on lespedeza were restricted by leaf veins, while those on lupines were not well defined. Lesions on hemp sesbania were more predominant on stem tissue. Soybean rust isolates from Brazil and Puerto Rico caused similar reactions and were more virulent on legume hosts other than soybean, while isolates from Taiwan were more virulent on soybean.

AN IMPROVED SELECTIVE MEDIUM FOR ISOLATION OF PSEUDOMONAS SYRINGAE PV. GLYCINEA FROM SEED, SOIL AND PLANT RESIDUE. D. J. Fieldhouse, M. Sasser, D. A. Burbage and C. F. Mucha. Department of Plant Science, University of Delaware, Newark, DE 19717-1303.

A modified and more selective recipe for the selective medium, BANQ, was compared with M71 (Leben, selective for P. syringae pv. glycinea), D4 (Kado and Heskett, selective for Pseudomonas SPP.) and nutrient agar (NA) for the isolation, identification and quantification of Pseudomonas syringae pv. glycinea, the causal agent for bacterial blight of soybean. The increased selectivity of the modified BANQ resulted in a 93% plating efficiency when compared to NA for 7 strains of P. s. pv. glycinea, and allowed growth of only small colonies of P. tabaci and P. tomato. M71 had a 117% plating efficiency, but allowed normal growth of 11 of 12 species of Agrobacterium, Erwinia, Xanthomonas and Pseudomonas tested. D4 had only a 73% plating efficiency and allowed normal growth of Erwinia carotovora and 6 Pseudomonas species.

ENCAPSULATION OF POTENTIAL BIOCONTROL AGENTS IN SODIUM ALGINATE AGGREGATES. D. R. Fravel[1], J. J. Marois[2], and W. J. Connick, Jr.[3]. [1]Univ. of Maryland, College Park, MD 20742, [2]USDA, ARS, Beltsville, MD 20705 and [3]USDA, ARS, New Orleans, LA 70179.

A method to encapsulate fungi that have potential to control plant diseases was tested. Melted and cooled 1% sodium alginate solutions were amended with 10% Pyrax® and either ascospores or conidia of Talaromyces flavus (isolate Tf1 or Tf1-1) or conidia of Penicillium oxalicum, Gliocladium virens, or Trichoderma viride (T-1-R9). The alginate-propagule-Pyrax® suspension was then dripped through Pasteur pipettes into a 0.25 M calcium chloride solution causing formation of solid aggregates. The aggregates were dried overnight and stored under room conditions. Populations of the fungi were estimated at 0, 2, 4, and 8 weeks after encapsulation by dissolving the aggregates in a mixture of 0.0087 M KH_2PO_4 and 0.07 M Na_2HPO_4 and plating onto potato dextrose agar amended with 0.3g chlorotetracycline and 1 ml Tergitol®/L. All fungi were viable after encapsulation. Populations ranging from 10^5 to 10^8/g declined over the test, resulting in approximately 10 to 100 fold losses after 4 weeks.

BEET LATENT ROSETTE DISEASE: CHEMOTHERAPY OF VECTORS. Monica Frosch and Karl Maramorosch. Department of Biology, Carleton University, Ottawa, Canada K1S 5B6, and Waksman Institute of Microbiology, Rutgers-The State University, Piscataway, New Jersey 08854.

Beet latent rosette disease, discovered in Germany by Schmutterer and Nienhaus in 1976, is caused by a rickettsia-like bacterium (RLB) transmitted by *Piesma quadratum*. Vectors are adversely affected by the RLB and usually die prematurely. Temporary remission of disease was obtained earlier by dipping roots of diseased plants in water solutions of penicillin or chloramphenicol (Frosch, 1983). Now we have tested the effect of tetracycline hydrochloride and amphothericin B methyl ester on vectors, fed on plants treated with these compounds, then transferred to untreated beet plants. The life span of vectors that had acquired tetracycline, but not amphothericin, was prolonged by several days and RLB transmission was interrupted or prevented.

USE OF ELISA TO DETECT POTATO LEAF ROLL VIRUS IN POTATO TUBERS. R.W. Goth and R.E. Webb, Vegetable Lab, USDA, ARS, Beltsville, Maryland 20705

Sensitive serological methods permit advances in detection of potato viruses. ELISA (enzyme-linked-immunosorbant-assay) was used to successfully evaluate potato tubers of a breeding population for potato leaf roll virus (PLRV). PLRV was not detected in any plants that developed from tubers classed as PLRV free. Use of this method to supplement the commonly used visual inspection procedures will reduce the number of PLRV plants that could serve as sources of PLRV infections in field test plots which could be an important factor is the epidemiology of this important insect transmitted-potato virus.

INTERACTION OF GLOBODERA SOLANACEARUM (=G. TABACUM SOLANACEARUM) AND PHYTOPHTHORA PARASITICA VAR. NICOTIANAE WITH FLUE-CURED TOBACCO. C.E. Grant, J.J. Reilly and A.P. Elliott, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

A tobacco cyst nematode, Globodera solanacearum (GS) and the black shank fungus, Phytophthora parasitica var. nicotianae (PPN) reduce yield and quality of flue-cured tobacco in Virginia. Types of interactions in greenhouse pots, microplots, and field plots were dependent on inoculum levels of pathogens, genotypes of cultivars, soil temperature and moisture. All combinations of inoculum levels of GS and PPN resulted in synergistic interactions on cv. Virginia 81 (highly resistant to GS and susceptible to PPN). On cvs. McNair 944 and Coker 319 (highly and moderately resistant to PPN and susceptible to GS), the interactions were synergistic at lower inoculum levels and antagonistic at high inoculum levels under greenhouse and microplot conditions. At high inoculum levels, under field conditions, all interactions were synergistic. Low soil moisture and high soil temperature contributed significantly to the interaction in cvs. with resistance to PPN.

DOSAGE EFFECTS OF BEET WESTERN YELLOWS VIRUS. A. D. Hewings, USDA-ARS Plant Disease Research Laboratory, Fort Detrick, Bldg. 1301, Frederick, MD 21701 and C. J. D'Arcy, Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Scarlet Globe radish and a parental sugarbeet line susceptible (SP 6322-0) and a line resistant (C17) to BWYV were inoculated with 1, 5, or 20 viruliferous aphids. When virus titer was measured by ELISA on radishes grown in a growth chamber, titer differences attributable to dose were distinct 4 days after inoculation. At 8, 12, and 16 days, dosage effects were more pronounced in all tissues except cotyledons. In a field study, no dosage effects were observed on sugarbeet. Acquisition efficiency of BWYV by Myzus persicae is 100% when individual late-instar aphids raised on BWYV infected plants are analyzed by ELISA. Absorbance is less variable from aphids analyzed from the same radish plant than from different plants. Transmission efficiency of BWYV by M. persicae averages 74% on radish and 40% on sugarbeet.

INTERACTIONS BETWEEN VIRULENT CANKERS OF ENDOTHIA PARASITICA AND SOURCES OF VIRULENT AND HYPOVIRULENT INOCULUM ON AMERICAN CHESTNUT. D. L. Hobbins and W. L. MacDonald, WVU Plant Path. and Ag. Micro., 401 Brooks Hall, P. O. Box 6057, Morgantown, WV 26506-6057.

This study was undertaken to determine the types of isolates that result after virulent (v) cankers are exposed to v or hypovirulent (hv) Endothia parasitica inoculum. Brown v strains were used as color markers to initiate cankers on American chestnut. After 8 wks. growth, resultant cankers were exposed to v or hv orange inoculum growing on bark pieces placed 10 cm above the canker. The cankers were sampled after a 10-week exposure to inoculum and the recovered isolates compared to the original strains. Cankers exposed to hv inoculum yielded over 50% brown v colonies; 35% of the samples resembled the orange hv strains in growth habit, but were pigmented light brown. Eighty percent of the isolates recovered from cankers exposed to v inoculum resembled the brown v cultures; others resembled orange v strains. Establishment of cytoplasmic agents in the v canker thallus suggests that transfer of hv agents is possible after a short exposure to naturally disseminated hv inoculum.

FUMIGANT NEMATICIDES TO CONTROL THE TOBACCO CYST NEMATODE (GLOBODERA SOLANACEARUM = GLOBODERA TABACUM SOLANACEARUM) IN FLUE-CURED TOBACCO. D.A. Komm, So. Piedmont Center, VPI&SU, Blackstone, VA 23824.

Fumigant nematicides; Soilbrom 90®, Terr-o-cide 54-45®, Terr-o-cide 30®, Telone C-17®, Telone II®, Vorlex®, and Vorlex® were applied preplant at 2 locations in formulated rates at 37, 37, 75, 112, 81, 37 and 47 l/ha, respectively. In addition, a non-fumigant nematicide, Nemacur 3®, was applied preplant at 6.7 kg ai/ha. The effect of the nematicides on the tobacco cyst nematode (Globodera solanacearum) was determined by measuring plant and root vigor, yield, plant height, and stalk diameter of flue-cured tobacco. All treatments increased yields and certain plant characteristics at both locations. Greatest yield increases resulted from Nemacur 3® followed by treatments of Soilbrom 90®, Terr-o-cide 54-45 and 30®, Telone C-17®, Telone II®, and Vorlex®.

EFFECTS OF FUNGAL CONTROL AGENTS AND MEDIA AMENDMENTS ON FORMATION AND SURVIVAL OF PHYTOPHTHORA CINNAMOMI PROPAGULES IN PINE BARK. S. Kularatne and W.H. Wills, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Pine bark (PB) was mixed with peat 9:1 (PP) with Weblite® and peat 1:1:1 (PW) and with clay loam soil 1:1 (PS) and treated, after steaming, with Truban®, Subdue®, Aliette® and Mortierella alpina (Ma). Nylon mesh squares (1x1 cm), colonized by Phytophthora, were put at three depths in 475 cc pots of the media and examined for propagules 2, 4, 8 and 16 days later. Sporangia and chlamydospores reached maximum numbers in 2 days in all media, then declined rapidly. Production and survival of both were highest in PB and least in PS where sporangia were almost eliminated in 16 days. Chlamydospores survived all treatments for 16 days, but only at low levels in Truban and Subdue treated PS. Truban and Subdue suppressed formation and survival of both propagules greatly, Aliette and Ma very little. Greatest suppression of both propagules was in PS at pH 5.7 and least in PB at pH 4.6. Significant three-factor interactions indicated complex relations among media, control agents and time.

FATTY ACID PROFILING OF PLANT RELATED BACTERIA. L. T. Miller, Hewlett-Packard Company, Route 41, Avondale, PA 19311 and M. Sasser, University of Delaware, Newark, DE 19717-1303.

Gas chromatographic profiling of bacterial fatty acids is a rapid and sensitive technique for identification of plant related bacteria. The saponification, methylation and analysis of the extract can be completed in less than 90 minutes. Recent advances in high performance gas chromatography, including fused silica capillary columns, have increased the confidence level of acid identification by improving retention time stability and increasing resolution to allow the separation of peaks which could not be resolved with a packed column. Due to the variety of unusual fatty acids and the stability of the acids within a related group of bacteria, it is often possible to distinguish bacteria to the subspecies or pathovar level.

MORPHOLOGICAL COMPARISONS OF TILLETIA INDICA (MITRA) FROM INDIA AND MEXICO. G. L. Peterson, M. R. Bonde, W. M. Dowler and M. H. Royer. Plant Disease Research Laboratory, USDA-ARS, Fort Detrick, Bldg. 1301, Frederick, MD 21701.

Karnal bunt of wheat, first described in 1931 near Karnal, India, recently became of concern to the USDA when the disease was discovered in Sonora, Mexico, and infected seed was intercepted in wheat entering the U.S. from Mexico. Questions arose as to whether the pathogen in Mexico is the same as that in India. Teliospore diameters for four isolates from Punjab, India, and three from Sonora, Mexico, were nearly identical (38.0-41.5 um). The ranges of mean lengths and widths of primary sporidia were 64.4-78.8 um, and 1.6-1.8 um, respectively. The ranges of mean lengths and widths of secondary sporidia were 11.9-13.0 um and 2.00-2.03 um, respectively. There were no significant differences among the isolates for any of these parameters. Morphologically, the pathogens from India and Mexico could not be differentiated.

Competitive pathogenicity and control of Sclerotinia minor and S. sclerotiorum on blight susceptible and resistant peanuts. P. M. Phipps, Tidewater Res. Ctr., VPI&SU, Suffolk, VA 23437.

Florigiant and VA 81B peanuts were planted in twin rows (18 cm apart) on 0.9 m centers in 1982. Plots were 1.8 m wide, 12.2 m long and replicated four times. Apothecia of Sclerotinia minor (Sm) and S. sclerotiorum (Ss) were abundant on the soil surface before land preparation, but only apothecia of Ss were found subsequently. Fungicides were applied in 327 L spray/ha on Aug 4 when initial blight symptoms appeared. A second spray was applied 4 wk later. Tissue assays indicated Sm caused ca 50 and 100% of symptoms on Florigiant and VA 81B, respectively. Disease foci in untreated plots at harvest averaged 42.5 and 8.8 on Florigiant and VA 81B, respectively. Sprays of vinclozolin (0.84 kg/ha), iprodione (1.12 kg/ha) and dicloran (4.2 kg/ha) on Florigiant suppressed disease counts 56, 49, and 22%, respectively. Yields of Florigiant were increased significantly by vinclozolin and iprodione. VA 81B yielded significantly less than Florigiant only in comparison of VA 81B untreated to Florigiant treated with vinclozolin.

DETECTION OF VIRAL-LIKE PARTICLES IN GRAPEVINE EXTRACTS. E. V. Podleckis and M. K. Corbett, Botany Dept., Univ.Maryland, College Park, MD 20742.

Previously we reported spherical and flexuous rod viral-like particles in ultrathin tissue sections of Vidal-256 grapevines exhibiting symptoms of "little grape" (Phytopathology 72:710). Extracts from young leaves or roots of grapevines were ground (1:5 w/v) in 0.01M phosphate buffer (pH 7.0) containing 2.5% nicotine. One drop of the extract placed on a carbon-coated collodion-covered electron microscope grid for about one min was rinsed dropwise with 2-5 drops of extraction buffer and negatively stained dropwise with 2-4 drops of 2% aqueous ammonium molybdate, pH 4.5. Residual liquid was removed with absorbent paper and the preparation air dried. Electron microscopy of such preparations showed membrane associated 29 nm spherical viral-like particles and flexuous rod viral-like particles about 11 X 800 nm with crossbanding subunits similar to those associated with closteroviruses. Closterovirus like particles were also detected in extracts from Mission and Emperor grapevines purported to be infected with leafroll.

A PATHOGENIC FLUORESCENT PSEUDOMONAD ASSOCIATED WITH BRASSICA SEEDS. P.S. Randhawa, Fruit Lab, BARC-W, Beltsville, MD 20705 and N.W. Schaad, Univ. of Idaho, Moscow, ID 83843.

During root colonization in seedling bioassay chamber collard seeds treated with washings of 2 cabbage seed lots resulted in poorly developed seedlings. A bacterium fluorescent on King's B agar was isolated from roots of these seedlings. The bacterium was Gram negative, rod shaped and oxidase negative. Plating seeds of these two seed lots on germination paper resulted in reduced germination and radicles of many germinated seeds rotted. Samples (400 seeds each) of untreated and surface disinfested (1:5 clorox for 10 min) seeds of one lot produced 6 and 14% infected seedlings, respectively. Soaking seeds of healthy collards for 1 min in 1.2×10^8 CFU/ml of the causal bacterium and sowing in the greenhouse resulted in reduction of germination by 21%. Furthermore, 16% of the resulting seedlings showed symptoms. The symptoms consisted of highly stunted seedlings with chlorotic or purple cotyledons for at least 2-3 weeks. Inoculum as low as 1.2×10^3 CFU/ml resulted in typical symptoms of disease within 2-5 days.

DISEASE DEVELOPMENT AND CYST REPRODUCTION ON TOBACCO SPLIT ROOT SYSTEMS INFECTED BY GLOBODERA SOLANACEARUM AND PHYTOPHTHORA PARASITICA VAR. NICOTIANAE. J. J. Reilly, C. E. Grant, A. P. Elliott, So. Piedmont Center, VPI & SU, Blackstone, VA 23824 and Plant Pathology, VPI & SU, Blacksburg, VA 24061, respectively.

Globodera solanacearum and Phytophthora parasitica var. nicotianae, were placed on either half or on both halves of a divided tobacco root system. Each half of the root system was grown in a separate pot. The nematode on one-half of a root system did not increase root necrosis of the fungus-infected half over root necrosis caused by the fungus alone. The fungus did increase root necrosis on the nematode-infected half compared to the nematode alone. Evidence indicated that both pathogens were needed on the same root system to cause severe root necrosis. When G. solanacearum and P.p. nicotianae were on separate halves of a split root system, the nematode population increased compared to G. solanacearum alone.

BIOLOGICAL CONTROL OF RHIZOCTONIA BLACK SCURF ON POTATO. Jean Beagle-Ristaino and G. C. Papavizas. Dept. of Botany, Univ. of Maryland, College Park, MD 20742; and Soilborne Diseases Laboratory, USDA, ARS, Beltsville, MD 20705.

The efficacy of several fungal antagonists for control of Rhizoctonia solani on potato was evaluated in greenhouse and field tests. Trichoderma viride (benomyl-resistant biotype T-1-R9) and Gliocladium virens (isolate G1-21) applied as dusts to sclerotia-infested seed potatoes before planting reduced disease incidence by 50% and 45%, respectively, in a Beltsville field. Disease incidence on potato with these antagonists was not significantly different from chemical control with PCNB. Isolates of T. hamatum (Tri-4, Tm-19, Tm-23) and T. harzianum (WT-6) significantly reduced disease severity in the field. Viability of sclerotia from seed pieces retrieved from the field was reduced 54% or more by antagonists. In the greenhouse, up to 88% reduction in germination of sclerotia was obtained by treating sclerotia-infested seed potatoes with antagonists before planting.

EPICHLOË TYPHINA IN COLONIES OF AGROSTIS PERENNANS. C.W. Roane and M.K. Roane, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

The fungus Epichloë typhina (Pers. ex Fr.) Tul. is suspected of being the toxicogenic fungus associated with fescue foot and summer syndrome of cattle grazing on Festuca arundinacea Schreb. In the summer of 1983, a high percentage of plants in colonies of Agrostis perennans (Walt.) Tuckerm. growing on the southern slope of Gap Mt. in Montgomery Co., Va., was found to have white wefts of mycelium characteristic of E. typhina, the cause of choke disease of grasses. Diseased host plants were in discontinuous but dense colonies growing in a logging trail about 0.5 km long. The white mycelia developed into yellow-orange, perithecium-bearing stromata. A strong, mushroom-like odor was associated with the developing stromata. In quiet air, this odor was detectable several feet from diseased grass colonies. Since inoculation attempts with E. typhina have been difficult in the past, A. perennans is a candidate for inoculation studies with the fungus.

ERWINIA CAROTOVORA SUBSP. CAROTOVORA DNA CLONED IN PLASMID pBR322 AND ISOLATION OF GENES FOR PECTOLYTIC ACTIVITY. D.P. Roberts, C. Allen and G.H. Lacy, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Erwinia carotovora subsp. carotovora EC14 DNA was cloned to study the genetics of pathogenicity. Fragments of EC14 DNA, from partial digests of chromosomal DNA restricted by endonuclease Pst I, were ligated using T4 ligase into restricted plasmid pBR322 dephosphorylated with calf intestinal phosphatase. Resultant chimeric plasmids were transformed into Escherichia coli DH1. Screening for cloned EC14 DNA was performed on media containing ampicillin and/or tetracycline. Pst I inserts had ampicillin-sensitive and tetracycline-resistant phenotypes. Clones containing genes mediating pectolytic activity were detected on pectate-yeast extract agar adjusted to pH 7.8 and pH 5.4. Pectolytic colonies were surrounded with clear halos after precipitating undecomposed polygalacturonic acid with 2N HCl.

PAECILOMYCES LILACINUS AS A BIOLOGICAL CONTROL AGENT OF MELOIDOGYNE INCOGNITA. E.R. Rodriguez and R.J. Young, WVU Plant Pathology & Ag. Micro., 401 Brooks Hall, P.O. Box 6057, Morgantown, WV 26506-6057.

Laboratory, greenhouse and field experiments were conducted to determine the potential of P. lilacinus as a biological control agent of Meloidogyne incognita, a root-knot nematode. Physiological studies showed that P. lilacinus had an optimum temperature for radial growth of 25-30°C., utilized a broad range of carbon and nitrogen sources and was not deficient for thiamine, biotin or pyridoxine. When P. lilacinus was introduced into greenhouse or field soil, root-knot galls on tomato roots were reduced. Microscopic examination of eggs, second stage larvae, and adult females from tomatoes grown in fungus-infested soil failed to show systemic hyphae, but hyphae were seen proliferating throughout egg masses, and surrounding individual eggs. Paecilomyces lilacinus was recovered from 76% of the egg masses. Its presence reduced the number of root-knot nematode juveniles collected from misted root systems by 76%. Evidence suggests that P. lilacinus parasitizes the root-knot nematode reducing second stage larvae and galling.

EXPRESSION OF AN ERWINIA CHRYSANTHEMI PECTATE LYASE ISOZYME GENE IN ESCHERICHIA COLI. David L. Roeder, Jeffrey L. Ried and Alan Collmer. Department of Botany, University of Maryland, College Park, MD 20742.

The production of pectate lyase (PL) was examined in Escherichia coli HB101 isolates containing recombinant plasmids with Erwinia chrysanthemi DNA inserts. Isoelectric focusing revealed that plasmids pCSR1 and pCSR6 encode an isozyme with an apparent pI of 8.3. Plasmid pCSR50, which contains the pCSR1 insert in reverse orientation, expresses PL at a level only ca. 2% of that of pCSR1. During logarithmic growth in a glycerol-casamino acids minimal medium, the rate of PL production by E. coli isolates carrying pCSR1 and pCSR6 was repressed up to 6-fold by addition of glucose but was unaffected by the addition of digalacturonic acid or galacturonan. PL assays of culture supernatants, osmotic shock fluids and intracellular extracts of mid-logarithmic phase cultures showed that over 95% of the total activity in E. coli cells carrying pCSR1 and pCSR6 was located in the periplasmic space.

A USER-FRIENDLY DATA RECORDING PROGRAM FOR THE TRS80 MODEL 100 PORTABLE COMPUTER. M. H. Royer, Plant Disease Research Laboratory, USDA-ARS, Ft. Detrick, Bldg. 1301, Frederick, MD 21701.

A BASIC language algorithm was written to prompt the person collecting data for input. The program assumes no previous computer exposure and allows for many input errors, with prompts for correct data entry. It will accommodate up to a six level factorial, with any number of levels for any one factor. The program allows for data inspection and editing at any point. The stored data and comments may then be sent to any other compatible computer with communications ability. The program stores data in a form that is directly compatible with many statistical packages. Use of the Model 100 can significantly reduce the time required to analyze data, and prevents human error in re-entering the data into a computer for analysis.

A COMPARISON OF TEMPERATURE AND PHOTOPERIOD REQUIREMENTS FOR TILLETIA INDICA TELIOSPORE GERMINATION. M. H. Royer and J. Rytter, Plant Disease Research Laboratory, USDA, ARS, Fort Detrick, Bldg. 1301, Frederick, MD 21701.

Karnal bunt, caused by Tilletia indica, has caused recent concern due to the appearance of the fungus in the Yaqui and Mayo valleys of Mexico. Should the disease spread to the U.S., serious deleterious effects to U.S. wheat production and export may result. Teliospores from Sangrur, India and the Yaqui Valley in Mexico were tested for their germination responses to 5, 10, 15, 20, 25, and 30 C on water agar over 30-40 days. Very little germination occurred at 5 or 30 C, whereas 15 to 20 C was optimal for both geographical sources. The maximum average percentage germination was 55%. The quadratic response function of germination over time was significantly different between the Mexican and Indian collection at 25 C, with germination of the Mexican collection being slightly higher. Germination was enhanced with a 12 or 24 hour daily exposure to light.

DECREASED FUNGICIDE SENSITIVITY IN TARGET PATHOGEN POPULATION ASSOCIATED WITH FAILURE OF METALAXYL TO CONTROL PYTHIUM BLIGHT ON TURFGRASS

P. L. Sanders, Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802

Sixty to seventy-five percent of the propagules of Pythium aphanidermatum recovered from a site where metalaxyl failed to control Pythium blight on turfgrass grew without inhibition on a medium containing 50 and 100 ppm metalaxyl. Representative isolates were pathogenic on pot-grown bentgrass, producing symptoms typical of Pythium blight. These isolates were more aggressive than a pool of wild-type P. aphanidermatum, and were not controlled by the commercial use rate of metalaxyl on pot-grown bentgrass. They were, however, adequately controlled in vivo by propamocarb and fosetyl aluminum.

EFFECT OF NITROGEN FORM ON VASCULAR DISCOLORATION AND XYLEM SAP COMPOSITION OF YELLOW-POPLAR INOCULATED WITH VERTICILLIUM ALBO-ATRUM. C. J. Sanford and A. L. Morehart, Plant Science Department, University of Delaware, Newark, DE 19717-1303.

Nutrient solutions containing different nitrogen forms (NO_3-N, NH_4-H, NH_4NO_3, or no N) were applied bi-weekly to one-yr-old yellow-poplar seedlings grown in sand. After 5 wk xylem sap was extracted from some plants while others were stem-inoculated with Verticillium albo-atrum. After 17 wk all plants were harvested, evaluated for vascular discoloration, and the xylem sap extracted. Xylem sap from both collections was analyzed for ninhydrin positive nitrogenous compounds. Inoculated seedlings which received NO_3-N exhibited greater ($p<.05$) vascular discoloration than those which received NH_4-N or no N but did not exhibit greater discoloration than those which received NH_4NO_3. Analysis of xylem sap revealed 22 free amino acids and NH_4^+ with no qualitative and few quantitative differences between treatments. Xylem sap amino acid level had no evident relationship to pathological vascular discoloration.

MOLECULAR CLONING OF AN ERWINIA CHRYSANTHEMI PECTATE LYASE ISOZYME GENE IN ESCHERICHIA COLI. Christianne Schoedel and Alan Collmer. Department of Botany, University of Maryland, College Park, MD 20742.

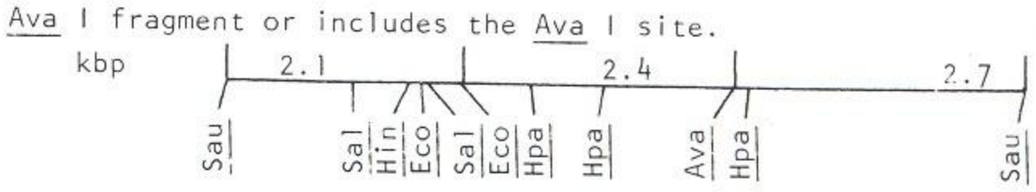
A gene coding for pectate lyase in the soft rot pathogen, Erwinia chrysanthemi, was cloned into Escherichia coli HB101 by inserting Sau 3A-generated DNA fragments of ca. 7 kb into the Bam H1 site of plasmid vector pBR322. Restriction mapping revealed that three distinct recombinant plasmids were present in the eight pectolytic clones originally recovered. Two of these plasmids, pCSR1 and pCSR50, have the same insert in reverse orientation. The insert in pCSR6 contains a ca. 4.5 kb segment that is also present in pCSR1 and pCSR50. The inserts in pCSR6 and pCSR50 have the same orientation. Pectate lyase-producing subclones were obtained by generating deletion derivatives of pCSR1 and pCSR6. Pectolytic, recombinant E. coli isolates induced limited maceration of potato tuber slices.

EVALUATION OF RESISTANCE TO MONILINIA FRUCTICOLA IN PEACH. Ralph Scorza and T. van der Zwet, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, West Virginia 25430

Twenty-one peach genotypes were evaluated for reaction to infection by M. fructicola. Fruit were sampled based upon average fruit pressure (maturity) of a subsample. Brix was also recorded. Forty undamaged fruit of each genotype were collected at each of 3 pressures. Fruit were washed, dried, evenly sprayed with 30 ml of a 2×10^5 conidial suspension, and incubated in a moist chamber at 27° C. Percent infected fruit was determined at 3, 6, and 9 days after inoculation. Fruit pressure, genotype, and length of incubation were significant sources of variability in infection. There was no relationship between brix and infection. Based on percent infection, significant differences in cultivar susceptibility were apparent following 3 days incubation. After 6 or 9 days, infection levels were greater than after 3 days, but cultivars were rated as equally susceptible. Determinations of resistance to M. fructicola in peach must take into account fruit maturity and duration of host-pathogen interaction.

RESTRICTION AND SUBCLONE ANALYSES OF THE ERWINIA CHRYSANTHEMI DNA FRAGMENT MEDIATING PECTATE LYASE PRODUCTION IN PLASMID pPL-1. V.K. Stromberg and G.H. Lacy, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Plasmid pPL-1, containing a 7.2 kbp Sau 3A fragment of E. chrysanthemi DNA with the pectate lyase (PL) gene, was a gift from Dr. A. Collmer, Dept. Botany, Univ. Maryland. Our endonuclease analyses with Ava I, Eco RI, Hpa I, Hind III and Sal I indicated the order of restriction sites on the fragment as shown below. The enzymes Bam HI, Kpn I, Pst I, Xba I and Xho I did not restrict the fragment. We subcloned the 5.1 kbp Sau 3A-Eco RI fragment and the 2.4 kbp Eco RI-Ava I fragment into plasmids pVS-1 and pVS-3, respectively. Since only pVS-1 mediates PL production, the PL locus is probably within the 2.7 kbp Sau 3A-Ava I fragment or includes the Ava I site.



FIELD INOCULATION AND SPREAD OF RUST, PUCCINIA CANALICULATA ON YELLOW NUTSEDGE. E. M. Sutker and S. C. Phatak. USDA, ARS, NER, PDRL, Bldg. 1301, Ft. Detrick, Frederick, MD 21701 and University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793, respectively.

Puccinia canaliculata (Schw.) Lagerh. has shown efficacy as a biocontrol of yellow nutsedge. Methods and time of introducing rust inoculum into field stands of yellow nutsedge were studied at Tifton, GA in 1982 and 1983. Introducing the rust in early April by transplanting heavily rusted plants from the greenhouse to a thick stand of yellow nutsedge resulted in secondary spread in 14-21 days and an epidemic within 8 weeks. Initial spread was primarily down wind. Spraying an urediniospore suspension in high performance liquid chromatography (HPLC) water with Triton B-1956® in June resulted in an average of 300 infected leaves/m^2 as compared to 2 leaves/m^2 in the non-inoculated plots. Secondary spread created an epidemic throughout the experimental area in approximately three weeks.

A NEW LEGUME STRAIN OF TOBACCO MOSAIC VIRUS FROM SOYBEAN IN YUGOSLAVIA. N. Taraku and S. A. Tolin. University of Kosova, Prishtinë Inst. Plant Production, Pejë., Yugoslavia and Dept. of Plant Path., Phys., and Weed Sci., VPI&SU, Blacksburg, VA 24061.

Soybean (Glycine max (L.)Merr.) plants growing in the Province of Kosova in southern Yugoslavia were infected with tobacco mosaic virus (TMV). Symptoms in soybean were veinclearing with mild chlorotic mosaic. On the basis of host range, symptomatology, light and electron microscopy, properties in vitro and serological tests, the isolate was identified as a new strain of TMV. It differed from both common (TMV-C) and bean (TMV-B) strains of TMV in host range and symptoms induced. In addition to soybean, Pisum sativum L. cv. Little Marvel and Trifolium pratense L. cv. Kenland were infected. Phaseolus vulgaris L., cvs. Pinto, Tendercrop, Topcrop and Bountiful, and Lycopersicon esculentum cv. Rutgers were not infected. Lesions distinctive from TMV-C were induced on Chenopodium, Datura and Nicotiana. In serological tests, the new strain was closely related to TMV-C but only distantly related to TMV-B. Virus purified from tobacco plants sedimented as several components.

EFFECT OF PATHOGEN VIRULENCE ON INHERITANCE OF PHYTOPHTHORA RESISTANCE OF SAFFLOWER. C. A. Thomas. Plant Pathology Lab., PPI, ARS, USDA, Beltsville, MD 20705.

The inheritance of resistance of safflower to isolates of Phytophthora drechsleri possessing three distinct levels of virulence was determined in the greenhouse. The isolates were highly virulent on 'Nebraska 10' safflower, weakly virulent on 'USB' safflower, and highly virulent, moderately virulent, and weakly virulent on the F_1 hybrid. Ten-week-old plants of Nebraska 10, USB, the F_1, and the F_2 and BC_1 populations were inoculated by smearing a plug cut from a lima bean agar plate culture into an incission made lengthwise in the middle of the stem. The inoculated area was covered with moist cheesecloth and aluminum foil. Resistant plants survived to maturity. The reactions indicated that resistance to the highly virulent, moderately virulent, and weakly virulent isolates is conditioned, respectively, by double recessive, single recessive, and single dominant factors.

RECOVERY OF EPIPHYTIC ERWINIA AMYLOVORA FROM APPARENTLY HEALTHY APPLE TISSUES IN THE ORCHARD. P. D. Van Buskirk, T. van der Zwet and M. Sasser. USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430; and Plant Science Department, University of Delaware, Newark, Delaware 19711.

Buds, blossoms, leaves, stems, and fruit were collected from 30-year old Rome apple trees at weekly intervals between early March and late June and were brought to the laboratory for processing. Tissues (10 stems, 25 buds, or fruit and 200 blossoms or leaves) were placed in flasks with 10-100 ml of distilled water, agitated for 3 min, and the liquid poured off. With a sterile pipet, 0.1 ml samples of a 1:1 and 1:100 dilution of these aliquots were plated on Miller-Schroth selective medium, incubated at 26 C for 48 hrs, and colonies of E. amylovora were counted. Cultures were tested for authenticity through gas liquid chromatography and through pathogenicity on young apple fruit and succulent Jonathan shoots in the greenhouse. A high percentage of the washings from the various apple tissues yielded high numbers of E. amylovora isolates, especially after early May.

SEVERE FIRE BLIGHT ON LOW CHILLING PEAR IN THE NILE DELTA. T. van der Zwet, USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430, and K. Y. Mickail, Plant Pathology Research Institute, Ministry of Agriculture, Cairo.

Following the initial outbreak of fire blight (Erwinia amylovora) on pear in Egypt in 1964, the disease apparently remained quiescent for two decades. Optimum weather conditions during bloom in 1982 and 1983 appear to have been responsible for the severe occurrence of blossom blight in April 1983. Loss of fruit per tree varied from 10-75%. The hot and dry desert climate, starting soon after bloom and continuing until November, initiated early canker formation and apparently reduced further spread of the disease. Young, non-bearing pear trees were not affected but fire blight was quite severe on bearing quince trees. Nearly all of the pear acreage in Egypt consists of the one low chilling variety "Le Conte' which is highly susceptible to fire blight. Recommendations for control during 1983-84 consist of three sprays of Bordeaux mixture between harvest and bloom and two applications of streptomycin during bloom.

THE DEVELOPMENT OF A PEST AND ORCHARD MANAGEMENT EXPERT SYSTEM USING PROLOG, A LOGIC PROGRAMMING LANGUAGE. R.S. Virkar, M.J. Weaver, J.W. Roach and C.R. Drake, Depts. Computer Science & Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

An orchardist's problems typically include diseases, insects, weeds, low temperature injuries and drought. PROLOG, a logic programming language, was used to build POMME (Pest and Orchard Management Expert system) on a VAX 11/780 computer as an aid to apple growers. An expert system emulates the way a human expert thinks and comes to a conclusion. The knowledge base of POMME was developed by multiple interviews with a human expert; in this case a plant pathologist. It was encoded as declarative frames in PROLOG rules. This knowledge base consists of a set of Structural Primitives and Blocks that can be used hierarchically. POMME's knowledge base is being supplemented with "deep models" to give POMME more sophisticated reasoning capabilities. POMME has been developed to evaluate the use of state-of-the art technology which delivers pest control information to growers and is feasible as an educational tool.

A METHOD FOR RAPID PRODUCTION OF PROTOPLASTS FROM USTILAGO MAYDIS SPORIDIA. W. F. Waterfield and H. D. Sisler, Botany Dept., Univ. Maryland, College Park, MD 20742

An enzyme preparation from culture filtrates of Trichoderma harzianum was used to prepare protoplasts of U. maydis sporidia. T. harzianum was grown for 7 days at 30 C on a low glucose (2.5 g/liter) nutrient medium containing 2 g/liter U. maydis sporidia previously extracted with ethanol, ethanol: hexane (1:1 v:v), and hexane. A twenty fold concentration of the enzyme in the culture filtrate was obtained by $(NH_4)SO_4$ precipitation (400 g/liter) and dialysis against distilled water for 24 hours. When 7.0g of sporidia (fresh weight) were added to 50 ml of the dialyzed preparation containing 0.6 M $(NH_4)SO_4$ as a protoplast stabilizing agent, protoplasts were released from 95-98% of the sporidia within 20 min at 30 C.

CHARACTERISTICS OF RESPIRATION IN A WILD TYPE AND AN ERGOSTEROL-DEFICIENT MUTANT OF USTILAGO MAYDIS. W. F. Waterfield and H. D. Sisler, Botany Dept., Univ.Maryland,College Park,MD 20742

Characteristics of respiratory activity of a sterol C-14 demethylation-deficient mutant of U. maydis (erg-40), which lacks ergosterol but contains C-14 methyl sterols, were compared with those of the wild type (WT). Respiratory cytochromes similar to those in the WT were detected in erg-40. Both strains contain an electron transport pathway sensitive to azide and antimycin A and a pathway insensitive to these inhibitors. The erg-40 mutant consumes O_2 and glucose more rapidly than the WT, but grows at a slower rate. O_2 uptake is stimulated by 4 µg/ml of the uncoupling agent, 4,5-dichloro-2-triflouromethylbenzimidazole, in the WT but not in erg-40. Oligomycin (2 µg/ml) strongly inhibits respiration of the WT but only slightly inhibits that of erg-40. It is possible that C-14 methyl sterols in the inner mitochondrial membrane affect oxidative phosphorylation efficiency of the erg-40 mutant or that the excess of free fatty acids which occur in this strain uncouple oxidative phosphorylation by acting as protonophores.

DEVELOPMENT OF A COMPUTERIZED PESTICIDE INFORMATION RETRIEVAL SYSTEM FOR VIRGINIA. M.J. Weaver, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Many states have computerized pesticide label data to increase the efficiency of information transfer to clientele. However, few have met the need for computer-assisted dissemination of newsletters, fact sheets, notices, and publications containing pest control-related subject matter. A system of this type has been created for use by Virginia extension and research personnel. The system was developed using EXEC2 protocols and XEDIT profiles for use in the CMS environment on an IBM VM/370 computer. Function-key activated, user-friendly menus and an on-line help facility enhance the system's performance. The addition of mechanisms to search pesticide label data and pest control recommendations are forthcoming.

INCIDENCE OF THIELAVIOPSIS BASICOLA ON SELECTED LANDSCAPE-GROWN JAPANESE HOLLY CV. 'HELLERI' IN VIRGINIA. R.L. Wick, Dept. Plant Path., Physiol. & Weed Sci., VPI&SU, Blacksburg, VA 24061.

Thirty-four landscape-grown Japanese holly cv. 'Helleri', 17 rated as healthy/17 as declining, were examined for the occurrence of Thielaviopsis basicola root disease. From each plant, one hundred feeder roots, 3 to 6 mm in length, were cultured on carrot discs and the recovery of T. basicola noted. Additionally, phytopathogenic nematodes were extracted from soil by elutriation/sugar flotation. Soil was also tested for P, K, Ca, Mg, pH and soluble salts. Thirty-three of 34 plants had root systems colonized by the fungal pathogen. The average number of roots colonized per plant, was 26%. There was a significant relationship ($p=0.05$) between the number of roots colonized and the disease rating. Plants rated as healthy, had an average of 17% roots colonized by T. basicola while declining plants had 34%. Decline, in this study, was not correlated with available soil nutrients, pH, soluble salts or phytopathogenic nematodes.

THE EFFECTS OF GLOMUS DIAPHANUM AND RHIZOBIUM TRIFOLII ON INFECTION OF RED CLOVER BY FUSARIUM ROSEUM 'ACUMINATUM'. Jane E. Yarger and Joseph B. Morton, WVU Plant Pathology & Ag. Microbiology, 401 Brooks Hall, P. O. Box 6057, Morgantown, WV 26506-6057.

Fusarium species are commonly isolated from roots of field-grown red clover. A greenhouse study was conducted to determine the effects of mycorrhizal and nodulated red clover plants on infectivity by two isolates of Fusarium roseum 'Acuminatum', differing in pathogenicity. Wounded roots of 8-week-old plants were inoculated with a condial suspension of the two Fusarium isolates. Treatments included plants that were mycorrhizal and nodulated without N and P fertilization, mycorrhizal with N fertilization, nodulated with P fertilization, or non-mycorrhizal and non-nodulated with and without N and P fertilization. After six weeks, colonization by G. diaphanum was good in all mycorrhizal treatments. The degree of necrosis was greatest in mycorrhizal plants with or without Rhizobium, and was similar for both Fusarium isolates. Cultural tests of symptomatic and asymptomatic roots indicated Fusarium was present in all treatments.