Abstracts of Presentations at the 1988 Annual Meeting

of The American Phytopathological Society
North Central Division

June 22-23, 1988
West Lafayette, IN

Alphabetized by first author's last name.

SODIUM BISULFITE-ENHANCED PEROXIDASE ACTIVITY IS ACCOMPANIED BY INCREASED SPORULATION OF BIPOLARIS MAYDIS RACE T (BMT). M. Akhtar and M. O. Garraway, Dept. of Pl. Path., OARDC and The Ohio State University, Columbus, OH 43210.

Sodium bisulfite, a reducing agent, increased peroxidase activity in maize (Zea mays L.). This observation prompted a study of its effect on sporulation of BMT on infected maize leaves. Detached leaves of cultivar W64A (susceptible and resistant isolines) were infiltrated with sodium bisulfite (250 or 500 ppm) or double distilled water in the dark for 24 h at 28 C. These leaves were inoculated with BMT and incubated for another 48 h at 28 C. Sporulation was measured in vivo. Sporulation was higher than the controls on either isoline treated with sodium bisulfite but more so on the susceptible one. Other chemicals such as 1-aminocyclopropane-1-carboxylic acid (ACC) and L-methionine which enhanced peroxidase activity also increased sporulation. Sporulation was more on nutrient media when supplemented with peroxidase. Sodium bisulfite appears to increase both peroxidase activity and sporulation of BMT on maize.

RESISTANCE TO *Helminthosporium carbonum* RACE 1 ON SUSCEPTIBLE MAIZE LEAVES CAUSED BY PRIOR INOCULATION WITH RACE 2. F. A. Cantone and L. D. Dunkle. USDA/ARS, Dept of Botany and Plant Pathology, Purdue Univ, W. Lafayette, IN 47907.

Helminthosporium carbonum race 1 produces large necrotic lesions (susceptible reaction) on leaves of susceptible maize, whereas infection by race 2 results in only small flecks (resistant reaction). The addition of HC-toxin, a host-specific toxin produced by race 1, to conidia of race 2 resulted in the formation of lesions identical to those caused by race 1 alone. Resistance to race 1 was obtained by inoculating susceptible leaves with conidia of race 2 for at least 12 hr prior to a challenge inoculation with race 1. Small flecks comparable to a resistant reaction were formed. However, the addition of HC-toxin to conidia of race 1 in the challenge inoculations resulted in susceptible lesions. HC-toxin was responsible for either creating a favorable environment for the growth of race 1 or the breakdown of resistant barriers induced by inoculation with race 2. We analyzed for the presence of phenolic compounds in infected tissue over a period of 60 hr. Variations in the levels of wall-bound ferulic and p-coumaric acids were observed in treatments that yielded both resistant and susceptible reactions. However, no consistent changes indicating a possible mechanism of induced resistance were detected.

SYNTHESIS OF HC-TOXIN DURING PATHOGENESIS OF MAIZE LEAVES BY *Helminthosporium carbonum*. L. M. Ciuffetti and L. D. Dunkle. Dept of Plant Pathology, Cornell Univ, Ithaca, NY 14853 and USDA/ARS Dept of Botany and Plant Pathology, Purdue Univ, W Lafayette IN 47907.

Highly virulent isolates of *H. carbonum* (race 1) produce a toxic cyclic tetrapeptide (HC-toxin) that is active against susceptible (S) genotypes of maize. A sensitive HPLC procedure was used to detect and quantify HC-toxin. The toxin was not detected in homogenates of ungerminated conidia, of 1- to 3-d germlings, or in the germination medium. To determine when HC-toxin accumulates during pathogenesis, extracts of leaf lesions were analyzed at various times after inoculation (PI). Although the S reaction could be distinguished histologically from the resistant (R) reaction by 16 to 24 hr PI and visibly by 36 hr, HC-toxin was not detected at 48 hr PI of S leaves. The toxin was present in extracts of lesions 72 hr PI and increased rapidly thereafter. Identity of HC-toxin was confirmed by dansylation and TLC of acid hydrolyzates of the peak from HPLC. Neither the toxin nor degradation products were detected in lesions of R leaves through 5 d PI. The results suggest that interaction of pathogenic hyphae with host cells is necessary for toxin production and that small quantities of toxin are sufficient during early stages of infection.

CLASSIFICATION OF PSEUDOMONAS SOLANACEARUM BY ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLP). Douglas Cook and Luis Sequeira, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706

RFLP analysis was used to study the taxonomic relationships among 62 strains of Pseudomonas solanacearum, representing three races and four biovars. When Southern blots of EcoR1- or EcoR1 + BamH1-digested genomic DNA were probed with nine unique EcoR1 cloned fragments from strains K60 or K2R, 28 distinct RFLP patterns were identified. Similarity coefficients revealed two major groups: group 1 contains two members of race 1 biovar 1 and all members of race 1 biovars 3 and 4; group 2 contains the additional members of race 1 biovar 1 as well as all members of races 2 and 3. Similarity coefficients within groups 1 and 2 are 81%±8% and 62%±17%, respectively. Similarity coefficient between the two groups is only 13.5%. Group 2 is composed of five distinct subgroups corresponding to race 1 biovar 1, race 3, and three subgroups of race 2. Some subgroups correspond to strains with distinct host ranges or geographical origins.

NUCLEAR MIGRATION MEDIUM FOR LACCARIA. N. S. Duncan, R. L. Doudrick, A. A. Alm[1], and N. A. Anderson[2]. [1]Forest Resources, [2]Plant Pathology, University of Minnesota, St. Paul, MN 55108.

The genus Laccaria includes important mycorrhizal fungi. Genetic studies of members of this genus symbiotic with black spruce are currently underway. Reciprocal exchange of nuclei between homokaryons defines compatible mating types. The purpose of this study was to identify a medium which would promote this interaction. Three levels of glucose or sucrose (2.5, 5.0, 10.0 g/l) were substituted in the modified Melin-Norkrans' medium (MMN), with pH adjusted to 5.5 or 6.1. Matings of three isolates representing two ecological populations were utilized. Preliminary indications are that nuclear migration is enhanced by reducing carbohydrate and increasing pH. Distinct color changes associated with compatible pairings were evident at 2.5 g/l glucose, pH 6.1.

INSIGNIFICANCE OF TOXIC EFFECTS OF CYCLIC TETRAPEPTIDES, CYL-2 AND CHLAMYDOCIN, TO PATHOGENESIS OF MAIZE BY *Helminthosporium carbonum*. L. D. Dunkle, F. A. Cantone, S-D. Kim, and H. W. Knoche. USDA/ARS, Dept of Botany and Plant Pathology, Purdue Univ, W. Lafayette, IN 47907, and Dept of Agricultural Biochem, Univ of Nebraska, Lincoln, NE 68583.

The pathogenic race (Race 1) of *H. carbonum* produces a cyclic tetrapeptide containing amino-oxo-epoxydecanoic acid (Aoe), which is essential for selective toxicity to genotypes of maize susceptible to *H. carbonum*. The non-pathogenic race (Race 2) lacks the ability to produce this host-specific toxin (HC-toxin). Addition of HC-toxin to conidial inoculum of Race 2 results in the formation of necrotic leaf lesions that are indistinguishable from those caused by Race 1. The structurally similar, Aoe-containing, cyclic tetrapeptides, chlamydocin and cyl-2, had toxic effects similar to HC-toxin; they inhibited root growth and induced the loss of electrolytes to a slightly

greater extent in susceptible than in isogenic resistant genotypes. However, addition of chlamydocin or cyl-2 to the inoculum did not alter the host reaction to Race 2. Lesions were small flecks identical to those incited by Race 2 alone. The results suggest that chlamydocin and cyl-2, although toxic in bioassays, do not elicit the host responses that are significant and essential to pathogenesis by *H. carbonum*.

THE INFLUENCE OF TEMPERATURE ON FORMATION AND RELEASE OF ALTERNARIA PORRI CONIDIA IN MICHIGAN. K. L. Everts and M. L. Lacy. Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

The influence of weather variables [temperature, vapor pressure deficit (VPD), hours of leaf wetness, daily rainfall, and daily solar radiation] on formation and release of conidia by Alternaria porri was investigated during two growing seasons. Strong positive correlations were found in 1985 and in 1987 between the natural logarithm (ln) of the conidia released during the current day (D-0) (independent variable), and average temperature during periods of low (<1 mb) VPD during the previous day (D-1), and the maximum VPD on D-0. In 1987 there was a strong positive correlation between ln of the conidia released on D-0 and average VPD on D-1. Using 1987 weather and spore trapping data, an equation to predict spore release on D-0 was constructed using average temperatures during periods of low (<1 mb) VPD between 0700 on D-1 and 0600 on D-0, and single maximum VPD values between 0600 and 2200 on D-0.

GLYCEOLLIN EFFECTS ON *PHYTOPHTHORA MEGASPERMA* F. SP. *GLYCINEA* PLASMA MEMBRANE H^+ AND Ca^{2+} CONDUCTANCE. John L. Giannini[1], Jana S. Holt[2], Jack Paxton[2], and Donald P. Briskin[1], Departments of [1]Agronomy and [2]Plant Pathology, University of Illinois, Urbana, IL 61801

A method has recently been developed for the isolation of sealed plasma membrane vesicles from Phytophthora megasperma f. sp. glycinea. Associated with these vesicles was a vanadate-sensitive proton pumping ATPase. These vesicles also were shown to have a nH^+/Ca^{2+} antiport likely responsible for calcium movement out of the fungal cell. The Km for this antiporter was found to be 7μM for calcium, well within the range of values found for calcium transporters and pumps in plant cells. The plant phytoalexin, glyceollin, was found to increase the proton and calcium conductance across the Phytophthora plasma membrane. Significant conductance increases were observed at glyceollin concentrations of less than 50μM. Unlike the plant tonoplast and plasma membrane ATPase, the fungal ATPase showed no glyceollin effect until concentrations greater than 100μM were used. These findings suggest that the soybean defense compound glyceollin interferes with the physiology of a fungal pathogen in at least two ways.

DEVELOPMENT OF PHIALOPHORA GREGATA IN SOYBEAN PLANTS. Lynn E. Gray. USDA/ARS, Univ. of Illinois, Urbana, IL 61801.

Colonization of the stem vascular system of Corsoy-79 soybean inoculated with Phialophora gregata was determined at various times after inoculation. The fungus colonized the stem vascular system and progressed upward in the vessels with time. Leaf vascular tissue was colonized soon after infection was established in the stems. Leaf symptom development was associated with leaf vascular tissue colonization by the fungus. Spores of Phialophora in the stem vascular tissue were recovered by centrifuging stem segments of infected plants. Spores were recovered from stems 3 weeks after inoculation. Phialophora spores were translocated into the leaf vascular tissue when plant roots were exposed to a spore suspension. Differences were observed in stem and leaf colonization patterns between susceptible and resistant soybean plants.

Verticillium dahliae as a potential biocontrol agent of velvetleaf Abutilon theophrasti R. J. Green, Jr. and G. L. Wiley, Department of Botany and Plant Pathology, Purdue University, W. Lafayette, IN USA 47907

Verticillium dahliae appeared naturally in stands of velvetleaf (VL) Abutilon theophrasti in soybean/weed competition studies. Within three years, suppression of VL was almost 100% and the weed was non-competitive with soybeans. VL plants with symptoms were 97% infected and 84% of all plants without symptoms yielded V. dahliae. The soybean/weed competition studies were based on different weed population densities, row width and tillages. None of these variables had a measurable effect on soil populations of V. dahliae. Greenhouse studies showed initial host specificity of VL isolates of V. dahliae, however, serial passage of isolates through a number of test plant species, including soybean, showed that host specificity could be altered. The implications of this instability will be discussed in consideration of V. dahliae as a potential biocontrol agent.

THE EFFECT OF SEED-BORNE FUSARIUM MONILIFORME ON THE EMERGENCE OF SWEET CORN INBREDS. J. M. Headrick and J. K. Pataky. Department of Plant Pathology, University of Illinois, Urbana 61801

Selfed ears of 115 sweet corn inbreds were silk-inoculated with F. moniliforme in 1986. Inoculated and noninoculated ears were harvested. The percentage of apparent infection was observed. The level of symptomless infection was determined by plating healthy-appearing kernels on PDA and PCNB media. Significant differences in apparent infection were observed due to inbreds and inoculation. A significant inbred x inoculation interaction was observed for in vitro incidence. Incidences of apparent and symptomless infection were 1 and 0% for the most resistant inbred (IL125b) and the most susceptible inbred (IL783a) was 100% infected. In 1987, symptomless and nonsorted seed lots of each inbred from 1986 were planted in the field to assess the effect of infection on emergence. A significant inbred x inoculation interaction was observed, as was a significant difference due to infection. Interaction terms involving infection were not significant. Thus, inbreds varied in their response to inoculation, but emergence was reduced in all infected inbreds.

MECHANISMS OF BIOCONTROL OF RHIZOCTONIA ROOT ROT OF SUGAR BEET BY BIOCONTROL AGENTS [BINUCLEATE RHIZOCTONIA SPP. (BNR) AND LAETISARIA ARVALIS]. L. J. Herr, Dept. of Plant Pathology, The Ohio State Univ., OARDC, Wooster, OH 44691.

Possible mechanisms of biocontrol involving competition, hyperparasitism and antibiosis were studied. Competition was investigated as comparative growth rates of biocontrol agents (BA) and Rhizoctonia solani (RS) on Difco PDA + 0.1% yeast extract. Growth rates in order were: highest L. arvalis; RS, BNR 5 & 6; BNR 1, 3, 4 & 10; BNR 2; and BNR 9. Of these BA, only L. arvalis, BNR 2 & 9 have failed to control root rot in most biocontrol tests made to date. No hyperparasitism was evident when tested by pairing BA with RS on water agar-coated slides. Antibiosis of BA to RS was ascertained by an inhibition test on PDA + YE agar plates and by mycelial yields of RS on wk-old culture filtrates of BN-1 and RS. There was no inhibition of RS and mycelial yields of RS from control soln (Czapek's salts + 0.3% sucrose) and RS filtrate did not differ, but RS growth was reduced by BN-1 filtrate.

NORTHERN STEM CANKER BIOTYPE OF DIAPORTHE PHASEOLORUM VAR. CAULIVORA ISOLATED FROM SYMPTOMLESS SOYBEANS. G. J. Holland and T. S. Abney, Dept. of Botany and Plant Pathology, Purdue University, and USDA-ARS, W.Lafayette, IN 47907.

Century 84 soybeans were evaluated in a rotation by tillage system at the R6 and R8 growth stages for infection by the "Diaporthe Complex" [Diaporthe phaseolorum var. caulivora (Dpc) and D. p. var. sojae (Dps)]. The northern stem canker biotype of Dpc was isolated routinely from pods of symptomless plants at both growth stages but less than 1% of the seed was infected. Total Diaporthe spp. infected 20% of the soybeans at the greenbean stage (R6); Dpc was the dominant isolate (69%). Infection increased to 30% at harvest maturity (R8); however, Dps was the dominant member. Pod infection by Dpc was more extensive in continuous soybean than in corn-soybean or wheat-corn-soybean rotations. The no-till system appeared to increase Dpc infection at the R8 growth stage compared to conventional and chisel tillage systems. This study suggests that data based on the visual assessment of soybean plants and seed infection do not reflect the importance of the northern stem canker pathogen or its role in premature dying.

INHIBITION OF PLASMA MEMBRANE AND TONOPLAST H^+-TRANSPORTING ATPASES BY GLYCEOLLIN. Jana S. Holt[1], John L. Giannini[2], Donald P. Briskin,2 and Jack D. Paxton[1], Departments of [1]Plant Pathology and [2]Agronomy, University of Illinois, Urbana, IL 61801

The soybean phytoalexin glyceollin, an autophytotoxic and antibiotic compound, inhibits ATP-dependent proton transport of membrane vesicles isolated from red beet storage tissue. Proton pumping of the tonoplast was more sensitive to glyceollin (50% inhibition at 50μM) than that of the plasma membrane (50% inhibition at 80 μM). When glyceollin was added to plasma membrane or tonoplast vesicles having a steady state pH gradient, proton influx ceased and the gradient slowly dissipated. The uncoupler gramicidin collapsed the gradient rapidly in the inhibited vesicles. Glyceollin directly inhibited tonoplast and plasma membrane ATPase activity with the difference in sensitivities parallel to that of proton pumping in the respective membranes. These results suggest that glyceollin could act to disrupt solute transport at both membranes through direct effects on the proton pumping ATPases.

LACZY GENE MODIFIES PEPTIDASE ACTIVITY IN PSEUDOMONAS AUREOFACIENS. K.D. Hughes, T.S. Roseman, B.C. Hemming, F.E. Lytle and D.M. Huber. Department of Botany & Plant Pathology and Chemistry, Purdue University, W. Lafayette, IN 47907; and Biological Sciences, Monsanto Co., St. Louis, MO 63198.

Pseudomonas aureofaciens (*P. fluorescens* biotype E) with and without the *lac*ZY marker gene were tested to determine the effect of the *lac*ZY gene on aminopeptidase activity. A twenty-four h culture ($5x10^{-5}$ cells) of the wild type *P. aureofaciens* 3732RN, pMON5003 modified 3732RN (*lac*ZY plasmid), L11 modified 3732RN (*lac*ZY genomic insert) or *P. phaseolicola* was suspended on a 3 mm 0.45 *u* filter attached to a suction flask. The immobilized cells were incubated three min in 100 *ul* beta-naphthylamide substrate (10^{-4} M), drained, washed with 700 ul THAM buffer (5 mM, pH 8.0) and the solution assayed for peptidase activity in a laser fluorometer. Characteristic profiles of *P. phaseolicola* and *P. aureofaciens* 3732RN were obtained. The *lac*ZY gene doubled HPRO activity and reduced ARG activity. The introduced plasmid, carrying multiple copies of the *lac*ZY gene, stimulated ALA, CYS, & HPRO peptidase activity but further reduced ARG, HIS, LEU, & LYS activity. The laser procedure provides a rapid means of detecting genetically modified *P. aureofaciens* and is being extended for direct fluorigenic evaluation of beta-galactosidase.

EFFECT OF FALL AND SPRING INFECTION OF WHEAT STREAK MOSAIC VIRUS ON WINTER WHEAT. R. M. Hunger and J. L. Sherwood, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078-0285.

Reaction of six hard red winter wheat cultivars (Century, Chisholm, Pioneer 2157, Siouxland, Tam 108, and Vona) to infection by wheat streak mosaic virus (WSMV) in the fall and spring was assessed by symptomatology and a protein-A sandwich ELISA. Rows of wheat (3.05 m long) were inoculated with WSMV on 10-21-86 or on 3-27-87 using a Devilbiss air gun at 60-75 psi. Symptoms were assessed and foliage samples for ELISA were collected on 4-16-87 and 5-13-87. Infection of seedlings in the fall significantly reduced yields of all cultivars. Spring infection significantly reduced yields of all cultivars except Siouxland and Pioneer 2157. ELISA confirmed the presence of WSMV, and results indicate the importance of preventing early infection by WSMV by controlling volunteer wheat on which viruliferous wheat curl mites thrive and can infect cultivated wheat seedlings.

SOIL FACTORS INFLUENCING THE DELETERIOUS EFFECTS OF ATRAZINE ON UNGERMINATED CONIDIA OF COCHLIOBOLUS SATIVUS. T. Isakeit and J.L. Lockwood, Dept. Botany & Plant Pathology, Michigan State University, East Lansing MI 48824.

The influence of soil organic matter and pH on the lethal effect of atrazine on conidia of Cochliobolus sativus incubated on soil was determined. Viability of conidia on Boyer sandy loam (SL) (-1 kPa matric potential) containing 25 µg/g atrazine was 7% after three weeks, as compared with 99% in the control. Decreasing the organic carbon (O.C.) of Boyer SL from 0.73% to 0.04% by H_2O_2 digestion or NaOH extraction before addition of atrazine nullified its lethal effect. The addition of 4 mg/g humic acid to atrazine-treated Boyer SL (0.04% O.C.) decreased viability to 60%. Increasing the pH of Boyer SL from 5.1 to 7.5 with $CaCO_3$ completely nullified the lethal effect of atrazine. Viability on Spinks SL containing atrazine remained at 99% after three weeks. The addition of 4 mg/g humic acid and decreasing the pH of Spinks SL from pH 6.6 to 5.3 with HCl reduced viability to 86% and 65%, respectively.

EFFECT OF CULTURE FILTRATES OF THE DIAPORTHE/PHOMOPSIS COMPLEX ON SOYBEAN SEEDS AND SEEDLINGS. M. Ivanovic and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana, IL 61801-4709.

Culture filtrates of three isolates each of Diaporthe phaseolorum var. caulivora (DPC), D. phaseolorum var. sojae (DPS), and Phomopsis longicolla (PL) grown on modified Czapek-dox broth in the dark for 21 days at 21°C and filtered through ashless Whatman no. 2 filter paper and Nalgene filters were used to treat soybean (Glycine max) seed and excised seedlings. All culture filtrates significantly (P=0.05) reduced seed germination after 36 and 48 hr compared to the control. Radical length of germinating soybean seeds was singificantly reduced with culture filtrates from PL significantly reducing radical length below that of DPC and DPS after 48 hr. Culture filtrates of all isolates caused irreversible wilting of excised soybean seedlings, with those of PL causing significantly more than those of DPC and DPS after 24 and 48 hr.

MODELING EARLY BLIGHT DISEASE PROGRESS WITH TERMS FOR INFECTION RATE, INCUBATION PERIOD, AND LESION EXPANSION WITHIN AN AGE-STRUCTURED ARRAY OF POTATO FOLIAGE. K. B. Johnson, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

A disease progress model for early blight (Alternaria solani) was coupled to an age-structured array of potato leaf tissue produced by a crop growth simulator. The disease progress model was a modified logistic (Berger & Jones, Phytopathology 75:792) and included terms for infection rate, variable incubation period, lesion expansion rate, and maximum severity at leaf senescence. In the field, incubation period depended on age of leaf tissue, and maximum disease severity depended on crop age. Observed lesion expansion rates were independent of leaf or crop age. In simulations, host leaf area expansion limited the rate of disease increase until midseason. Control strategies can be simulated by modifying model parameters; however, the indeterminant lesion expansion parameter is unrealistic, and incidence/severity relationships among similarly aged cohorts of leaf tissue need to be incorporated.

INDUCTION OF INFECTION STRUCTURES AND GERMINATION OF THE TELIOSPORES IN PHAKOPSORA PACHYRHIZI. E. Koch and H. H. Hoppe. GhK, Nordbahnofstr. 1a, 3430 Witzenhausen, Fed. Rep. of Germany.

The direct mode of infection of P. pachyrhizi (soybean rust) suggests that the stimuli involved in infection structure formation differ from those of the stomate penetrating rusts. Following germination of uredospores, appressoria were not formed on water agar or after a heat shock. On dialysis membranes, appressoria were formed at high proportions. On membrane filters induction of appressoria was related to the pore size of filters. Scanning electron microscopy showed that only the fine structured filters provided sufficient contact necessary for appressoria formation. Further development of infection structures apparently required additional stimuli and was only observed on paraffin containing collodion membranes. P. pachyrhizi forms telia on soybean and other hosts. A sequence of wetting and drying induced germination of teliospores and formation of basidia and basidiospores.

SCREENING FIELD ISOLATES OF ERWINIA STEWARTII USING POLYCLONAL AND MONOCLONAL ANTIBODIES IN ELISA TESTS. G. L. Lamka, D. C. McGee, J. H. Hill, and E. J. Braun, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011.

Samples of maize leaves showing symptoms of Stewart's Wilt were collected from hybrid seed production fields in Iowa in 1987. Tissue samples were ground in 2 ml of sterile 0.02 M phosphate buffered saline, pH 7.2, and streaked on nutrient agar plates. Single colonies with cultural characteristics typical of E. stewartii, the causal organism, were subcultured and inoculated into A632 inbred maize seedlings in the greenhouse. Twenty-seven isolates from different fields were found to be pathogenic. The 27 pathogenic isolates and 10 nonpathogenic isolates were screened in a ds-ELISA using polyclonal antibodies produced in rabbits and in an indirect ELISA using a specific monoclonal antibody derived from BALB-c mice. All pathogenic isolates reacted positively in both assays while the non-pathogenic isolates did not react in either assay.

ISOLATES OF RHIZOCTONIA SOLANI AG2-II PATHOGENIC TO SOYBEANS. Z. Liu and J. B. Sinclair, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Two isolates of R. solani, 61D-3 and 65L-2, obtained from IL-grown soybeans, caused pre- and postemergence damping-off, and stem, crown and root rot of soybeans at ED_{50} = 100 ug fresh mycelium/g soil. They also caused leaf and bud blight. Isolate 65L-2 was more pathogenic than 61D-3 and produced more of a toxic metabolite in liquid culture. Isozyme pattern and hyphal-anastomosis studies placed them in AG2-II. Both isolates survived as thick-walled, hyphal cells and sclerotiumlike structures. Such hyphal cells functioned as spores, germinating uni- or bipolarly. Maximum, optimum, and minimum growth was at 40, 30, and 8°C, respectively, on PDA. Isolate 65L-2 overwintered in soil in the field and maintained pathogenicity to soybeans. Both isolates had a narrow host range compared to isolates of R. solani AG4 and another ungrouped isolate. Both produced pectate lyase and proteolytic enzymes. This is the first report of AG2-II being isolated from and pathogenic to soybeans.

BIOLOGICAL CONTROL OF NECROTIC RING SPOT OF POA PRATENSIS
B.P. Melvin, J.M. Vargas, Jr. and W.L. Berndt. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI. 48824

Bimonthly enumeration of bacteria, actinomycete and fungi populations in a necrotic ring spot (Leptosphearia korrae) infected Kentucky bluegrass turfstand indicated increases in numbers of bacteria and actinomycetes may be responsible for disease reduction. Stimulization of bacteria and actinomycetes in the thatch and soil was accomplished through daily irrigation and application of bioorganic turf amendments. Dilution plate counts using selective media was used to determine the effects of three different irrigation practices and the use of bioorganic treatments on microbial populations. The differences in soil moisture, due to the irrigation practice, had a pronounced effect on bacteria and actinomycete populations, while

fungi populations remained relatively constant. Increases in bacteria and actinomycete populations in test plots were accompanied by a reduction in number of active necrotic ring spots, while plots with low populations of these organisms showed an increase in number of active ring spots.

RESPONSES OF SOYBEAN CELLS TO CULTURE FILTRATE OF PHIALOPHORA GREGATA. Damares C. Monte-Neshich[1], Lynn E. Gray[2], Angus G. Hepburn[1], Departments of 1. Agronomy and 2. Plant Pathology, USDA-ARS, University of Illinois, Urbana, Il 61801.

A cDNA library was constructed using poly(A)+RNA from SB1-M soybean cells treated for 12 h with culture filtrate of Phialophora gregata. Recombinant plaques were screened by differential hybridization using ^{32}P-labelled first strands cDNA prepared from RNA extracted from either treated or untreated cells. 3 classes of cDNA clones were selected, which showed greater hybridization with the probe prepared from treated cells than with that prepared from untreated cells. Northern blot analysis demonstrated that each clone selected represents a mRNA species which increases substantially in abundance during exposure of the cells to fungal metabolites. Slot blot analysis of total RNA extracted from stem and leaves of soybean plants, 5 weeks after inoculation with the fungus shows that these cDNA clones selected from cultured cells treated with the fungal culture filtrate correspond to genes that are also induced in the plant upon infection with the fungus.

ISOLATION AND CHARACTERIZATION OF A NON-PATHOGENIC, ACONIDIAL MUTANT OF *Colletotrichum graminicola*. D.G. Panaccione, J.B. Rasmussen, and R. M. Hanau. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

A mutant of *Colletotrichum graminicola* strain M1001 was isolated and experiments showed that it failed to produce conidia and mucilage in response to light when grown on a variety of agar-based media. In liquid culture, the mutant was unable to produce conidia unless it was wounded. When the mutant was grown on an agar-based medium that contained polygalacturonic acid as the sole source of carbon, it was lacking in a negative-phototropic response that is characteristic of strain M1001. In addition to being deficient in its ability to respond to light, greenhouse studies showed that the mutant was non-pathogenic to a highly-susceptible maize inbred (Mo-940) whereas strain M1001 and a conidiating, spontaneous revertant were both extremely pathogenic. Appressoria formed by the mutant were pigmented and produced penetration pegs. Moreover, leaves of seedlings (Mo-940) that were wounded prior to inoculation maintained their resistance to the mutant, suggesting that the non-pathogenic phenotype is not due to a failure of the fungus to penetrate host tissue. Attempts are being made to complement the mutant phenotype by transformation with a cosmid library of genomic DNA from strain M1001.

TIMING OF FUNGICIDE APPLICATIONS FOR CONTROL OF COMMON RUST OF SWEET CORN. J. K. Pataky. Department of Plant Pathology, University of Illinois, Urbana 61801

Four replications of four hybrids and six fungicide treatments were arranged in a split-plot to evaluate timing of fungicidal control of common rust. Hybrids differed in reactions to Puccinia sorghi from susceptible (S) to partially resistant (R). Zero to five applications of Dithane M-45 were made at weekly intervals from the 4-5 leaf to the early tassel stages. All treatments (except 0) included applications at the 4-5 leaf stage but only the 5 application treatment included the early tassel stage. Rust severity was measured weekly. The hybrid by fungicide interaction term was significant. Rust severity 1 wk before harvest was not different within hybrids for 3, 4 and 5 fungicide applications and was 6, 6, 3 and 1% on S, MS, MR and R hybrids, respectively. For the check treatment (0 applications), severity was 28, 16, 15 and 4%, respectively. These preliminary results indicate that sweet corn hybrid adult plant reactions to P. sorghi supersede the need for fungicidal control at mature plant stages.

EFFECT OF FOUR ISOLATES AND A WILD TYPE OF CERCOSPORA KIKUCHII ON SOYBEAN SEED QUALITY. M. A. Pathan, J. B. Sinclair and R. D. McClary, Dept. of Plant Pathology, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin, Urbana, IL 61801-4709.

Soybean (Glycine max) seed lots of six cultivars: Amsoy, Bragg, Davis, Hood 75, Tracy and Williams, uninoculated or inoculated with one of four distinguishable isolates of C. kikuchii were studied. Seed lots from each combination and 1 from naturally-infected Sieben brand plants (WT) were kept separate and the following parameters determined: 300-seed weight; seed volume; free fatty acid (FFA), oil, and protein content. From analysis of combined data for all cultivars, seed from plants inoculated with isolate PR had significantly (P=0.05) reduced seed weight, increased FFA and protein, and reduced oil content compared to all other isolates, followed by those from plants inoculated with isolates (descending order) ATCC-36864, WT, IN-C4, and IL-ATCC. There was no significant difference in volume among seed lots from plants inoculated with isolates ATCC-36864, IN-C4, PR, or WT.

BIOCHEMICAL AND PATHOLOGICAL ANALYSES OF MELANIN-DEFICIENT MUTANTS OF *Colletotrichum graminicola*. J.B. Rasmussen, D.G. Panaccione, and R. M. Hanau. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

UV-mutagenesis was used to recover two melanin-deficient mutants (Mel1$^-$ and Mel2$^-$ phenotypes) from an isolate of *Colletotrichum graminicola* (strain M1001) which causes leaf-anthracnose in maize. The disease ratings determined in greenhouse studies for M1001, the Mel1$^-$ mutant, and the Mel2$^-$ mutant were 4.4, 0.6, and 1.2, respectively (5.0 = dead plant). Both mutants were less efficient than strain M1001 in causing lesions on detached leaves. In contrast, wounding the leaves prior to inoculation with either mutant always resulted in lesion formation. No differences were found between the mutants and strain M1001 in rates of growth, production of conidia, germination, and the ability to form appressoria when cultured *in vitro*. However, the appressoria of both mutants were colorless whereas those produced by strain M1001 were darkly pigmented. Tests have indicated that exogenously applied scytalone, an intermediate in the polyketide pathway, is capable of restoring pigmentation in appressoria of both mutants.

MICROELEMENT IMMOBILIZATION PREDISPOSES WHEAT TO TAKE-ALL. T. S. Roseman, J. D. Phillips, and D. M. Huber. Department of Botany & Plant Pathology, and Agronomy; Purdue University, W. Lafayette, IN 47907.

Seed bacterization of wheat with fluorescent Pseudomonads (in a peat carrier) reported to reduce take-all root, crown and foot rot in naturally infested soils increased disease severity. This study was initiated to determine if the increase in take-all with seed bacterization could be explained as induced immobilization of Mn since Mn deficiency is known to increase take-all, and seed treatment with Mn is known to reduce take-all. Five different peats were evaluated for their ability to absorb Mn by incubating one gm peat with 20 ml of 0, 10^{-4}, $2.5x10^{-4}$, or $5x10^{-4}$ M aqueous concentrations of $MnSO_4$ for 11-hr, decanting the supernatant through a 0.45 mu millipore filter, and assaying it for Mn by atomic absorption. Michigan muck, the most absorptive peat, absorbed 526 ug/g Mn while the Indiana sphagnum, the least absorptive, absorbed up to 164 ug/g Mn. Although the five peats differed greatly in absorption capacity, their ability to immobilize Mn could explain their influence on wheat's predisposition to take-all.

PARTIAL PURIFICATION OF HOST SPECIFIC PATHOTOXIN PRODUCED BY SEPTORIA GLYCINES. H. Song, and S. M. Lim, Dept. of Plant Pathology, USDA-ARS, University of Illinois, IL 61801.

Previously we reported host specific pathotoxicity in culture filtrates of Septoria glycines. Culture filtrates of Septoria glycines grown in a modified Czapek's liquid medium for 21 days were mixed with 2% charcoal to remove the melanin pigments. Various organic solvents were used to extract the pathotoxin from filtrates, but toxicity was only detected in water-soluble phases. Filtrates were treated with XAD-7 resin to remove organic substances, and then were concentrated in vacuo to one-fiftieth of their original volume. Concentrated filtrates were dialyzed against Tris-HCl buffer (0.05M, pH 8.5) to remove salts and applied to an ion-exchange filtration column (DEAE-Cellulose anion exchanger) at pH 8.5. Anion portions of filtrates exhibited pathotoxicity when assayed on detached soybean cotyledons. Partially purified pathotoxin is being applied to soybean organogenic tissues to select survival tissues through repeated applications of the pathotoxin. Further purification of the pathotoxin is in progress.

UNUSUAL "SCORCH DECLINE" SYMPTOMS IN ASH TREES CAUSED BY VERTICILLIUM DAHLIAE. R. Spear, G. L. Worf, and M.F. Heimann. Department of Plant Pathology, University of Wisconsin-Madison 53706.

Nearly 50% of green (Fraxinus pennsylvanica) and white (F. americana) ash in two nurseries and many landscape trees have shown symptoms of a disease of undetermined cause. Initial foliage color on affected branches is light green to chlorotic. Later symptoms include irregular marginal necrosis, curling and defoliation. Stems and roots, including cross sections, appear normal. Branches die; trees sometimes decline further. After many earlier attempts failed, V. dahliae was isolated from some leaf petioles of <50% of suspect trees using PDA containing 100 ppm iprodione + 200 ppm chloramphenicol. Thirteen of 20 one-yr green ash seedlings showed characteristic symptoms

within 60 da after stem inoculations. V. dahliae was re-isolated from all seedlings showing symptoms, but not from symptomless or control trees.

DEVELOPMENT OF PHYTOPHTHORA ROOT ROT ON SOYBEAN TAPROOTS IN AEROPONIC CULTURE. R.E. Wagner and H.T. Wilkinson. Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL, 61801-4709.

An aeroponics system was developed for nondestructive investigations of disease progress on soybean roots inoculated with Phytophthora megasperma f. sp. glycinea (Pmg). Roots of 4-day-old plants were suspended in a chamber and misted with a noncirculated nutrient solution. Plants were removed easily for inoculation and measurements of root growth and lesion length. Roots were inoculated 12-24 hr after transplantaion. Taproot elongation ceased and lesions were observed on roots (cv. Williams) inoculated with Pmg race 1 within 24 hr at 25 C and between 24 and 48 hr at 20 and 15 C. Lesion length after 48, 96, and 144 hr was 0.2, 4.3, and 5.6 cm at 15 C; 4.1, 6.8, and 8.7 cm at 20 C; and 6.0, 7.4, and 10.0 cm at 25 C, respectively. Root lengths of noninoculated and inoculated plants after 144 hr averaged 37.1 and 10.5 cm at 25 C; 26.8 and 12.3 cm at 20 C; and 14.6 and 10.5 cm at 15 C, respectively.