

# APS North Central Division

## Abstracts

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### Alphabetized by first author's last name

DETECTION OF *CLAVIBACTER MICHIGANENSE* SUBSP. *SEPEDONICUM* WITH SOUTHERN HYBRIDIZATION AND THE POLYMERASE CHAIN REACTION. Ahmed L. Abdel-Mawgood and Thomas L. German. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

The genes for the 16s rRNA from *sepedonicum* (CMS) and *michiganense* (CMM) of *Clavibacter michiganense* were cloned. Sequence comparison between the two subspecies indicated a hypervariable region, from which probes and primers specific for Southern hybridization (#6034) and polymerase chain reaction (PCR) specific to CMS were developed to distinguish between the two subspecies. The oligonucleotide (#6034) and another oligonucleotide with the upstream sequence of the CMS were used in the PCR to specifically amplify a 0.9-kb-fragment from the CMS isolates. In addition, the DNA probe obtained from CMS (#6034) hybridized only with the 1.5-kb PCR amplified fragment of the 16s rRNA gene from 11 out of 12 CMS isolates. This probe did not hybridize with the DNA from any other subspecies of *Clavibacter* tested.

EVALUATION OF FOUR FUNGICIDES AND A BIOLOGICAL AGENT TO CONTROL TWO WINTER PATHOGENS OF WHEAT. R.E. Baird<sup>1</sup>, D. M. Huber<sup>1</sup>, and C.W. Mansfield<sup>2</sup>, Botany & Plant Pathology Department<sup>1</sup>, Agronomy Department<sup>2</sup>, Purdue University, Southwest Purdue Agricultural Program, Vincennes, IN 47591.

Winter-kill of wheat (*Triticum aestivum* L.) caused by *Rhizoctonia cerealis* usually occurs during the late winter and early spring but *Fusarium graminearum* also may be associated with winter injury. In 1992, up to 95% stand loss occurred in Indiana winter wheat fields; with loss dependent upon variety, soil type, and environmental conditions. Treatments applied to a moderately resistant wheat cultivar (Pioneer Brand 2548) at two locations (clay soil and sandy soil) near Bicknell, Indiana included 1) Fluazinam 50WP, 2) Bayleton 50WP, 3) ASC 66820, 4) Tilt 25EC, 5) Bravo 720, and 6) nontreated control. Several of the fungicides and the biological agent (treatment 3) effectively limited growth of both pathogens on PDA in an *in vitro* assay; however, results obtained in an incubator test were variable and inconsistent with the *in vitro* assay. None of the treatments were significantly different from the nontreated control in field trials; however, stand densities in the nontreated plots often were higher than treated plots and indicated that the treatments may have been phytotoxic or reduced natural biological control agents antagonistic to the winter kill pathogens.

DEVELOPMENT OF POTATO EARLY DYING IN RESPONSE TO INFECTION BY TWO PATHOTYPES OF *VERTICILLIUM DAHLIAE* AND CO-INFECTION BY *PRATYLENCHUS PENETRANS*. Despina D. Botseas and Randall C. Rowe, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Isolates of *V. dahliae* (Vd) from vegetable compatibility group (VCG) VCG 4A and 4B, that had shown differences in virulence on potato (cv. Superior) when uprooted plants were inoculated with conidia, were tested in field microplots containing soil infested with Vd microsclerotia and/or *Pratylenchus penetrans* (Pp) vermiforms. No differences in virulence were observed between VCG 4A and 4B isolates when tested alone, but VCG 4A isolates exhibited higher virulence than VCG 4B isolates when Pp was also present. A synergistic interaction between VCG 4A and Pp resulted in increased symptom development and lower yield. Tuber numbers were higher with Vd-infected plants regardless of VCG 4 subgroup, due to a larger number of small and medium sized tubers.

EVALUATION OF F1 CORN HYBRIDS FOR RESISTANCE TO *ASPERGILLUS FLAVUS* AND AFLATOXIN. K.W. Campbell, D.G. White, J. Toman, and T. R. Rocheford. Plant Pathology Department, University of Illinois, N-519 Turner Hall, 1102 South Goodwin Avenue, Urbana, IL 61801

The most effective control of *A. flavus* Link ex. Fries and aflatoxin production in corn is the development of resistant corn hybrids. The objective of this study was to identify sources of resistance in various F1's using a modified pinboard inoculation technique. In 1991, 1,189 and 978 crosses with the susceptible MO17 and B73, respectively, were evaluated in single row plots. Twelve to 18 plants per each of two replicates were inoculated 20-24 days following mid silk. Thirty to 40 days following inoculation, all ears were husked and a visual rating of 1-10 (1=10%, and 10=100%) *A. flavus* colonization of the inoculated area was determined. Eighteen F1 crosses with B73 and 17 F1 crosses with MO17 were selected for further study. Aflatoxin analyses were performed on the 35 selections. Several inbreds provide an extremely high level of resistance to *A. flavus* with low apparent colonization of kernels. Three of the inbreds (LB31, CO158, and 75-R001) provided resistance to both B73 and MO17 in F1 crosses.

ENTRY OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* INTO TOMATO PLANTS THROUGH HYDATHODES. W.M. Carlton, M. L. Gleason, and E. J. Braun. Dept. of Plant Pathology, Iowa State University, Ames, Iowa, 50011.

Entry of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) into tomato seedlings was assessed by inoculating guttation droplets. Guttation was induced by placing 8-wk-old seedlings (cv. 'Jet Star') in a growth chamber, saturating the soil with water, and enclosing the plants in plastic bags. Plants were inoculated by touching a 1.0 µl droplet of a mixture of 3 rifampicin-resistant strains of Cmm to guttation droplets on the terminal leaflets of 2 leaves/plant. Lesion development was assessed weekly after

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inoculation with  $10^3$ ,  $10^6$ , or  $10^9$  cfu/ml of the pathogen. Initial symptoms appeared as small chlorotic areas 2 wk after inoculation. The chlorotic areas became necrotic and expanded along the leaflet margins. Eight wk after inoculation, inoculated leaflets were completely necrotic and *Cmm* was detected in the leaf petiole 6 cm below the base of the leaflet. Inoculum concentration influenced infection efficiency, but not lesion size or expansion rate. After inoculation with  $10^9$  cfu/ml of *Cmm*, populations increased from  $10^6$  cfu/leaflet at 1 wk to  $10^8$  cfu/leaflet at 8 wk.

**DIVERSITY OF TYPES OF RESISTANCE TO NORTHERN LEAF BLIGHT IN THE BS19 MAIZE SYNTHETIC.** M. L. Carson, USDA-ARS, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

During the process of recurrent selection for resistance to northern leaf blight (NLB) in the maize synthetic BS19, several different types of resistance were observed. Inbred lines exhibiting these resistances have been isolated and are being characterized. A chlorotic lesion form of resistance was found in several selections and is probably controlled by the *Ht1* gene, although no known sources of *Ht1* were used in the synthesis of BS19. Other selections under study have an unusually long latent period that appears to be under polygenic control. A third type of resistance observed is expressed as a highly pigmented necrotic spot surrounded by large circular chlorotic halo.

**A COMPARISON OF THREE TECHNIQUES FOR INOCULATING CHINESE CABBAGE WITH *PLASMIDIOPHORA BRASSICAE*.** L.A. Castlebury and D.A. Glawe, Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801.

Three techniques for inoculating Chinese cabbage (*Brassica pekinensis* Rupr.) roots with *Plasmidiophora brassicae* Woron. were compared. Fourteen-day old seedlings were inoculated by: 1) dipping the roots in a suspension of  $10^8$  resting spores/ml; 2) coating the roots with a  $10^8$  resting spores/cc soil mixture; or 3) placing a thin slice (approx. 1 cm diam., 1 mm thick) of infected cabbage root next to the seedling roots. Pots were arranged in a randomized complete block design and harvested after five weeks. The root slice technique produced equivalent or superior results and required less preparation time (approx. 15 min.) than the other two methods (approx. 3 hours each). The root slice technique is a rapid and effective method for inoculating cabbage roots with *P. brassicae* for research purposes, classroom experiments and maintaining populations in the greenhouse.

**RAPD FRAGMENT DIVERSITY AMONG *STEMPHYLIUM* SPECIES FROM ALFALFA.** C. Chaisriook, D. Z. Skinner, and D. L. Stuteville, Department of Plant Pathology, Kansas State University, Throckmorton Hall, Manhattan, KS, 66506-5502. USDA-ARS and Department of Agronomy, Kansas State University, Manhattan, KS 66506-5501.

Genomic polymorphisms among *Stemphylium* species from alfalfa from diverse geographic areas were detected by the random amplified polymorphic DNA method. Principal component analysis of 205 RAPD fragments generated from 11 oligodeoxynucleotide 10-mers grouped 26 *Stemphylium* monoconidial isolates, including five morphology-based taxonomy species, into two clusters. One cluster included *S. botryosum* and *S. globuliferum*. The other cluster included *S. alfalfae*, *S. herbarum*, and *S. vesicarium*. *Pithomyces chartarum* and *P. atro-olivaceus*, included in the analysis as outgroups, were widely separated from *Stemphylium* and from each other. One major RAPD fragment was common to all *Stemphylium* species but absent from the *Pithomyces* species. Phylogenetic analysis indicated that *S. botryosum*, and *S. globuliferum* were divergent from *S. vesicarium*, *S. herbarum*, and *S. alfalfae*. Of the 12 *Stemphylium* isolates from alfalfa from the United States, one isolate each from New Hampshire and Pennsylvania was identified as *S. botryosum* and the other eight, from California, Idaho, Kansas, Utah, Washington and Wisconsin, were *S. alfalfae*.

**MOLECULAR MARKERS IDENTIFYING THREE HETEROTHALLIC *PYTHIUM* SPECIES.** W. Chen, Illinois Natural History Survey, 607 East Peabody Drive, Champaign, IL 61820

Identification of heterothallic *Pythium* species requires mating with tester strains. Mating compatible strains may not always result in sexual reproduction. Molecular markers are needed to facilitate identification. Three heterothallic *Pythium* species, *P. heterothallicum*, *P. splendens*, and *P. sylvaticum*, were compared by means of restriction digestions of PCR-amplified nuclear small-subunit rDNA, internal transcribed spacer (ITS), 5' portion (F63/R635) of large-subunit rDNA and a portion (ML1/ML4) of mitochondrial large subunit rDNA. Except for the ITS region, amplified products were uniform in length among the three species. Two length variants were observed in the ITS region, 850 bp long for *P. heterothallicum* and *P. splendens*, and 1020 bp long for *P. sylvaticum*. Restriction digestions of the PCR products showed species-specific banding patterns and little intraspecific variation was observed. The restriction digestion profiles are characteristic of and could be used to identify these species.

**RESISTANCE TO GRAY LEAF SPOT OF CORN.** S. T. Coates and D. G. White, Department of Plant Pathology, University of Illinois, Urbana, IL, 61801.

In 1989 more than 1300 inbreds were screened for resistance to gray leaf spot. A low volume overhead mist/irrigation system was used to create a favorable environment for disease development. In 1990, the 65 most resistant inbreds were evaluated as F1 hybrids with the susceptible parents FR1141 and FR20. In 1991, the available generations of the most resistant crosses from 1990 were evaluated at Urbana, IL., and Andrews, N.C.. Plants were rated individually and means were calculated for each generation. Generation mean analysis was used to determine genetic effects. For five inbreds, CI7, CI43, T222, and Illinois inbreds B37HTN and 198, dominance was important at one of the locations. Dominance was important at both locations for Illinois inbred DS:74:1071. These preliminary results indicate that dominance for gray leaf spot resistance exists in certain inbred lines. More testing is necessary to determine the importance of this dominance.

**ORGANIZATION OF THE CONJUGAL TRANSFER REGION TRAIL ON THE *Agrobacterium tumefaciens* PLASMID pTYC58.** David M. Cook and Stephen K. Farrand, Department of Plant Pathology, University of Illinois, Urbana, IL 61801

Three regions, TraI, TraII, and TraIII, on the Ti plasmid pTYC58 are essential for conjugal transfer. The TraI region encodes both negative and positive functions that are responsible for the regulation of conjugal transfer. TraII contains the origin of conjugal transfer or *oriT*. A 58 bp sequence represents the smallest functional *oriT*. To better characterize the TraII-encoded functions, we mutagenized the region with the transposons Tn3HoHo1 and Tn5. Tn3HoHo1 *lacZ* gene fusions in this region show that transcriptional units flank the *oriT* on either side and that the direction of transcription is away from the *oriT* site. TraII *lacZ* transcriptional gene fusions are active in strain NT1(pTYC58Tra) which is derepressed for conjugal transfer but are not in strain C58. Furthermore, *lacZ* fusions are not active in strain NT1. These results suggest that expression of TraII genes is positively regulated by the TraI-encoded positive regulator. DNA sequence analysis shows that a number of potential open reading frames (ORFs) exist within TraII surrounding the *oriT* site. The N-terminal portion of a deduced amino acid sequence encoded by the open reading frame designated ORF3 is highly homologous to those of endonucleases involved in mobilization encoded on plasmids pTF1 and RSF1010.

**TOMATO AND PEPPER SEED ASSAYS FOR THE DETECTION OF BACTERIAL DISEASES.** T.L. Cutting and J.P. Hubbard, The Asgrow Seed Company, 5300 N. 28th Street, Richland, MI 49083

Bacterial spot *Xanthomonas campestris* pv. *vesicatoria*, bacterial speck *Pseudomonas syringae*, pv. *tomato*, and bacterial canker *Clavibacter michiganensis* subsp. *michiganensis* of tomato *Lycopersicon esculentum* Mill. and bacterial leaf spot *Xanthomonas campestris* pv. *vesicatoria* of pepper *Capsicum annuum* L. may be seed transmitted. Tomato and pepper seed are almost exclusively high value, hand pollinated hybrids and growers frequently use labor-intensive, high input stake culture to grow crops from those seed. Also, seed are often planted in plug transplant greenhouses where risk of spread of foliar pathogens is very high. In recognition of these epidemiological and economic factors the Asgrow Seed Company has developed and instituted a selective medium based seed assay on a bulk seed sample of 30,000 seed for detection of the presence of these bacterial disease organisms in tomato and pepper seed lots. This presentation will describe the assay procedures which serve as basis for the claim that each seed lot offered for sale has been assayed for the presence of the pathogens that cause bacterial spot, speck and canker of tomato or bacterial leafspot of pepper and that none has been detected in the seed sample tested.

**DISEASE PRESSURE ON SOYBEANS IN ILLINOIS.** S.R. Eathington, Dept. of Agronomy; S.M. Lim, Dept. of Plant Pathology; Univ. of Arkansas; C.D. Nickell, Dept of Agronomy; and J.K. Pataky, Dept. of Plant Pathology; Univ. of Illinois; Urbana, IL 61801

Development of disease resistant cultivars is a primary emphasis of soybean (*Glycine max*) breeding programs. To determine disease pressure on soybeans in Illinois, 13 years of disease monitoring data consisting of 89 environments and 52 cultivars were analyzed. Of the 12 diseases observed, brown spot (*Septoria glycinis*) was the predominate foliar disease while brown stem rot (BSR) (*Phialophora gregata*) was the most frequent soilborne disease. The distribution curve of brown spot severity ratings taken at the R6 growth stage, was partitioned based on a z score of  $\pm 0.43$  from the sample mean. This showed that a given year in the northern third of Illinois has a 23% chance of having brown spot symptoms in the high range (greater than 46% of the total leaf area), while central Illinois has a 31% chance and the southern third of Illinois only a 15% chance. Brown stem rot occurred at 63% and 38% of the environments in northern Illinois and central Illinois, respectively. BSR was not observed in southern Illinois. Brown spot caused yield reduction statewide, while localized yield reduction was observed by other diseases.

**INTEGRATION OF RESISTANCE WITH A WEATHER-BASED FUNGICIDE SCHEDULING PROGRAM FOR CONTROL OF ANTHRACNOSE ON PROCESSING TOMATOES.** B.A. Fulling, E. C. Tigchelaar, and R. X. Latin. Purdue University, West Lafayette, IN 47907.

A weather-based fungicide scheduling program (Tom-cast) was compared against calendar-based spray schedules on tomato cultivars resistant or susceptible to anthracnose in experimental field plots. The performance of the weather-based program was evaluated at several predetermined action thresholds based on recorded temperature and leaf wetness durations. All weather-based spray treatments tested on the resistant cultivar Ohio 8245 were as effective against anthracnose as the seven-day spray treatment. The treatment with the lowest action threshold was comparable to the seven-day treatment on the susceptible cultivar Murrieta, whereas treatments with higher action thresholds resulted in significantly greater levels of anthracnose. The weather-based fungicide application program provided effective disease control with 4 sprays on the resistant cultivar and 7 sprays on the susceptible cultivar, compared to 10 sprays with the seven-day spray interval.

WHAT'S IN A NAME? *EUTYPA ARMENIACA* VS. *EUTYPA LATA*. D.A. GLAWE, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

In 1957, M.V. Carter and C.G. Hansford described *Eutypa armeniaca* as the species causing a canker and dieback disease of apricot (*Prunus armeniaca*). Although not clearly differentiated from previously described *Eutypa* species, the name served as an effective means of designating strains pathogenic on this and other hosts. Recently *E. armeniaca* and other species have been synonymized with *Eutypa lata*, although anamorphic and other characters important in *Eutypa* taxonomy are usually indeterminable in herbarium collections. This situation exemplifies important problems confronting taxonomists working with plant pathogenic Ascomycetes: Should fungal names be based on teleomorphic characters without determining the range of anamorphic, physiologic or pathogenic traits? Are morphological characters sufficient to characterize fungi which differ in host ranges and pathogenic capabilities? At what taxonomic level should non-morphological characters be reflected in fungal names? Such questions must be dealt with if fungal classification is to serve the needs of plant pathologists and other scientists studying fungi.

DEVELOPMENT OF SEMI-SELECTIVE MEDIA FOR *SCLEROTINIA SCLEROTIUM*. Jana M. Hansen and James R. Venette. Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

*Sclerotinia sclerotiorum* can cause devastating losses to beans, sunflowers, and other crops in North Dakota. Epidemiological and etiological studies on *Sclerotinia* are hampered by lack of suitable selective media. This study was an attempt to improve medium selectivity. Fourteen isolates from various regions in the state were screened for growth on plates of modified Czapek's medium (without sucrose, with CaCl<sub>2</sub> at 9.3 g/L, and layered with polypectate). All isolates grew on the pectate-layered medium. To select fungicides that allowed mycelial growth, one isolate was tested on media containing each of 22 fungicides incorporated into the pectate layer. From these preliminary results, five selected fungicides at six different concentrations were tested against the 14 field isolates. Mycelial growth rate varied among isolates. Because of this variability, development of a useful semi-selective medium has been difficult.

THE AGROCIOPINE CATABOLIC REGION OF *AGROBACTERIUM TUMEFACIENS* TI PLASMID pTiC58 ENCODES AT LEAST FIVE GENES REQUIRED FOR OPINE AND AGROCIIN 84 TRANSPORT. G.T. Hayman<sup>1</sup>, S. Beck von Bodman<sup>1</sup>, P. Jiang<sup>1</sup>, H. Kim<sup>1</sup>, and S.K. Farrand<sup>2,3</sup>. <sup>1</sup>Nat. Ctr. for Agric. Utilization Res., USDA, ARS, Peoria, Illinois, and Depts. of <sup>2</sup>Plant Pathol. and <sup>3</sup>Microbiol., Univ. Illinois, Urbana, Illinois.

Crown gall caused by certain strains of *Agrobacterium tumefaciens* can be controlled by *A. radiobacter* strain K84. Efficient control depends upon the pathogen being susceptible to an antiagrobacterial antibiotic, called agrocin 84, produced by strain K84. Susceptibility to agrocin 84 is encoded by the Ti plasmid, and is associated with the catabolism of a class of sugar phosphodiester opines called agrocinopines. The locus for opine catabolism and agrocin 84 sensitivity, called *acc*, has been characterized. A minimal subclone containing seven kb is sufficient for both phenotypes. Complementation analysis showed *acc* to be between 5.3 and 6.9 kb, and to be divisible into at least five groups, *accA* through *accE*. All five are required for sensitivity to the antibiotic, and at least four are required for transport of the opine and agrocin 84. DNA sequence analysis predicts that *accA* encodes a periplasmic binding protein (PBP) related to the PBP associated with dipeptide transport in *Escherichia coli*. Similar analyses suggest that *accE* encodes a phosphodiesterase. The sequence shows strong homology with *glpQ*, an *E. coli* gene encoding glycerolphosphodiester phosphodiesterase. Maxicell and cell fractionation experiments showed that *accA* encodes a 60 kDa protein located in the periplasmic compartment.

APPARENT CONFORMATIONAL DIFFERENCES AMONG VIRIONS OF SYMPTOM-MODULATING TURNIP CRINKLE VIRUS COAT PROTEIN MUTANTS. L. A. Heaton, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Several spherical plant viruses swell under conditions that deprotonate carboxyl groups and/or remove divalent cations. Swollen turnip crinkle carmovirus (TCV) and tomato bushy stunt tobamovirus particles migrated more slowly than contracted particles during agarose gel electrophoresis (AGE). Symptom-modulating coat protein mutants of TCV were assayed by AGE. One mutant was like the wild-type virus, two mutants appeared to be always swollen and less stable at elevated pH, and one mutant appeared to be always contracted and more stable at elevated pH. A fifth mutant was a mixed population of swollen and contracted particles. The possible correlation between the symptoms elicited by each mutant and its conformation will be discussed.

HOST SOURCES, VIRULENCE AND OVERWINTERING OF *RHIZOCTONIA SOLANI* AG FROM FIELD LETTUCE IN OHIO. L. J. Herr, Dept of Plant Pathology, Ohio State Univ., Wooster, OH 44691.

Field lettuces with *Rhizoctonia* bottom rot were collected (June-August) from organic soils at Celeryville in 1989 and Hartville in 1990. Samples of 10 plants/lettuce type/date included, Boston, bibb, green leaf, red leaf, romaine, escarole and endive. At Celeryville 58% of *Rhizoctonia solani* anastomosis group, intraspecific group (AG, ISG) isolates were from green leaf, escarole 20%, red leaf 14% and romaine 8%. 93% of green leaf isolates were AG-1-1B and 82% of escarole isolates were AG-1-1C. At Hartville, 30% of isolates were from romaine, while Boston, endive and bibb had 20, 20 and 19%, respectively. AG-1-1B made up 52% of all isolates. Most AG-2-1 isolates were from Boston and bibb. AG-1-1B, AG-1-1C and AG-4 survived overwinter better on the soil surface than when buried. An AG-4 isolate did not survive as well and was less virulent than other overwintered AG. Despite dominance of AG-1-1C isolates on escarole, an AG-1-1B isolate was more virulent on escarole than an AG-1-1C isolate.

GENETIC AND PHYSICAL ANALYSIS OF THE MANNITYL OPINE CATABOLIC REGION FROM pTi15955. S.B. HEN<sup>1</sup>, Y. DESSAIX<sup>2</sup>, W.S. CHILTON<sup>3</sup>, and S.K. FARRAND<sup>1</sup>. <sup>1</sup>Univ. of Illinois, Urbana, IL., <sup>2</sup>Institut des sciences végétales, France, and <sup>3</sup>North Carolina State Univ. N. C.

We have isolated and characterized Tn<sub>3</sub>-HoHo1- and Tn<sub>2</sub>-induced mutants of a cosmid clone, pYDH208, which encodes the mannopine cyclase-associated catabolism of mannopine (MOP) and agropine (AGR). Characterization of the transposon mutants by  $\beta$ -galactosidase activity and mannityl opine utilization patterns identified at least six transcription units. Catabolic functions for MOP and mannopinic acid (MOA) are encoded by a 16.4 kb region, whereas those for AGR are encoded by a 9.4 kb region within the MOP catabolic locus. The induction pattern of catabolism shown by Tn<sub>2</sub> insertion derivatives suggests that expression of pYDH208-encoded catabolic pathway for MOP, AGR and MOA is regulated by at least two independent control elements. Kinetic assays of [<sup>14</sup>C]MOP and [<sup>14</sup>C]AGR uptake indicate that clone pYDH208 confers two uptake systems for MOP and AGR, one constitutive and slow, and the other inducible and rapid. Activities of  $\beta$ -galactosidase of *lacZ* reporter gene fusions were much higher in the absence of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, suggesting that several genes encoded by pYDH208 are under nitrogen control. Assays of  $\beta$ -galactosidase and MOP cyclase activities in the presence of sugars indicate that mannityl opine catabolic genes are not strongly regulated by sugar catabolite repression.

DIURNAL AND SEASONAL PERIODICITY OF *CERCOSPORA ZEAE-MAYDIS* IN IOWA. J. H. Jenco and F. W. Nutter, Jr., Department of Plant Pathology, Iowa State University, Ames, Iowa, 50011

*Cercospora zeae-maydis*, the causal agent of gray leafspot, has become increasingly prevalent in the Midwest as a result of the trend towards the use of reduced-tillage management systems. In Iowa, severe epidemics have occurred most in the Southeastern tier of counties. Studies concerning the aeromycology of *C. zeae-maydis* were performed in 1991 at Ames and Crawfordsville, Iowa. Burkard volumetric spore traps were placed at each location prior to the initial appearance of symptoms. Final disease severity for both locations resulted in 30 to 40 percent of the available leaf area being affected by gray leafspot. The number of spores trapped/m<sup>3</sup>/hour was recorded at both locations up until 2-3 wk prior to harvest and from these data, diurnal and seasonal periodicities for *C. zeae-maydis* were determined. Hourly spore catches indicated a diurnal peak occurring between 1600 and 1800 hr at both locations. An additional peak occurred at 1100 hr at the Ames location. The seasonal periodicity of *C. zeae-maydis* showed a peak spore concentration of 182 spores/m<sup>3</sup> of air sampled on day 217 at Crawfordsville and 284 spores/m<sup>3</sup> on day 244 at Ames.

EBCDs AND ALTERNATIVE FUNGICIDES FOR CONTROL OF EARLY BLIGHT IN POTATOES. Janell Stevens Johnk and Roger K. Jones, University of Minnesota, St. Paul, 55108.

The proposed cancellation by the Environmental Protection Agency of ethylene bis dithiocarbamate (EBDC) fungicides for use on potatoes (*Solanum tuberosum*) spurred studies in 1990 and 1991 to compare the effectiveness of currently registered alternative fungicides. EBDC fungicides are commonly used in Minnesota for control of early blight caused by *Alternaria solani*. Experimental plots were established in both irrigated and dryland sites representing the major potato-growing areas (Sand Plains and Red River Valley regions) of the state. Chlorothalonil and triphenyltin hydroxide proved to be cost effective alternatives to EBDC. Use of these fungicides resulted in an estimated average net return of \$52/ha and \$75/ha, respectively, as compared to an average net return of \$41/ha with use of EBDCs. Iprodione was effective in control of early blight, but was not a cost-effective alternative (average net return of -\$31/ha). Copper hydroxide fungicides were ineffective in controlling the disease.

COMPARISON OF RACE 2 AND RACE 4 OF *COCHLIOBOLUS CARBONUM* BY PCR AMPLIFICATION. Margaret J. Jones, and Larry D. Dunkle. USDA/ARS. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907-1155.

To determine the genetic relatedness of the recently described race 4 of the maize pathogen *Cochliobolus* (*Helminthosporium*) *carbonum* to the commonly occurring race 2, we compared DNA polymorphisms generated by PCR amplification of bulked DNA from ten isolates of each race. Analyses with over 70 primers (10-mers) failed to distinguish the two races, although the DNA fingerprints varied slightly among isolates of each race when analyzed individually. Races 2 and 4 were clearly differentiated from race 3 and from the nonpathogenic race 0 by the consistent presence of two amplified products. When the isolates were analyzed with 17-mer primers located within the *Tox2* locus, which is essential for host-specific toxin synthesis, races 2 and 4 were differentiated from race 1 by the absence of *Tox2* sequences. The results suggest that the increased virulence of race 4, which causes larger leaf lesions on several maize lines, is due to the accumulation of virulence determinants.

GENES INVOLVED IN CATABOLISM AND THE REGULATION OF THE CATABOLISM OF MANNOPINE AND AGROPINE FROM TI PLASMID pTi15955. Kun-Soo Kim and Stephen K. Farrand. University of Illinois, Department of Microbiology, Urbana, IL 61801.

*Agrobacterium tumefaciens* strain 15955 carries Ti plasmid pTi15955, which confers the utilization of mannitol opines as specific growth substances. pYDH208 containing a 21 kb *HindIII* insert from pTi15955 encodes properly regulated utilization of MOP and AGR. Cells carrying a 14 kb *BamHI* subclone in pSaB4 from the pYDH208 can also grow well on AGR but grow slowly on MOP. Complementation analysis showed there are at least five distinct complementation groups in the 21 kb fragment. At least, Group II, III, IV, and V are inducible by MOP. While the growth of cells harboring pSaB4 is slower than cells harboring pYDH208 on MOP, Tn5seq1 insertions in region IV result in growth as fast as cells containing pYDH208. Some spontaneous mutants which grow on MOP as fast as cells carrying pYDH208 contain derivatives of pSaB4 with an IS element located in region IV. These mutants also grow well on AGR and show derepressed expression of MOP cyclase activity. However, cells containing Tn3HoHo1 and Tn5 insertions mapping to the same region in pYDH208 grow slowly on MOP and can not grow on AGR. Furthermore, the expression of MOP cyclase remains inducible. The DNA sequence of the region showed strong homology with that of an ORF believed to encode a repressor in plasmid pRiA4 associated with agropine catabolism. A gene in complementation group V appears essential for the utilization of MOP. A 2.7 kb subclone containing the region was sufficient to confer the utilization of MOP in NT1 strain.

THE RELATIONSHIP BETWEEN EARLY-SEASON VIRULENCE FREQUENCIES AND SUBSEQUENT POWDERY MILDEW EPIDEMICS ON WHEAT. Steven Leath, USDA, ARS, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616.

Frequency as well as presence of virulence genes corresponding to host resistance genes may be important in epidemic development. Fourteen wheat lines, ten with one resistance gene, three with two resistance genes, and one with no known genes were planted in a total of five environments in three years. In late-winter the presence and frequency of virulence genes in the local *Blumeria graminis* f. sp. *tritici* population was determined and correlated with subsequent epidemic development. Disease severities ranged across lines from none to greater than 15% of the flag leaves covered with mildew colonies. Significant correlations (> 0.60) existed between winter virulence frequencies and subsequent disease levels. However, virulence thresholds appear to differ for different resistance genes and high virulence frequencies did not always result in epidemics even in disease conducive

MISMATCH PCR YIELDS SPECIFIC PRODUCTS AT AN ANNEALING TEMPERATURE OF 35°C. K.-N. Li, T. L. German, and D. I. Rouse. University of Wisconsin, 1630 Linden Dr., Madison, WI 53706

In the process of developing a specific probe for *Verticillium* sp., we used a pair of primers that amplify a region of about 700 bp in the mitochondrial small rRNA gene of fungi. However, PCR was not consistent under conventional conditions. We often obtained no specific amplification, only a very wide smearing on agarose gels. Conventional manipulations with PCR conditions to increase specificity did not give sufficient improvement. Data base searches revealed that among the known fungal sequences there are 1-5 base pair mismatches with the primers, and mismatch positions were not conserved. By lowering the annealing temperature, the specificity of the PCR was improved and at annealing temperature of 35°C, our PCR results became very consistent. We have successfully amplified the desired region from six fungal genera and dozens of isolates of *Verticillium* spp. with divergent host and geographical origins. The specificity of the PCR products was verified through sequencing of the cloned PCR product and Southern hybridization. This finding has broadened the possibilities for designing specific DNA probes using PCR.

DNA RESTRICTION MAPPING OF NUCLEAR RIBOSOMAL RNA GENES OF *RHIZOCTONIA SOLANI* ANASTOMOSIS GROUP 2. Z. L. Liu, and J. B. Sinclair, Department of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

Restriction maps of 2.5 kb PCR-amplified DNA fragments representing 18 S and 5 S nuclear ribosomal RNA genes, and internal transcribed spacers of five intraspecific groups (ISG) of *R. solani* AG 2 were constructed. Comparisons of the 18 S regions of the maps supported the separation and close relationships among the five ISG based on molecular differentiations previously reported. Many restriction sites were shared by the five ISG including one *EcoRI*, five *HinfI*, two *MboI*, two *TaqI*, and most of *HaeIII* and *MspI* sites. No sites for *BamHI*, *EcoRV*, *KpnI*, *PstI* and *XhoI* were found. Variations in the lengths of restriction fragments produced with *HaeIII* and *MspI* differentiated ISG 2C, and 2E from ISG 2A, 2B and 2D. In addition to length mutations presented in the ITS, the absence or presence of certain *HaeIII* and *MspI* sites suggested possible site mutations involved in the 18 S rDNA regions among different populations.

EFFECT OF SOIL ORGANISMS ON GROWTH RATE AND MANGANESE OXIDATION ABILITY OF *GAEUMANNOMYCES GRAMINIS*. T.S. McCay-Buis and D.M. Huber. Botany & Plant Pathology Department, Purdue University, W. Lafayette, IN.

Temperature significantly influences the Mn oxidation ability of *Gaeumannomyces graminis* and preliminary observations indicated Mn oxidation is further affected by various microbial interactions. The effects on growth rate and Mn oxidizing ability of *G. graminis* isolates were determined on 4% PDA amended with 25 µg/g reduced Mn at 15 & 25 C. Microbial interactions were evaluated by first placing an agar disc with actively growing *G. graminis* hyphae in the center of the agar plate followed by inoculation with a "challenge" soil organism, at either the same time or after 3 days, in the center of the *G. graminis* inoculum. The size of the *G. graminis* colony was measured and Mn oxidizing ability was determined weekly after inoculation with the various challenge organisms. Some challenge organisms greatly stimulated or inhibited growth and/or Mn oxidizing ability of *G. graminis* isolates, while others had no or only slight effects. Interactions with these characteristics were further affected by temperature. One *Pseudomonas* sp. nearly totally inhibited Mn oxidation and growth of *G. graminis* at 15 C when inoculated 1 or 3 days after placing the *G. graminis* on the plate. At 25 C the effects were variable. Since virulence of *G. graminis* has been correlated with its Mn oxidation ability, and both are conditioned by temperature, these results indicate that rhizosphere microbial interactions are significant additional interactive factors in the take-all disease of wheat.

A HYPHOMYCETOUS STATE OF *EUTYPA ARMENIACA* IN ARTIFICIAL CULTURE. J. M. McKemy, D. A. Glawe, and G. P. Munkvold, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709, and Dept. of Plant Pathology, University of California, Davis, CA 95616.

*Eutypa armeniacae* Hansf. & Carter [= *E. lata* (Pers.: Fr.) Tul. & C. Tul. fide Rappaz] causes serious canker and dieback diseases of species of *Vitis*, *Prunus*, and other plants throughout the world. The anamorph has been described as a *Cytosporina* or *Libertella* state, and produces filiform conidia from sympodially and percurrently proliferating conidiogenous cells. In the present study, isolates from the western U.S. produced a hyphomycetous state in addition to pycnidia. Isolates were cultured on Difco potato dextrose agar at 19C under fluorescent light for 12 hr followed by 15C in darkness for 12 hr, for approximately 4 wks. Hyphomycetous conidiogenous cells arose from aerial and appressed hyphae. Conidia and conidiogenous cells of the hyphomycetous state resemble those formed within pycnidia.

**PATHOGENICITY OF FUSARIUM SPP. ON SOYBEAN IN THE RED RIVER VALLEY.** B. D. Nelson and C. E. Windels\*, North Dakota State University, Fargo, 58105 and \*University of Minnesota, Northwest Experiment Station, Crookston 56716.

*Fusarium* spp. were isolated from soybean (*Glycine max* [L.] Merr.) plants collected from 348 fields in the Red River Valley of Minnesota and North Dakota. Of 476 cultures of *Fusarium* isolated, 24% were *F. oxysporum*, 10% *F. solani*, 6% *F. equiseti*, 2% *F. acuminatum* and 1% other *Fusarium* spp. Pathogenicity of selected cultures of *F. oxysporum* and *F. solani* were evaluated in the greenhouse by placing *Fusarium*-colonized oat kernels in contact with the upper taproot of 14-day-old McCall soybean. After 3 wk, plants were evaluated for discoloration and decay of tap roots. Five of 54 cultures of *F. oxysporum*, and 23 of 32 cultures of *F. solani*, caused lesions >5 mm or lesions that partially girdled tap roots. Only two cultures of *F. solani* produced foliar symptoms typical of sudden death syndrome. *Fusarium* spp., particularly *F. solani*, may be important root rot pathogens of soybean in the Red River Valley.

**DEVELOPMENT OF CRITICAL-POINT YIELD LOSS MODELS TO ESTIMATE YIELD LOSSES IN CORN CAUSED BY CERCOSPOORA ZEAE-MAYDIS.** F. W. Nutter, Jr., and J. H. Jenco, Department of Plant Pathology, Iowa State University, Ames, Iowa 50011

Gray leafspot epidemics of different severities were obtained by inoculating four corn hybrids at different stages of crop development at 2 locations in Iowa. Disease severity (lesion area/total leaf area) was assessed weekly on 5 randomly selected plants per plot beginning in late June and ending in early September. Mean disease severity assessments on the lower-third (leaves 1-5), middle-third (leaves 6-10), upper-third (10-15), and overall mean (all 15 leaves) were regressed against yield to develop critical-point models. Disease severity assessments on the mid- and upper- canopy leaf layers had a better relationship to yield than lower-leaf assessments. The best critical-point models, based on the coefficient of determination ( $R^2$ ) and standard error of the estimate, occurred for assessments made at the late dough stage of growth with  $R^2$  values ranging from 38 to 91% at Crawfordsville, Iowa and 30 to 78% at Ames, Iowa.

**POSITIVE REGULATION OF CONJUGAL TRANSFER OF TI PLASMID pTIC58 IN *Agrobacterium tumefaciens*.** KEVIN R. PIPER, SUSANNE BECK VON BODMAN, STEPHEN K. FARRAND. 382 PABL, 1201 W. Gregory, Univ. of Illinois, Urbana, IL 61801.

Conjugal transfer of the nopaline/agrocinopine-type Ti plasmid, pTIC58, requires three regions, Tral, Trall, and Tralll, and is regulated at three levels. Transfer is normally repressed, but is inducible by addition of the conjugal opines, agrocinopines A&B. This level of regulation is mediated by a repressor, AccR, which negatively regulates conjugal transfer and agrocinopine catabolism functions. Upon derepression by conjugal opines, transfer is positively regulated by an activator encoded within the Tral region. The activator is required for expression of genes in Tral and possibly Tralll. Conjugal transfer frequencies of opine-induced donors are maximized by a third regulatory component called conjugation factor (CF). This diffusible factor is produced by opine-induced donor cells and is apparently an integral part of the conjugal regulatory system. We located and identified the gene in Tral encoding the activator, determined that the CF effect is Tral dependent, and localized genes necessary for CF production. Transposon mutagenesis and analysis of subclones of Tral in *trans* to a Tral::lacZ fusion plasmid demonstrated that the activator is encoded by a 1.8 Kb EcoRI fragment within Tral. CF increased  $\beta$ -galactosidase expression in strains harboring a Tral::lacZ reporter plasmid in *trans* to a Tral activator clone but, had no effect on a strain lacking the activator clone. This demonstrated that the CF effect is mediated through the Tral region. Transposon mutagenesis of Tral disrupted CF production. Analysis of subclones demonstrated production of CF by a 21 Kb KpnI fragment which contains the Tral region but not by a smaller subclone of Tral alone. We conclude that the activator gene is encoded within EcoRI-33, is necessary for expression of the transfer genes and that CF interacts with the activator to increase transfer gene expression. Production of CF appears to be regulated by Tral but is not encoded by genes within Tral.

**THE WHEAT GENETICS RESOURCE CENTER - GERM PLASM CONSERVATION, EVALUATION, AND UTILIZATION.** W. J. Raupp and B. S. Gill, Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506-5502.

Established in 1984, the Wheat Genetics Resource Center collects, maintains, evaluates, and documents the genetic resources of wheat; studies host-pathogen co-evolution; and develops genetic and cytogenetic stocks for rapid and efficient gene transfer for breeding superior wheat cultivars. With over 3500 accessions, the WGRC has one of the most extensive collections of wild wheat species and genetic stocks in the United States. These accessions are evaluated for resistance to leaf, stem, and yellow rust; Septoria; tan spot; powdery mildew; wheat streak and barley yellow dwarf viruses; Hessian fly; greenbug; Russian wheat aphid; and wheat curl mite. Useful genes are identified and incorporated into wheat lines available for use in breeding programs. The WGRC has pioneered the development of new cytogenetic stocks and chromosome and DNA-based assays for plant genome analysis and efficient germ plasm development. These research methodologies, along with the fostering of cooperation of an international team of investigators, are the basis of the conservation and utilization of the world's germ plasm of wheat.

**VARIATION IN VIRULENCE AND EFFECTS OF ASSOCIATED ORGANISMS ON RHIZOCTONIA WINTER-KILL OF WHEAT.** C. Riegel and D.M. Huber. Botany & Plant Pathology Department, Purdue University, West Lafayette, IN 47907.

Approximately 50% of the 312,000 ha of winter wheat planted in the fall of 1991 in Indiana were abandoned by mid-April because of severe winter killing caused by *Rhizoctonia solani* (BN). Several potential pathogens were isolated with *Rhizoctonia* and were evaluated in a growth chamber to see if the increased disease severity in the field was the result of synergism with the other pathogens, especially *Fusarium graminearum* and *Pythium* spp., or increased virulence of *Rhizoctonia* as the primary organism. Wheat seedlings grown for 2 weeks in a greenhouse soil mix were inoculated by pouring 20 ml of 1 wk old PDA agar culture of each organism that was blended with water (1:3 V/V) over the soil surface prior to applying a wet cotton covering on top of the plants to maintain moisture and proximity to the inoculum. Plant survival was evaluated after a three-week incubation period at 10 C. Isolates of *R. solani* differed in virulence. Some caused complete plant kill with total maceration of plant tissue; others caused complete plant kill with limited maceration of the basal part of the stem. Neither *Pythium* nor *Fusarium*, in combination with *Rhizoctonia*, increased the severity of maceration; however, the disease was less severe when *Fusarium* was also present with *Rhizoctonia*. The severe winter wheat kill experienced in the 1992 winter season is attributed to *R. solani*, optimum conditions for disease, and limited resistance of commonly grown commercial wheat cultivars.

**MINIMAL GROWTH RATE NEEDED FOR FULL PATHOGENICITY OF COCHLIOBOLUS HETEROSTROPHUS ON MAIZE.** P. R. Thorson, S. K. Souhrada, and C. R. Bronson, Dept. of Plant Pathology, Iowa State University, Ames, Iowa 50011-1020.

Mutants of *Cochliobolus heterostrophus* are being identified which have reduced growth rate on agar media to determine whether normal growth is needed for full pathogenicity on maize. Mutants are also being examined for autotrophy and colony morphology. Thus far, 20 out of 3,838 survivors have growth rates ranging from 32% to 95% of wild-type; 12 have growth rates of 15% or less. Tests of these mutants suggest that a growth rate of 58% of wild-type may be sufficient to cause full-sized lesions. Some mutants with growth rates of 32% to 49% of wild-type cause slightly smaller lesions than wild-type; all mutants with growth rates of 15% or less cause flecks or no lesions. These results suggest that fast (wild-type) growth is not required for full pathogenicity. Additional mutants are being identified and tested.

**DISINTEGRATION OF PROTEINS IN SOYBEAN SEEDS ASSOCIATED WITH CERCOSPOORA KIKUCHII AND PHOMOPSIS LONGICOLLA.** R. K. Velicheti, K. P. Kollipara, J. B. Sinclair, and T. Hymowitz, Univ. of Illinois at Urbana-Champaign, 1102 S. Goodwin, Urbana, IL 61801.

Seed coats (sc) and cotyledons (ct) of cv. Hack soybean seeds without or with symptoms caused by *C. kikuchii* (Ck) or *P. longicolla* (Pl); and mycelia of Ck and Pl (controls) were tested for storage and functional proteins. Ck-infected sc had partial, and Pl-infected sc had substantial protein disintegration. Pl-infected ct showed selective disintegration of proteins at MW 91.5, 81.5, 77, 73, 70, 56.5, 62, 22 and 16 kd; tentatively identified as conglycinins  $\alpha$ ,  $\alpha'$ ,  $\beta$ ,  $\beta'$  and glycinins A<sub>1</sub> and A<sub>4</sub>. Lipoxigenase-1 was disintegrated in Ck-infected sc and Pl-infected ct. Seed lectins were present in Pl-infected sc, remaining unchanged in ct affected by either fungus.  $\beta$ -amylase activity was reduced in Pl-infected sc and ct, but was not detected in Ck-infected sc. This is the first report on disintegration of soybean seed proteins associated with fungal infection.

**CERCOSPORIN PRODUCTION AND COLONIZATION OF SOYBEAN SEED COATS BY CERCOSPOORA KIKUCHII.** R. K. Velicheti and J. B. Sinclair. University of Illinois at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana, IL 61801-4709.

*C. kikuchii* isolates from purple-stained soybean seeds were placed into three groups (% recovery); Ckcp (75%) isolates produced cercosporin (CR) as purple nonanhydroCR (NCR); Ckcy (15%) isolates produced excess fatty acids, triglycerides, and CR; and Ckcc (10%) isolates produced only CR on potato-dextrose agar (PDA). All isolates inoculated onto seed coats (sc) produced NCR. Cercosporin changes to NCR with the loss of -OH and -OCH<sub>3</sub> groups. Production of CR by these three isolates on PDA was Ckcc>Ckcy>Ckcp; and on sc was Ckcc>Ckcp>Ckcy. Purple pigmentation on sc was always associated with mycelial spread from hilar region to mesoderm. A correlation ( $r=0.96$ ;  $p=0.0001$ ) was recorded between the extent of purple stain and the quantity of CR/100 ug of sc. The enhanced sc colonization by higher CR producing isolates, and association of CR throughout the colonization correlates well with increased *C. kikuchii* colonization of soybean sc.

ROLE OF FATTY ACIDS AND TRIGLYCERIDES IN SOYBEAN SEED COLONIZATION OF *CERCOSPORA KIKUCHII*. R. K. Velicheti and J. B. Sinclair. University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave. Urbana, IL 61801-4709.

*C. kikuchii* cultures grown on potato-dextrose agar (PDA) were extracted with diethyl ether. Seed coats (sc) from purple-stained seeds infected with *C. kikuchii*, and seeds inoculated with *C. kikuchii in vitro* were extracted with acetic acid, mixed with dichloromethane, and then washed with water. Components of all extracts were resolved by TLC (silica gel G) using ethyl acetate:benzene (2:3) solvent system. *C. kikuchii* grown on PDA produced cercosporin, isocercosporin and lipids. However, lipids were not detected from either naturally-infected purple-stained soybean sc or from those sc inoculated with *C. kikuchii*. The lipids were identified as a mixture of fatty acids and triglycerides by infrared spectrometry. Thus, fatty acids and triglycerides produced by *C. kikuchii* in culture (analogous to *C. beticola* toxin) were not involved in seed colonization.

SPECTRAL PROPERTIES OF CERCOSPORIN ASSOCIATED WITH PURPLE SEED STAIN OF SOYBEANS. R. K. Velicheti and J. B. Sinclair. Department of Plant Pathology, University of Illinois at Urbana-Champaign. Urbana, IL 61801-4709.

*Cercospora kikuchii* causes purple seed stain of soybeans. Cercosporin (CR) dissolved in polyprotic acids produced nonhydrocercosporin (NCR) by the elimination of -OH and -OCH<sub>3</sub> groups. Absorption peaks in visible region of CR were 470 and 563, and of NCR were 490 and 590. NCR changed to CR when dissolved in acetic acid. CR dissolved in acetic acid plus H<sub>3</sub>PO<sub>4</sub> (1:1) showed absorption peaks at 479 and 579 indicating an intermediate compound. The role of this conversion of NCR and CR in plant disease will be discussed. Fluorescence emission spectra showed peaks at 610 nm for CR and 620 nm for NCR, when excited at 470 nm and 490 nm, respectively. Cercosporin fluoresced red in mycelia of *C. kikuchii* in culture and in soybean seed coats. Some isolates of *C. kikuchii* produced NCR in culture. However, all our *C. kikuchii* isolates produced NCR in soybean seed coats.

MULTI-LEVEL REGULATION OF pTIC58 CONJUGAL TRANSFER. S. Beck von Bodman, K. Piper, and S. K. Farrand. University of Illinois, Departments of Plant Pathology and Microbiology, Urbana, IL 611801.

Conjugal transfer of the nopaline Ti plasmid pTIC58 requires functions that map to three regions on the Ti plasmid. Transfer is repressed in absence of agrocinopines A and B. This primary negative regulation is achieved through the transcriptional repressor, AccR. The structural gene for AccR is linked to the agrocinopine catabolic locus, acc, which is also regulated by AccR. AccR is a DNA binding protein with structural similarity to the DeoR class of transcriptional repressors. Gel shift assays demonstrate that AccR binds to its own 5' regulatory region. This same intergenic region when cloned in the opposite orientation drives the expression of a promoterless  $\beta$ -galactosidase gene. This expression is also subject to negative regulation by AccR. Thus AccR regulates expression from divergent promoters. In one direction it down-regulates expression of accR and acc, and in the opposite direction it represses genes that are part of Tra region I. The distal region of Tra I encodes functions that are involved in secondary regulation of tra. Expression of lacZ reporter fusions located within Tra region II are dependent upon the presence of a 1.8 kb EcoRI fragment (E33) contained within Tra region I. Maximal activation provided by E33-encoded functions, however, requires a diffusible conjugation factor (CF). The production of CF is encoded within a 21 kb fragment that overlaps Tra region I acc. Our current model predicts that primary regulation of pTIC58 conjugal transfer is mediated by the conjugal opines through the negative gene regulator, AccR. Under induced conditions, Tra region I expresses a positive gene regulator. This activator requires a third Ti plasmid-encoded factor, CF, for full conjugal activity.

Hemagglutination of swine blood cells by tomato bushy stunt virus (TBSV) suggests saccharide binding by the virus. M. H. Walter, L. A. Heaton, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

TBSV and turnip crinkle virus coat protein and the lectin concanavalin A (Con A) are structurally similar (Argos et al., 1980. J. Mol. Evol. 15:169-179). Hemagglutination assays (HAs) were used to investigate possible saccharide binding by tomato bushy stunt virus (TBSV). In HAs using different animal red blood cells (RBCs), TBSV agglutinated swine RBCs at virus concentrations as low as 11 micrograms/ml (HA titre of 512). TBSV HA titers differed from titers of mock-inoculated controls by 8 to 512 fold. Specificity was demonstrated by hemagglutination inhibition using TBSV pre-incubated with polyclonal antibody specific to TBSV. Non-specific antisera did not inhibit hemagglutination. Hemagglutination by TBSV suggests functional similarities to Con A and possible specific recognition events in virus infection.

SOIL-INCORPORATION OF GREEN OAT LEAVES AND ROOTS FOR REDUCTION OF APHANOMYCES DAMPING-OFF OF SUGAR BEET. C. E. Windels and J. Neilsen, N.W. Expt. Stat., Univ. Minnesota, Crookston, 56716.

Soils collected from three fields naturally infested with *Aphanomyces cochlioides* were planted to oat or left fallow in the greenhouse (18 C). After 4 wk, plants were removed at soil level and cut into 1-2 cm long pieces (referred to as leaves). Soil treatments where oats had grown included: 1) leaves + roots, 2) leaves only, 3) roots only, and 4) leaves and roots removed; fallow soil was supplemented with 5) oat leaves, or 6) not treated. Moist soil was incubated at 24-27 C for 3 wk and then planted to sugar beet. After 4 wk, root rot indices (RRI) (0-100 scale) were significantly lower in soil from two fields when oat leaves + roots were incubated in soil (means = 52) than for the other five treatments (means = 86). In the third field soil, RRI of treatments 1-3 did not differ significantly (53, 50, and 31, respectively) but were significantly different from treatments 4-6 (99, 97, and 99, respectively). *Aphanomyces* damping-off was most consistently reduced when oat leaves and roots were incubated in soil where the crop had grown.

RESIDUAL RESISTANCE TO CROWN RUST IN TWO-GENE (F1) OAT HYBRIDS. J.M. Windes and W.L. Pedersen. Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Four F1 hybrids were obtained by crossing nearly-isogenic oat lines, *Avena sativa* L.; each isolate carried an ineffective resistance gene to crown rust, *Puccinia coronata* Cda. f.sp. *avenae* Eriks (P.c.a.). Fourteen-day-old seedlings of the F1 hybrids and the isolines' recurrent parent were grown in a growth chamber at 20 C with 16 hr of light and inoculated in a settling tower using urediniospores of a naturally occurring isolate of P.c.a. from Illinois. Inoculated plants were placed in a mist chamber at 100% relative humidity for 12 hours and returned to the growth chamber. Number of pustules per leaf and urediniospores per pustule were determined daily starting six days after inoculation and continuing for eight days. Lesion length was determined four days after pustules were visible. The experiment was repeated twice.

CHARACTERIZATION OF GENETIC VARIATION IN POPULATIONS OF *HYPOXYLON TRUNCATUM* USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS. C.-S. Yoon and D.A. Glawe, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

*Hypoxyylon truncatum* is a common pyrenomycete on *Quercus* spp. in North America. Genetic variation within and among populations was assessed by characterizing Random Amplified Polymorphic DNA (RAPD) markers. Six populations were studied: one from Louisiana, one from Mississippi, one from southern Illinois, two from central Illinois, and one from northern Illinois. Nine isolates from each population were characterized. Twenty arbitrarily chosen 10-mer primers (Operon) were used in conjunction with the polymerase chain reaction. Data were analyzed phenetically using the software package NTSYS-pc. Results suggested that RAPD are useful genetic markers for studying genetic relationships among and within populations of this fungus, and can serve as useful taxonomic characters.

INOCULATION TECHNIQUES TO PRODUCE GALLS OF COMMON SMUT ON EARS OF SWEET CORN. S. A. Zimmerman and J. K. Pataky, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

Several methods of inoculating sweet corn with *Ustilago maydis* were evaluated in 1991. Injecting sporidia into silk channels at the full- and early-silk stages resulted in 50 and 20% incidence of ear galls, respectively, on 'Sweetie Bicolor 76' and 'Yankee Belle'. Injecting teliospores at the full- and early-silk stages resulted in 24 and 21% smutted ears. Less than 5% of the ears had galls when sporidia or teliospores were injected into ears prior to emergence of silks or into stalks prior to emergence of ear shoots. Spraying sporidia or teliospores on to ear shoots wounded by sandblasting did not increase smut substantially. In another trial, incidence of ear galls was 40 and 44% on Sweetie Bicolor 76 when teliospores or sporidia were sprayed on wounded ear shoots at the early- and mid-silk stages, respectively. Other treatments, including the control, had about 20% ear galls.

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## To Complete the Grids, Please Use the Classification Codes Provided Below.

### Principal Area of Expertise

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(Choose one)

- A Agronomy
- B Bacteriology
- C Biochemistry
- D Botany
- E Breeding
- F Cell Biology
- G Ecology
- H Entomology
- I Genetics
- J Horticulture
- K Microbiology
- L Molecular Biology
- M Mycology
- N Nematology
- O Plant Pathology
- P Plant Physiology
- U Seed Pathology
- Q Soil Science
- R Viticulture
- S Virology
- T Other \_\_\_\_\_

### Title/Function

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(Choose up to two)

- 001 Agronomist
- 002 Bacteriologist
- 003 Biochemist
- 004 Botanist
- 005 Breeder
- 006 Cell Biologist
- 007 Chairman
- 008 Chief
- 009 Dean
- 010 Department Head
- 011 Director
- 012 Ecologist
- 013 Epidemiologist
- 014 Geneticist
- 015 Graduate Student
- 016 Group Leader
- 017 Horticulturist
- 018 Instructor/Lecturer
- 019 Leader
- 020 Librarian
- 021 Manager
- 022 Molecular Biologist
- 023 Mycologist
- 024 Nematologist
- 025 Plant Pathologist
- 026 Asst. Plant Pathologist
- 027 Assoc. Plant Pathologist
- 028 Extension Plant Pathologist
- 029 Plant Physiologist
- 030 Post Doctoral
- 031 Private Practitioner/Tech. Advisor
- 032 President
- 033 Exec. Vice President
- 034 Asst. to President
- 035 Project Leader
- 036 Professor
- 037 Asst. Professor
- 038 Assoc. Professor
- 039 Professor Emeritus
- 040 Provost
- 041 Retired
- 042 Scientist
- 043 Asst. Scientist
- 044 Assoc. Scientist
- 045 Soil Scientist
- 046 Superintendent
- 047 Supervisor
- 048 Technician
- 049 Technical Sales/Service
- 050 Virologist
- 051 Viticulturist
- 052 Other \_\_\_\_\_

### Commodity Specialization

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(Choose up to three)

- 001 All crops
- 002 Alfalfa
- 003 Almond
- 004 Apple
- 005 Avocado
- 006 Barley
- 007 Banana/Plantain
- 008 Bean
- 009 Blueberry
- 010 Cane Fruits
- 011 Cereal Crops
- 012 Cherry
- 013 Christmas Trees
- 014 Citrus
- 015 Clover
- 016 Cole Crops
- 017 Corn
- 018 Cotton
- 019 Cranberry
- 020 Cucurbits
- 021 Field Crops
- 022 Flax
- 023 Forages
- 024 Forest/Nurseries
- 025 Forest Tree
- 025 Fruit
- 026 Grape
- 027 Grasses
- 028 Hardwood Tree
- 029 Legumes
- 030 Market and Storage
- 031 Mint
- 032 Nursery Crops
- 033 Oats
- 034 Ornamentals
- 035 Pea
- 036 Peach
- 037 Pear
- 038 Peanut
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- 040 Potato
- 041 Rice
- 042 Root Crops
- 043 Rye
- 044 Safflower
- 045 Shade Tree
- 046 Softwood Tree
- 047 Soybean
- 048 Strawberry
- 049 Sugar Beet
- 050 Sugarcane
- 051 Sunflower
- 052 Sweet Corn
- 053 Sweet Potato
- 054 Tobacco
- 055 Tomato
- 056 Tropical/Subtropical Fruits
- 057 Tropical Root Crops
- 058 Turf Grasses
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- 060 Walnuts
- 061 Wild Rice
- 062 Wheat
- 063 Other \_\_\_\_\_

### Interest/Research Special Area

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(Choose up to three)

- 001 Abiotic Diseases
- 002 Administration
- 003 Aero and Space Biology
- 004 Air Pollution
- 005 Bacterial Genetics
- 006 Bacteriology and Bacterial Diseases
- 007 Consulting
- 008 Control Plant Diseases - Biological
- 009 Control Plant Diseases - Breeding Resistance
- 010 Control Plant Diseases - Chemical (General)
- 011 Control Plant Diseases - Chemical (Bactericide)
- 012 Control Plant Diseases - Chemical (Fungicide)
- 013 Control Plant Diseases - Chemical (Nematicide)
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- 028 Nematology and Nematode Diseases
- 029 Parasitic Seed Plants
- 030 Physiology of Microorganisms
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- 032 Plant Disease Losses
- 033 Plant Disease Survey
- 039 Seed Pathology
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- 035 Teaching
- 036 Vector Mediated Transmission of Plant Disease
- 037 Virology and Virus Diseases
- 038 Other \_\_\_\_\_

### Purchasing Responsibility

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(Choose up to two)

- 001 Scientific Books
- 002 Culture Media, Microbes
- 003 Microscopes
- 004 Molecular Biology Supplies, Enzymes, Plasmids, etc.
- 005 Microbiology Equipment
- 006 Radioisotope Detectors
- 007 Radiochemicals
- 008 Plant Growth Chambers
- 009 Plant Tissue Culture Media
- 010 Oligodeoxyribonucleotide Synthesis
- 011 Peptide Synthesis
- 012 Antibodies
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- 014 Seeds
- 015 Chromatographic Equipment
- 016 Other \_\_\_\_\_

## Other Professional Memberships

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(Choose up to three)

- 01 American Society of Agronomy
- 04 Alliance of Crop Consultants
- 07 American Association for the Advancement of Science
- 10 American Horticultural Society
- 13 American Institute of Biological Sciences
- 16 American Society for Microbiology
- 19 American Society for Horticultural Science
- 22 American Society of Plant Physiologists
- 25 Australasian Society of Plant Pathology
- 28 Botanical Society of America
- 31 British Mycological Society
- 34 British Society of Plant Pathology
- 37 Canadian Society of Plant Pathology
- 40 Crop Science Society of America
- 43 Entomological Society of America
- 46 Indian Phytopathological Society
- 49 International Society for Molecular Plant Microbe Interactions
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