The American Phytopathological Society

NORTH CENTRAL DIVISION

Annual Meeting
June 16-18, 1986

ABSTRACTS

MONOSACCHARIDE FERMENTATION BY SELECTED *FUSARIUM* STRAINS AND MUTANTS. A.A. Antonopoulos and E.G. Wene, Argonne National Laboratory, Argonne, IL 60439.

More than 200 *Fusarium* isolates and UV-mutants were tested, and all were able to ferment glucose and xylose to ethanol to some degree. These fermentations were conducted in 250-mL shake flasks and in 5-L fermentors. The ability of *Fusaria* to produce ethanol under a variety of conditions was also studied. Yields under fermentor conditions were improved by increasing inoculum size and controlling aeration. Of the isolates tested, several yielded 4.3 mg/mL of ethanol within 48 hr in 1% glucose solutions, a conversion efficiency approximately 83% of the theoretical. Experiments also focused on xylose fermentation. Several strains and mutants produced up to 4.2 mg/mL ethanol from 1% xylose broths in 48 hr, and up to 8 mg/mL from 2% xylose in 48 hr. Fermentations with 3% xylose required 72 hr to equal the 48 hr ethanol production rate from 2% solutions. *Fusaria* were able to grow and produce ethanol in up to 30% xylose broth. Addition of glucose to higher xylose concentrations appeared to increase the ethanol yield.

Biological control of Pythium ultimum on Red Kidney bean by bacterial and fungal biocontrol agents. S. M. Bissonnette, B. J. Jacobsen. Dept. of Plant Pathology, Univ. of Illinois, Urbana, IL 61801.

Biocontrol candidates were isolated from the rhizosphere of Phaseolus vulgaris (cv. California Light Red Kidney bean) grown in field soil naturally infested with P. ultimum. Field soil was obtained from a field which had been in Kidney bean monoculture for over 10 yrs. Twenty-eight candidates, including fungi, bacteria (including actinomycetes), were screened for control of P. ultimum. Isolates were applied as seed treatments. Treated seeds were grown in field soil in the glasshouse for 30 days using a CRD, r=4. Three bacterial candidates designated S3, S3a, and S17, and three fungal candidates designated S6, S9 and S13, exhibited significant control of P. ultimum as measured by oospores/g dry root. There were significant increases in total plant dry weight, total height, and root length. In steamed soil the treatments provided a significant increase in root length, and no other effects were found. Isolates are being identified and tested in the field.

REACTION OF SUNFLOWER LINES TO PHOMA MACDONALDII. P. A. Donald, J. F. Miller, J. R. Venette, Dept. Plant Pathology and USDA/ARS Dept. of Agronomy, North Dakota State Univ., Fargo 58105.

Death of sunflower (Helianthus annuus L.) can occur when plants are parasitized by the fungus Phoma macdonaldii Boerema. The pathogen can cause stem girdling lesions that lead to premature ripening of flowering plants. Immunity to P. macdonaldii has not been identified in commercial sunflower hybrids. Spray inoculation with conidial suspensions of P. macdonaldii of greenhouse grown seedlings was used to test 18 inbred and hybrid lines for response to the pathogen. Disease severity was measured by rating lesion intensity 10 days after inoculation. All hybrids and inbreds were susceptible; however, significant differences occurred among lines. Hybrids generally were less susceptible than their inbred parents. Hybrid performance could not be predicted by inbred parental reaction. Results from this study indicate that resistance factors are complex.

POPULATION DYNAMICS OF FOUR DIABROTICINA BEETLE VECTORS OF BACTERIAL WILT OF CUCURBITS. L. Einemann and J. Steadman, Dept. Plant Pathology, Univ. of Nebraska, Lincoln, NE 68583-7022.

Four beetle species, southern (spotted) cucumber, western corn rootworm, striped cucumber and a new vector, Acalymma gouldi Barber carried cucurbit bacterial wilt. Censusing (25 times) in 1984 from June to October and censusing (10 times) in 1985 from July to October indicated westerns and A. gouldi were less than 5% and 1% of the total beetle population, respectively. The pathogen was harbored 4% (1984) and 9% (1985) of westerns and 41% (1984) and 20% (1985) of A. gouldi. Striped beetles varied from 55% to 6% of the population while their frequency of carrying wilt was 28% to 37%. Southern (spotted) beetles comprised 40% to 90% of the population, but only 8% carried the pathogen. Vector potential was determined by extract injection into cucumber seedlings.

A COMPARISON OF METHODS TO STORE ISOLATES OF PYRENOPHORA TRITICI-REPENTIS. R. M. Hunger, Plant Pathology Dept., Oklahoma State University, Stillwater, OK, 74078-0285.

Four, single-ascospore isolates were stored continuously on clarified V-8 juice agar (CV-8) at 4 C, on CV-8 at 4 C but transferred monthly, on CV-8 at -70 C, in 10% DMSO in liquid nitrogen, and on autoclaved straw at room temperature and at -10 C. Isolates were evaluated after 0, 4, 8, 12, and 16 wk of storage for growth and sporulation on potato dextrose agar and CV-8, and for pathogenicity on wheat seedlings. Growth and pathogenicity were not affected by storage on CV-8, but isolates sporulated sparsely after 16 wk at 4 C. One isolate stored on straw showed abnormal and decreased growth after 12 wk of storage. Growth and pathogenicity of the other isolates was not affected by storage on straw, but sporulation declined after 16 wk. No changes in the isolates occurred when stored in liquid nitrogen and results indicate that storage in liquid nitrogen is best to maintain isolates of P. tritici-repentis.

LABORATORY IDENTIFICATION OF PATHOGENIC ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *APII* RACE 2. Karen F. Ireland and Melvyn L. Lacy, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI. 48824.*

Fourteen isolates of *Fusarium oxysporum* f. sp. *apii* (FOA) race 2 and one FOA race 1 isolate were grown on potato sucrose agar containing 15 g $KClO_3$/ l. Mutants that could not utilize NO_3^- as a nitrogen source were recovered for each isolate. Two complimentary mutants were selected from each isolate and all mutants were paired in all possible combinations. Mutants from four FOA race 2 isolates formed nitrate-utilizing heterokaryons with at least one mutant from all other FOA race 2 isolates. No FOA race 2 mutants formed heterokaryons with the FOA race 1 mutants. Mutants from 11 other forma speciales of *F. oxysporum* did not form heterokaryons with FOA mutants. This procedure can identify FOA race 2 isolates in half the time it takes for pathogenicity tests, but success depends upon the production of good tester strains. Partial characterization revealed the FOA mutants were lacking either nitrate or nitrite reductase activity.

* = Student Paper Competition

OVERWINTERING POPULATIONS OF PSEUDOMONAS SYRINGAE PV. TOMATO AS A SOURCE OF SPRING INOCULUM IN MICHIGAN. D. J. Jardine and C.T. Stephens, Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS 66506 and Dept. of Botany and Plant Pathology, Michigan State Univ., E. Lansing, MI 48824

Studies were undertaken to determine if Pseudomonas syringae pv. tomato (Pst) could overwinter and serve as a spring inoculum source in Michigan. Pst was not found in association with leaves or roots of weed species sampled from fields in which infected tomato plants had grown the previous year. The pathogen also was not detected in nonrhizosphere soil of infested fields. Pst was recovered in April from overwintered surface debris inoculated with a rifampicin resistant isolate the previous year. The ability of overwintered Pst to serve as a source of spring inoculum was related to the tillage system used. Leaf infection by the resistant Pst occurred when tomato seedlings were planted into a previously infested field that was either spring-plowed or left untilled. Plants did not become diseased when planted into areas that were fall plowed.

CHARACTERIZATION OF WHEAT SPINDLE STREAK MOSAIC VIRUS AND TIME COURSE STUDY OF VIRAL DISEASE PROGRESSION BY WESTERN BLOT ANALYSIS. T. L. Kendall and S. A. Lommel, Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506.

The molecular weight of a field isolate of wheat spindle streak mosaic virus (WSSMV) capsid protein was determined to be 36 kd by SDS-polyacrylamide gel electrophoresis. Antiserum made against denatured WSSMV capsid protein was found to be specific when compared with a known WSSMV antiserum and with antiserum to wheat soilborne mosaic virus (WSBMV). Viral disease progression in the source field was monitored at two-week intervals with Western blots using WSSMV, WSBMV, and wheat streak mosaic virus (WSMV) antisera. WSSMV decreased over time and was not detected in the last collection. WSMV was detected at the second collection and increased over time. WSBMV was not detected at any time.

MODIFICATION OF DNA BY THE HOST-SELECTIVE TOXIN FROM HELMINTHOSPORIUM CARBONUM, RACE 1. Shin-Duk Kim, Stephen J. Danko, and Herman W. Knoche, Department of Agricultural Biochemistry, University of Nebraska, Lincoln, NE 68583-0718

Maize lines susceptible to Helminthosporium leaf spot, caused by Helminthosporium carbonum, race 1, are also very sensitive to HC-toxin, a cyclic tetrapeptide produced by the fungus. Because the toxin contains an epoxide group essential for biological toxicity, HC-toxin may damage sensitive maize by modification of nucleic acids or proteins. This hypothesis is supported by the observation that HC-toxin requires an extended incubation period with sensitive maize tissue to cause damage. When HC-toxin was incubated with deoxyguanosine, dGMP, poly-d(G-C), or DNA isolated from susceptible or resistant maize leaves, a toxin-substrate adduct was formed, as evidenced by the appearance of new fluorescence absorbtion and emission bands. The modification appears to be covalent and non-specific. If the basis for sensitivity of maize to HC-toxin involves modification of DNA in vivo, resistance may be due to detoxification, differential uptake, or DNA repair.

ENVIRONMENTAL AND GENETIC FACTORS AFFECTING POD INFECTION OF SOYBEANS BY PHOMOPSIS SP. G. L. Lamka and D. C. McGee, Department of Plant Pathology, Seed and Weed Sciences, 351 Bessey Hall, Iowa State University, Ames, Iowa 50011.

Weekly inoculations of soybeans with conidia of Phomopsis longicolla showed that higher levels of pod infection, as measured at growth stage R6, occurred in plants inoculated at reproductive compared to vegetative growth stages. Individual environmental parameters measured following the inoculation of reproductive stage plants, were not highly correlated with pod infection. When moisture events in the 48 hour period following inoculation were defined by combining moisture parameters, wet events (minimum relative humidity >60%, greater than 25 hours of >90% relative humidity, and greater than 30 hours of leaf wetness) were associated with increased pod infection. Twenty cultivars were screened for susceptibility to Phomopsis pod infection under field conditions and in a greenhouse. No differences in susceptibility to pod infection by P. longicolla were detected in the 20 soybean cultivars tested in the field and the greenhouse.

PITTED SCAB OF RUSSET BURBANK POTATOES. D.A.R. McQueen, N.A. Anderson, and A.G. Peterson, Departments of Plant Pathology and Entomology, University of Minnesota, St. Paul, MN 55108.

Deep pitted lesions were observed on Russet Burbank potatoes grown in short rotations on sandy soils in central Minnesota. Although 66% of the lesions contained insects, mites, or annelids, no decrease in the numbers of lesions occurred when replicated plots in production fields were treated with Aldicarb, Dyfonate, or Phorate. Plots in the same field treated with 20 and 30 lbs PCNB per acre decreased the number of lesions significantly. Isolates of Streptomyces were obtained from the lesions. In inoculation studies in the greenhouse, pitted lesions occurred on tubers only from plants growing in inoculated soil.

INFECTION OF ASPEN BY Hypoxylon mammatum THROUGH TREEHOPPER WOUNDS. M. E. Ostry and N. A. Anderson, USDA Forest Service and Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

During oviposition female treehoppers (Family: Membracidae) cut slits into small diameter ($\bar{x}$ = 5 mm) twigs, often killing the distal portion. We have found hypoxylon cankers associated with these wounds. Small cankers ($\bar{x}$ = 3.5 cm)--many not extending beyond the wound--were usually found on 2-year-old branch wood in the upper crowns of trees. Hyphal pegs were present on several of the cankers examined. Cankers do not advance to the main stem because the small twigs dry and fall off before the fungus reaches a major branch. Treehoppers make wounds deep into the wood similar to wounds made by saperdas and cicadas. This is further evidence that a deep, incompletely closed wound is necessary for infection.

PECTOLYTIC BACTERIA IN SORGHUM STALK ROT TISSUE. J.E. Partridge and P.T. Nordquist, Depts. of Plant Path. and Agron., UNL, Lincoln, NE 68583.

Organisms were isolated from the second internode of sorghum plants standing in the field throughout the Nebraska winter. As previously reported in the literature, Fusarium graminearum and F. moniliforme were the predominant microorganisms in matured sorghum plants before frost. However, after frost the predominant microorganisms present were bacterial species. Many of the bacterial isolates possessed pectolytic activity as judged by their growth on pectin containing media. The abundance of pectolytic bacteria continued until only the rind and vascular tissues remained. After the tissues were "shelled out", the bacterial predominance waned and the major microorganism was F. graminearum. The rapid rise in bacterial population with the concomitant masceration of stalk tissue leads to a postulation that, in sorghum, pectolytic bacteria may play a major role in post-frost stalk decomposition (stalk rot).

ESTIMATION OF EFFICACY OF FOLIAR FUNGICIDES ON BARLEY BY MULTISPECTRAL RADIOMETRY. V. D. Pederson, North Dakota State University, Fargo 58105.

Fungicide treatments were applied at the boot stage and boot plus 10 days to barley (Hordeum vulgare L.) cultivars 'Larker' at Fargo and 'Robust' at Langdon, ND. Prevalent foliar diseases were Spot Blotch (Helminthosporium sativum) at Fargo and Scald (Rhyncosporium secalis) at Langdon. Fungicides included Mancozeb (Dithane M-45), Propiconazol (Tilt) and Ortho XE779 applied at recommended rates. A multispectral radiometer with wavelengths of 500 to 850 nm at 50 nm intervals was used to record percent reflection of radiation from plot canopies at the soft dough stage of kernel development. Highly significant positive correlations between percent reflection of the IR wavelengths and mean disease proportion on the upper 3 leaves were obtained at both locations. Prime advantages of the multispectral radiometer over visual observations for estimation of foliar disease control are speed and objectivity of measurements.

NUTRITIONAL REQUIREMENTS FOR SEXUAL REPRODUCTION BY PYRENOPHORA TRITICI-REPENTIS. W.F. Pfender and S.L. Wootke, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Pyrenophora tritici-repentis, causal agent of wheat tan spot, was inoculated onto plates of purified cellulose amended with various combinations of inorganic salts and urea, then incubated under conditions favorable for production of pseudothecia and ascospores. Sexual reproduction was dependent on nitrogen (N), phosphorus (P) and potassium (K) levels. Given P and K at adequate levels, 900 ppm N (1.9 mg urea/g cellulose) supported

abundant sporulation. At 90 ppm N sporulation was reduced 60%. At 9 ppm N very few ascocarps or ascospores were produced. In the presence of 900 ppm N, requirements for P and K were clearly demonstrated. When supplied with K alone, no ascocarps were produced; with P alone a few ascocarps were produced, but none developed ascospores. When supplied with 200 ppm P and 500 ppm K together, abundant sporulation occurred. Reducing both simultaneously to 1/10 these levels had no significant effect, but at 2 ppm P + 5 ppm K sporulation was virtually eliminated.

MULTIVARIATE ANALYSIS TO COMPARE MICROBIAL COMMUNITIES OF WHEAT STRAW UNDER DIFFERENT SIMULATED TILLAGE PRACTICES. W.F. Pfender and S.L. Wootke, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Winter wheat straw naturally infested with Pyrenophora tritici-repentis (Pt) and Septoria nodorum (Sn) was buried in the soil, placed directly on the soil surface, or placed 2 cm above the soil within a straw layer to simulate different tillage practices. Periodically between June and April straw from each treatment was sampled and assayed by dilution plating to determine microbial populations. A data matrix of samples X species was constructed and analysed by detrended correspondence analysis (DCA). The major DCA axis ran from newly-colonized and above-soil straw (occupied by primary colonizers such as Pt and Sn) to on-soil and buried straw (occupied by secondary colonizers such as Trichoderma and actinomycetes). Pt and Sn were absent from most on-soil and buried straws. Laboratory studies on degradative abilities of isolates showed that chitin degradation and low-temperature cellulolysis were associated predominantly with secondary and primary colonizers, respectively.

VARIATION IN A RUST-RESISTANT REACTION OF PHASEOLUS VULGARIS L. DUE TO LEAF AGE. M. Shaik and J.R. Steadman, Dept. of Plant Pathology, Univ. of Nebraska, Lincoln, NE 68583-0722.

Leaf age at the time of inoculation has been reported to influence the degree of susceptibility to bean rust (Uromyces appendiculatus (Pers.) Unger var. appendiculatus). In a resistant reaction, manifested by small pustules surrounded by necrotic areas, the presence of necrosis decreased when older leaves were inoculated. Inoculation of 2- to 8-day-old leaves resulted in the development of necrosis around all the pustules. On 10- to 14-day-old leaves, necrosis surrounded no more than 40% of the pustules and on 16-21 day-old leaves necrosis did not develop around any pustules. The morphological and microscopic manifestation of this variation and its importance in evaluating resistance to the bean rust pathogen is discussed.

EFFECT OF SELECTING FOR RESISTANCE TO PERONOSPORA TRIFOLIORUM IN ALFALFA ON FORAGE SAPONIN CONTENT. D. L. Stuteville and D. Z. Skinner, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Selecting in alfalfa to alter forage saponin content has affected resistance to P. trifoliorum (Crop Sci. 16:193-199; Can. J. Plant Sci. 58:893-894). 'CUF 101' alfalfa and germplasms UC 123 and UC 143 derived from it by one and two cycles, respectively, of recurrent phenotypic selection for resistance to P. trifoliorum were evaluated for forage saponin content. The percent of plants resistant (symptomless) to P. trifoliorum isolates I-7 and I-8, respectively, were CUF 101, 28 and 3; UC 123, 62 and 3; and UC 143, 61 and 72. Saponin indexes (greater values=lower saponin content) were CUF 101, 101; UC 123, 92; UC 143, 100; 'Lahontan' (low saponin check), 98; and 'Unita' (high saponin check), 35. Saponin apparently played no role in the resistance to P. trifoliorum as the highly resistant UC 143 germplasm was derived from the very low saponin CUF 101 cultivar without affecting the saponin index.

ISOZYME DIFFERENCES BETWEEN GEOGRAPHIC SOURCES OF ENDOCRONARTIUM HARKNESSII. G. A. Tuskan and J. A. Walla, North Dakota State University, Fargo, ND 58105.

Starch gel electrophoresis was used to study isozyme variability of E. harknessi from two geographic sources in North Dakota. Spores from multiple galls on ponderosa pine at each source were bulked. Spore subsamples (50 mg) were prepared using three techniques; 1) 10 hr digestion in 10 ml of 1% cellulase, 1% chitinase and 1 M mannitol, followed by maceration in phosphate buffer, 2) maceration alone in phosphate buffer and 3) 24 hr germination, followed by maceration in phosphate buffer. Samples were then electrophoresed and screened for 33 enzyme systems using 3 buffers (morpholine-citrate, histidine-citrate and lithium-borate), with 11 enzymes/buffer. Twenty-one enzymes displayed isozyme activity. Spore preparation technique affected response of 11 enzymes. Five of the enzymes showed isozyme banding differences between the geographic sources. It appears that isozyme banding using spore preparation techniques 1 and 2 is sufficient to separate geographic sources of E. harknessii.

ISOLATION AND SCREENING OF CELLULOLYTIC *FUSARIUM* STRAINS. E.G. Wene and A.A. Antonopoulos, Argonne National Laboratory, Argonne, IL 60439.

Various techniques were used to isolate more than 3,500 *Fusarium* isolates from a wide variety of locations. This work involved the isolation and development of potential cellulolytic *Fusarium* strains able to economically degrade and ferment biomass to ethanol. Several cellulase enzyme screening assays were compared. Most of the isolates were screened for cellulolytic activity with a rapid test-tube-cellulose-agar clearing assay. Potential cellulolytic strains were further tested in 250-mL shake flasks and 5-L fermentors. Nearly all the isolates showed some degree of cellulolytic activity in the rapid screening technique. *Fusarium oxysporum* was the most often isolated species and generally showed a higher degree of cellulolytic activity. Several isolates produced up to 1.2 IU/mL cellulase when grown in 5-L fermentors. Aeration and/or addition of nitrogen, pH control, and temperature influenced cellulase production.

FOUR YEAR FIELD EVALUATION OF FUNGICIDE SEED TREATMENTS ON WHEAT SEEDLING EMERGENCE. Ervin Williams, Jr. Plant Pathology Department, Oklahoma State University, Stillwater, OK 74078-0285.

Five commercial fungicide formulations (Carboxin-Captan, Carboxin-Thiram, PCNB, TCMTB; Metalaxyl) labeled for treating wheat seed were compared with untreated seed for consistency in improving seedling emergence from 1982-85. Percent seedling emergence was determined from plantings of 200 seeds in 12 ft rows in 10-15.5 C soil with adequate moisture. Bipolaris sorokiniana was the most prevalent pathogen isolated in 1982-84 with Pythium spp more prevalent in 1985. Certain fungicides significantly increased seedling emergence 2 of 4 years. No fungicide gave consistently high readings all 4 years. No post emergence plant mortality was observed from readings 4 weeks and 7 months after planting. Significant increases in seedling emergence occurred 50% of the time, however emergence increases from a given fungicide for a given year were unpredictable.

FIELD EVALUATION OF SUGAR BEET SEED TREATMENTS. C. E. Windels, Northwest Expt. Stat., Univ. of Minn., Crookston, MN 56716.

Sugar beet 'Maribo Ultramono' seed was treated with fungicides active against Pythium (metalaxyl, oxadixyl), Rhizoctonia solani (chloroneb, quintozene, furmecyclox, thiophanate), broad-spectrum fungicides (captan, thiram, quintozene+etridiazole) or with Quantum™-4000 (Bacillus subtilis), singly and in combination. In a field naturally infested with Pythium spp., R. solani and Aphanomyces cochlioides, oxadixyl and metalaxyl gave 911% and 848% increases in stand, respectively, over untreated seed. Metalaxyl+furmecyclox, captan, thiram, and thiram+quintozene+etridiazole gave 762%, 713%, 576% and 413% increases in stand, respectively, over untreated seed ($P>0.05$). B. subtilis, quintozene+etridiazole and fungicides active against R. solani gave stands equal to untreated seed. Stand loss was 41% for treated and untreated seed 8 wk after planting. Thus, in a field with soilborne pathogens, fungicides active against Pythium gave the best stands, but seed treatment did not affect post-emergence damping off.

FROST DAMAGE TO TURF MIMICS SEVERAL PATHOGENIC DISORDERS. G.L. Worf, J.S. Stewart, and M.F. Heimann, O.S.F. Department of Plant Pathology, University of Wisconsin-Madison, WI 53706.

Subfreezing temperatures in early May caused injury resembling several pathogenic disorders to Kentucky bluegrass. Several mistaken diagnoses were later observed. White leaf tips, often accompanied by white transverse bands across the leaves, were the primary effects of frost injury. Of 24 cultivars examined, 11 showed no color demarcation between green and white tissue, 'Merit' and 'Glade' had distinct purple margins, and the remaining 11 showed occasional purpling. These symptoms resembled infections caused by Ascochyta, Nigrospora or Lanzia/Moellerodiscus. Ascochyta was isolated from some spots, suggesting a saprophytic relationship. Shoots and tillers were damaged in pockets, especially where closely mowed. Their gray-brown coloration suggested Helminthosporium infections. Effects of low temperature were confirmed by growth chamber tests at -5 C.