Evaluation of *Fusarium graminearum* Associated with Corn and Soybean Seed and Seedling Disease in Ohio

K. D. Broders, P. E. Lipps, P. A. Paul, and A. E. Dorrance, Department of Plant Pathology, The Ohio State University, Wooster 44691

ABSTRACT

Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:1155-1160.

Fusarium graminearum is an important pathogen of cereal crops in Ohio causing primarily head blight in wheat and stalk and ear rot of corn. During the springs of 2004 and 2005, 112 isolates of *F. graminearum* were recovered from diseased corn and soybean seedlings from 30 locations in 13 Ohio counties. These isolates were evaluated in an in vitro pathogenicity assay on both corn and soybean seed, and 28 isolates were tested for sensitivity to the seed treatment fungicides azoxystrobin, trifloxystrobin, fludioxonil, and captan. All of the isolates were highly pathogenic on corn seed and moderately to highly pathogenic on soybean seed. Fludioxonil was the only fungicide that provided sufficient inhibition of mycelial growth; however, several fludioxonil-resistant mutants were identified during the sensitivity experiments. These results indicate that *F. graminearum* is an important pathogen of both corn and soybean seed and seedlings in Ohio, and that continued use of fludioxonil potentially may select for less sensitive isolates of *F. graminearum*.

Additional keywords: Dynasty, *F. acuminatum*, *F. equiseti*, *F. oxysporum*, *F. semitectum*, *F. subglutinans*, *F. verticillioides*, Maxim, SHAM, Trilex

Several species of *Fusarium* have been associated with diseases of corn at all growth stages, causing seed, root, stalk, and ear rot (5,7). In many cases, these species are either soilborne (7,28) or seedborne pathogens (7,16). Multiple species of *Fusarium* can be found in most agronomic crop soils in Ohio and throughout the United States (18). Additionally, multiple species of *Fusarium* can be isolated from a single diseased seedling, making accurate diagnosis difficult. Diagnosis can be complicated even more by the many primarily saprophytic *Fusarium* spp. commonly found in soil and on plant roots.

Of the many species of *Fusarium* that may be present in the soil and on plant tissues, perhaps the most important in Ohio, and much of the wheat- and corn-growing region of the United States, is *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwien.) Petch). *F. graminearum* is an economically important pathogen of cereal crops that can cause substantial yield and quality losses in corn and wheat, resulting from ear and stalk rots, and head scab, respectively. *F.*

graminearum also is an important seedling pathogen of both corn (4) and wheat (13), and the degree of pathogenicity on wheat and corn seedlings is highly correlated (4).

Most producers in Ohio use a corn-soybean or corn-soybean-wheat rotation in combination with reduced-tillage or no-tillage systems, which help to conserve soil, prevent erosion, and increase organic matter. These production practices may favor the survival of *F. graminearum* because *Fusarium* spp. survive on both corn (6,18,36) and soybean (2,18) debris. Reduced-tillage practices leave a substantial amount of corn and soybean residue on the soil surface, resulting in an increase in the amount of initial inoculum for the subsequent year's crop. In addition, high levels of plant residue on the soil surface in the spring prevent the soil from drying and warming as rapidly as soil that has been tilled or plowed using conventional methods (10,34), thereby increasing the amount of time required for seed to germinate. This predisposes seed and seedlings to infection by seed and soilborne pathogens.

Fungicide seed treatment is the most common practice for managing soilborne, seed, and seedling pathogens, including species of *Fusarium* (21). Recently, fludioxonil and two strobilurins, trifloxystrobin and azoxystrobin, were introduced to the seed-treatment market to improve the efficacy of seed treatment combinations against a wide range of soilborne pathogens, including *Fusarium* spp. Fludioxonil

differs from trifloxystrobin and azoxystrobin in the mode of action, but all three fungicides have site-specific modes of action. Prior to the introduction of fungicides with site-specific modes of action, fungicides or fungicide combinations, such as captan or pentachloronitrobenzene, with a very broad spectrum of activity were used to treat seed for protection against *Fusarium*, *Rhizoctonia*, and *Pythium* spp.

There is little data on sensitivity of *Fusarium* spp. to fludioxonil, but it appears to be effective against *Sclerotinia sclerotiorum* (Lib.) de Bary on infected soybean seed (23). *Botrytis cinerea* Pers., however, is reported to develop resistance to fludioxonil under both laboratory and field conditions (35). There are several recent reports of *Fusarium* spp. (11) and *Alternaria alternata* (Fr.) Keissl. (22,30) with decreased sensitivity to different strobilurin fungicides; hence, there may be a risk of fungicide resistance or insensitivity developing due to widespread use of fungicides with site-specific modes of action (1,33). In the case of resistance to the strobilurin fungicides, it also appears that some soilborne pathogens constitutively express cytochrome and alternative oxidase respiratory pathways (14,15), enabling them to continue production of ATP and, hence, appear resistant to strobilurin fungicides.

Pre- and post-emergence diseases have increased in Ohio despite the changes in fungicide chemistries used to treat both corn and soybean seed. This may be due, in part, to timing of planting beginning earlier in the year, which likely predisposes the seed to damage by increasing the amount of time required for germination at the lower temperatures, and lengthening the infection period. In Ohio, *Pythium* spp. are primary seed and seedling pathogens of corn and soybean (3); however, *F. graminearum*, as well as many other *Fusarium* spp., have been isolated from diseased corn and soybean seed and seedlings at relatively high frequencies. This high incidence of *Fusarium* spp. from diseased seedlings in combination with a shift in fungicide chemistries has led to the current investigation.

The objectives of this study were to (i) isolate and identify the *Fusarium* spp. associated with seed and seedling disease of corn and soybean, (ii) evaluate the pathogenicity of each isolate on corn and soybean seed, and (iii) determine the sensi-

Corresponding author: A. E. Dorrance
E-mail: dorrance.1@osu.edu

Accepted for publication 10 April 2007.

tivity of *F. graminearum* to four commonly used commercial seed-treatment fungicides.

MATERIALS AND METHODS

Isolation and identification. Diseased corn and soybean seedlings were collected from fields with reports of emergence problems during spring 2004 and 2005. Sections of root or shoot tissue from diseased seedlings were placed between two paper towels and pressed using a rolling pin to remove excess water, then plated onto water agar (WA; 20 g of agar/liter). Isolates producing conidia distinctive to *Fusarium* spp. were transferred to carnation leaf agar (CLA) (26) and allowed to colonize and produce conidia for 8 days. Sterile distilled water was used to dislodge the conidia, and a 5-mm wire loop was used to spread conidia onto plates containing Komada's selective media (17). Conidia were allowed to germinate for 24 h, and then single germinated conidia from each isolate were transferred to separate plates containing either CLA or potato dextrose agar (PDA) for identification. Isolates were identified by morphology according to the key by Nelson et al. (26) and, in the case of *F. verticillioides* (Sacc.) Nirenburg, as described by Nirenburg (27). For long-term storage, isolates were grown on CLA for 8 days, and then 2 ml of a 10% glycerol solution was pipetted onto the plate to dislodge conidia; the resulting conidial suspension was removed from the plate with a pipette, transferred to a 2-ml Eppendorf tube, and stored at -80°C (19).

Pathogenicity and greenhouse assays. All isolates collected were tested for pathogenicity on both corn and soybean seed. This test was conducted using a petri plate assay, similar to that used previously to evaluate fungicide seed treatments on corn for the protection against *Fusarium* spp. (24) as well as pathogenicity and aggressiveness of *Pythium* and *Rhizoctonia* spp. on soybean and corn (3,8,25,37). Each isolate was grown on CLA for 4 days and a 3-mm-diameter colonized plug was transferred to the center of an 8-cm-diameter petri plate containing 15 ml of 2% WA. The isolates were allowed to colonize the plate for 4 days. Ten seeds of either corn hybrid LG2540 (LG seeds; Elmwood, IL) or soybean cv. Sloan were surface disinfested in a 0.525% sodium hypochlorite solution and then equally spaced around the plate, approximately 1 cm from the edge. Plates were incubated at 22°C in the dark for 7 days; then, healthy seed were counted. Plates were scored by counting the number of seed that successfully germinated. Seed were considered to have successfully germinated if the radicle was >1 cm long and had $>50\%$ healthy tissue. Each isolate was scored for pathogenicity based on the following rating scale: 0 = 100% germination, with no symptoms of infection; 1 = 70 to 99% germination, with

no lesion formation on the roots; 2 = 30 to 69% germination, with coalesced lesions; and 3 = 0 to 29% germination, where all seed and root tissue was colonized (3). The experiment was a randomized complete block design with replicates as individual blocks. There were three replicate plates for each isolate in each experiment and the entire experiment was conducted three times.

Ten isolates of *F. graminearum* were used in a greenhouse experiment to evaluate their pathogenicity on both corn and soybean. Inoculum was produced using a modified protocol from Munkvold et al. (24) by adding 2 ml of a conidial suspension (1×10^6 spores/ml) of each isolate to a previously sterilized 3,000-ml flask containing 1,425 ml of sand, 285 ml of cornmeal, and 110 ml of deionized H_2O . Flasks were allowed to incubate for 7 days in the dark at 22°C and were shaken every day to evenly distribute inoculum. The sand-cornmeal inoculum was mixed with sterile soil at a rate of 3:1 (vol/vol), soil:inoculum. In all, 10 soybean (Sloan) and 10 corn (LG2540) seeds each were planted in 4-inch pots containing 500 ml of infested soil. The test was a randomized complete block design with three replicate pots per isolate. There was one pot for each isolate in each block and the experiment was conducted three times. Seed were allowed to germinate and grow for 14 days, at which time seedlings were scored for germination and the presence of lesions. Seedlings with lesions on either the seed, root, or shoot were plated on WA, and isolates were identified in the same manner as described previously.

Because pathogenicity was evaluated using an ordinal rating scale, the data was analyzed using a nonparametric approach as described by Shah and Madden (31). In separate analyses using PROC MIXED of SAS (SAS Institute, Cary, NC), species and isolates were treated as fixed effects and the relative pathogenicity among isolates within species was compared using contrasts.

Fungicide sensitivity. A subset consisting of 28 isolates of *F. graminearum*, representing all the collection sites, was used to assess baseline sensitivity to fludioxonil (Maxim4FS; Syngenta Crop Protection Inc., Greensboro NC), azoxystrobin (Dynasty; Syngenta Crop Protection Inc.), trifloxystrobin (Trilex; Gustafson LLC Plant, TX), and captan (Captan 400; Gustafson LLC). All fungicides were dissolved in deionized H_2O to achieve the proper concentration of active ingredients and added to potato carrot agar (PCA) after autoclaving, when the media had cooled to approximately 55°C . Commercial-grade fludioxonil was evaluated at 0.01, 0.1, 1, 10, and 100 μg a.i./ml, and nonamended PCA was used for the control. Trifloxystrobin and azoxystrobin (commercial-grade product) were evalu-

ated at 1, 10, and 100 μg a.i./ml. For the strobilurin fungicides, each concentration also was amended with salicylhydroxamic acid (SHAM) at 150 μg /ml to inhibit the action of the alternative oxidase respiratory pathway. Two controls were included for comparison with the strobilurin fungicides; the first was nonamended PCA and the second was PCA amended with SHAM at 150 μg /ml. A preliminary experiment was executed to determine the amount of SHAM that would provide 20 to 40% inhibition of mycelial growth of all isolates. SHAM was dissolved in a 1:1 concentration of dimethylsulfoxide (DMSO) and methanol, amended to PCA at 0, 50, 100, 150, and 200 μg /ml, and evaluated in the same manner as the fungicides. Captan (commercial-grade product) was evaluated at 50, 100, 200, and 400 μg a.i./ml, and nonamended PCA was used as the control.

For each isolate, a 3-mm-diameter colonized plug from the edge of a 4-day-old colony was transferred to the center of each plate for each concentration of the fungicide evaluated. The colony diameter was measured in two places after 96 h. For each isolate at each concentration, the percent growth-inhibition was calculated by dividing the average colony diameter minus 3 mm for the agar plug by the average colony diameter on the nonfungicide amended media, multiplied by 100. For the strobilurin fungicides, the colony diameter at each concentration was compared with the colony diameter on PCA amended with SHAM at 150 μg /ml to calculate the percent mycelial growth of the control. The experiment was a randomized complete block design with replicates representing individual blocks. Each isolate was evaluated at all fungicide concentrations and each isolate-concentration combination was considered a replication. Three replicates were separated over time, with at least 24 h between replications. Variation in sensitivity among isolates to the four different fungicides and the effect of fungicide concentration on the efficacy of the four fungicides were analyzed using PROC GLM of SAS.

Fludioxonil-resistant mutants. During the fludioxonil sensitivity experiments, several isolates were able to grow on the amended media. These isolates were used in further experiments to evaluate fludioxonil resistance. A small piece of mycelia from the edge of the colony of each of these isolates was transferred to CLA. Isolates were single spored as previously described and then tested again to determine whether they retained their ability to grow on fludioxonil-amended media. Isolates were evaluated on PCA amended with fludioxonil 1, 10, and 100 μg /ml as previously described, and those that retained the ability to grow at a rate of at least 50% of the control on fludioxonil at 100 μg /ml were considered to be fludioxonil-resistant mutants. These mutant

isolates then were used in further evaluations to determine whether they retained pathogenicity and were able to infect seed treated with fludioxonil.

Five *F. graminearum* fludioxonil-resistant mutants, K80R-FM, 39-FM, 216-FM, 292-FM, and 320-FM, identified during the fungicide sensitivity assays, as well as the *F. graminearum* wild-type (WT) isolates K80R, 39, 216, 292, and 320 (the mutants they were derived from, respectively), were evaluated to determine whether isolates retained their pathogenicity. Petri plate assays were used to determine whether fludioxonil-resistant mutants had the same level of pathogenicity on corn seed as the isolates from which the mutants were derived. Seed were treated with 80 μ l of Maxim4FS that was dissolved in 8 ml of deionized H₂O, and 4 ml of this suspension was applied to 454 g of seed in a plastic bag to give a final application rate of 40 μ l per 454 g of seed, which is equivalent to 0.1 ml/kg of seed, the maximum rate on the label. The seed were shaken vigorously for 5 min to assure that all seed were treated equally and no fungicide remained in the bag. Both sets of petri plate assays were prepared and scored as previously described. For the petri plate pathogenicity assay, the same 0-to-3 rating scale was utilized and, for the fungicide sensitivity assays, PCA was amended with fludioxonil at 1, 10, and 100 μ g/ml to determine whether fludioxonil-resistant mutants retained their capacity to grow on fludioxonil-amended media.

RESULTS

Isolation and identification. In all, 53 isolates of *F. graminearum* were recovered from 24 locations across 5 counties during 2004, and 62 isolates from 25 locations across 11 counties during 2005 (Fig. 1). All of the isolates were recovered from diseased corn and soybean seed and seedlings from fields with stand establishment problems. In addition to *F. graminearum*, isolates of *F. oxysporum* Schltdl., *F. equiseti* (Corda) Sacc., *F. acuminatum* Ellis & Everh., *F. avenaceum* (Fr.) Sacc., *F. semitectum* Berk. & Ravenel, *F. subglutinans* (Wollenw. & Reinking) P. E. Nelson, Tousoun & Marasas, and *F. verticillioides* also were recovered. *F. oxysporum* and *F. graminearum* were the species most frequently recovered from diseased plant tissue.

Pathogenicity and greenhouse assays. Isolates with pathogenicity scores <1, between 1 and 2, or >2 in the petri plate assay were considered to have a low, moderate, or high level of pathogenicity, respectively. All 105 isolates of *F. graminearum* evaluated had a moderate to high level of pathogenicity on both corn and soybean (Fig. 2). *F. graminearum* was significantly ($P < 0.0001$) more pathogenic on both corn and soybean than all other species of *Fusarium* (Fig. 2). Other than *F. graminea-*

rum isolates, only *F. equiseti* and *F. verticillioides* had significantly ($P < 0.05$) greater pathogenicity scores on corn seed than the noninoculated control. None of the *F. oxysporum* isolates evaluated in the pathogenicity assay were pathogenic on either corn or soybean seed.

In the greenhouse assays, *F. graminearum* was able to successfully infect both corn and soybean seedlings in infested soil experiments. All isolates were able to significantly ($P < 0.01$) reduce germination of both corn and soybean seed compared with

the control, and the germination of corn seed was significantly ($P < 0.001$) less than soybean seed (Fig. 3). Additionally, there was a significant ($P < 0.001$) effect of isolate on the germination of both corn and soybean seed (Fig. 3). All 10 isolates of *F. graminearum* were recovered from diseased tissue of corn and soybean. The isolates also caused necrotic lesions on both the roots and seed of soybean. Lesions were brown and ranged from small elongated to large coalesced lesions and, in several instances, the entire root system

Fig. 1. *Fusarium* isolates recovered from diseased corn and soybean tissue from 16 Ohio counties and one Indiana county during 2004 and 2005.

Fig. 2. Mean pathogenicity of 55 and 50 isolates of *Fusarium graminearum* collected during 2004 and 2005, respectively, in addition to six other species of *Fusarium* on corn and soybean seed in a petri-plate assay. The number in parentheses represents the number of isolates of each species evaluated. Pathogenicity was scored on a 0-to-3 rating scale based on percent germination compared with the control, in which 0 = 100% germination with no symptom of infection, 1 = 70 to 99% germination with lesion formation on roots, 2 = 30 to 69% germination with coalesced lesions and reduced root growth, and 3 = 0 to 29% germination with colonization of all seed.

was colonized and decayed or the seed did not germinate. In the case of corn, the isolates caused lesions on both the coleoptile and the roots and, in some cases, infected the seed and prevented germination.

Fungicide sensitivity. In the statistical analysis of the four fungicide seed treatments, there was a significant ($P < 0.001$) effect of isolate on the efficacy of trifloxystrobin, based on relative mycelial growth, and no effect of isolate on the efficacy of captan, azoxystrobin, and fludioxonil. Additionally, dose had a significant ($P < 0.001$) effect on mycelial growth as expected, and fludioxonil provided the greatest level of inhibition of mycelial growth at the lowest concentration of active ingredient. Azoxystrobin and trifloxystrobin were only able to inhibit mycelial growth to 25 and 35% of the control, respectively, at 100 μg a.i./ml (Fig. 4). Captan provided greater inhibition than azoxystrobin and trifloxystrobin, but even at the highest label rate of 200 μg a.i./ml, the mean mycelial growth was still 14% of the control (Fig. 4). In contrast, fludioxonil at a rate of 1 μg a.i./ml provided complete inhibition of mycelial growth (Fig. 4). However, in 12 situations, mycelial growth at a rate of 35 to 65% of the control was observed on media amended with fludioxonil at either 1, 10, or 100 μg a.i./ml. These isolates were considered fludioxonil-resistant mutants. All fludioxonil-resistant mutants evaluated in a second fungicide sensitivity assay retained the ability to grow at a rate of at least 80% of the control on media amended with fludioxonil at 100 μg a.i./ml (Fig. 5).

Fludioxonil-resistant mutants. In the petri plate pathogenicity assays with untreated corn seed, there was no significant ($P = 0.76$) difference in pathogenicity between the fludioxonil-resistant mutants and the WT isolates from which they were

derived (Fig. 6). There was a significant ($P < 0.001$) difference in the pathogenicity of WT isolates of *F. graminearum* on untreated seed versus seed treated with fludioxonil, indicating that the fludioxonil was efficacious on the WT isolates. There was no significant difference between the pathogenicity of the WT isolates of *F. graminearum* on seed treated with fludioxonil and the noninoculated controls (Fig. 6).

In the case of fludioxonil-resistant mutants, there was a significant ($P < 0.001$) effect of isolate on pathogenicity when tested on fludioxonil-treated seed, but no significant effect on nontreated seed. The pathogenicity of fludioxonil-resistant mutants K80R-FM, 292-FM, and 320-FM on fludioxonil-treated seed was not significantly different from that of nontreated seed, indicating that the fludioxonil was not effective against these isolates. In contrast, the pathogenicity of fludioxonil-resistant mutants 216-FM and 39-FM on treated seed was significantly ($P < 0.01$) less than that on nontreated seed (Fig. 6). However, the pathogenicity of all the fludioxonil-resistant isolates was significantly ($P < 0.01$) greater than the WT isolates on fludioxonil-treated seed and the noninoculated control (Fig. 6).

DISCUSSION

In Ohio, and across the corn- and soybean-growing regions of the United States, several *Fusarium* spp. have been implicated in causing seed and seedling damage (24). This study was able to demonstrate that there are multiple species of *Fusarium* present in the soil of many crop fields in Ohio, but most are not pathogenic. The exception was *F. graminearum*, which appears to be an important seed and seedling pathogen of corn and soybean. Additionally, several species of *Fusarium* have been associated with seedling and root

diseases of wheat, including *F. graminearum* (13,32).

The petri-plate pathogenicity assay in combination with the greenhouse assay provided an evaluation of the probable role of *F. graminearum* as both a seed and seedling pathogen of both corn and soybean. *F. graminearum* previously was implicated as a seedling pathogen of corn (7,16), and this study confirms previous findings (29) that *F. graminearum* is a pathogen of soybean. More importantly, *F. graminearum* was found in corn (18,20,36), soybean (2), and wheat (12) debris left on the soil after harvest, providing sufficient inoculum for seedlings planted into the debris. These previous studies and information from this study confirm that *F. graminearum* is present in the soil on diseased and dead seedlings as well as on soybean debris, providing additional evidence that soybean residue may provide inoculum for the succeeding wheat and corn crops.

Most corn seed is treated with fungicides for protection against many soilborne pathogens, including *Fusarium* spp. Currently, captan, trifloxystrobin, azoxystrobin, and fludioxonil are labeled for use on corn

Fig. 3. Germination of corn and soybean seed when planted in soil inoculated with 10 different isolates of *Fusarium graminearum*. The potting mix consisted of a sand-cornmeal substrate colonized by the *F. graminearum* isolates that was distributed equally with sterile soil at a rate of 3:1 (vol/vol) soil:inoculum.

Fig. 4. Growth of 28 isolates of *Fusarium graminearum* evaluated on media amended with different concentrations of azoxystrobin, trifloxystrobin, captan, and fludioxonil.

seed. Results from the fungicide sensitivity experiments indicate that there is little variability in sensitivity of *F. graminearum* isolates to the fungicides evaluated, with the exception of trifloxystrobin. The two strobilurin fungicides tested inhibited mycelial growth of *F. graminearum* only to 25 to 35% of the control at 100 µg a.i./ml, even with the addition of SHAM to inhibit the action of the alternative oxidase. This agrees with previous reports that *Fusarium* spp. have reduced sensitivity to strobilurin fungicides (11). Captan, at 200 µg/ml, an equivalent amount of active ingredient applied per seed when using the maximum label rate, provided greater inhibition of mycelial growth than the strobilurin fungicides. However, captan did not provide complete inhibition of mycelial growth. In contrast, fludioxonil at 1 µg/ml provided complete control of mycelial growth, with the exception of the fludioxonil-resistant mutants identified during these experiments.

All of the fludioxonil-resistant mutants retained the ability to grow on media amended with fludioxonil and also retained the ability to infect corn seed, not only nontreated seed but also corn seed that were treated with fludioxonil. In contrast, fludioxonil-treated seed germinated and produced a healthy root system when challenged with the WT isolates of *F. graminearum*. Thus, pathogenicity of these isolates was not impaired by this mutation. The mechanism and the rate at which these isolates became resistant to fludioxonil has not been determined. Resistance to fludioxonil previously was identified in both lab and field isolates of *B. cinerea*, and this was influenced by at least two genes (35).

F. graminearum was readily isolated from diseased corn seed and seedlings from the field and proved to be pathogenic on both corn and soybean seed and seedlings in pathogenicity experiments. We cannot determine precisely the cause of such a high level of *F. graminearum* recovered from these diseased seedlings. Changes in fungicide chemistries may have played a role because *Fusarium* spp. have demonstrated decreased levels of sensitivity to several of the newer fungicide compounds (11,35). Two other possible factors include an increase in inoculum as a result of reduced or no-tillage practices, and the effect of the soil environment on both seed germination and pathogen growth at earlier planting dates. The residue left on the soil surface provides a place for *F. graminearum* to survive, thus providing more initial inoculum in the form of mycelia or perithecia of the teleomorph *G. zae*, at the beginning of the growing season. This increase in inoculum may have allowed for the establishment of several isolates of *F. graminearum* that were pathogenic on soybean.

The temperature and moisture at the time of planting also may be very impor-

tant factors in the ability of *F. graminearum* to infect seed or seedlings. Seed planted early in the season generally are exposed to low soil temperatures with more moisture. This increases the time required for germination of the seed and favors many soilborne pathogens, in particular *F. graminearum*. Dufault et al. (9) found that both moisture and temperature had an effect on the production of perithecia of *G. zae*. High moisture levels (–0.45 Mpa) and temperatures as low as 16°C can increase the production and size of perithecia (9) and, therefore, may increase

the amount of inoculum available to infect young seedlings. However, infection of seedlings may be occurring at even lower soil temperatures, and primary inoculum sources probably would be large amounts of conidia on residues buried in the soil.

The cooler temperatures and high moisture levels at planting in the spring of 2004 and 2005, in combination with reduced tillage and new fungicide chemistries, all likely contributed to the higher frequency of *F. graminearum* recovered from diseased seed and seedlings in Ohio. The fact that *F. graminearum* causes multiple dis-

Fig. 5. Growth of five fludioxonil-resistant mutants on media amended with fludioxonil at 1, 10, and 100 µg/ml.

Fig. 6. Pathogenicity on corn of five wild-type isolates of *Fusarium graminearum* and the fludioxonil-resistant mutants derived from them. Corn seed was treated with Maxim4FS (40 µl per 454 g) and used in a petri-plate assay. Pathogenicity was scored on a 0-to-3 rating scale based on percent germination compared with the control, where 0 = 100% germination with no symptom of infection, 1 = 70 to 99% germination with lesion formation on roots, 2 = 30 to 69% germination with coalesced lesions and reduced root growth, and 3 = 0 to 29% germination with colonization of all seed.

eases on each of the crops in the rotation and readily sporulates on crop residues also may have contributed to the build-up of inoculum, especially in conservation tillage systems. Most importantly, in the current three-crop system, corn-soybean-wheat rotation may contribute very little to the reduction of inoculum of *F. graminearum*, because the pathogen has the capacity to infect all three hosts. These results indicate that *F. graminearum* is an important pathogen of both corn and soybean seed and seedlings in Ohio, and that continued use of fludioxonil potentially may select for less sensitive isolates of *F. graminearum*. Thus, some form of tillage may be needed to reduce inoculum and, consequently, minimize seed and seedling disease in early-planted corn and soybean.

ACKNOWLEDGMENTS

Salaries and research support were provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center (OARDC), The Ohio State University. This work was supported in part by Pioneer Crop Management Research Awards Program and OARDC Matching Grants Program. We thank A. Johnston, S. A. Berry, and F. Cruz for technical assistance; and J. Belizzi for collection of isolates in 2004.

LITERATURE CITED

- Avila-Adame, C., and Köller, W. 2003. Impact of alternative respiration and target-site mutations on responses of germinating conidia of *Magnaporthe grisea* to Qo-inhibiting fungicides. *Pest Manage. Sci.* 59:303-309.
- Baird, R. E., Mullinix, B. G., Peery, A. B., and Lang, M. L. 1997. Diversity and longevity of the soybean debris mycobiota in a no-tillage system. *Plant Dis.* 81:530-534.
- Broders, K. B., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. *Plant Dis.* 91:727-735.
- Carter, J. P., Rezanoor, H. N., Holden, D., Desjardins, A. E., Plattner, R. D., and Nicholson, P. 2002. Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. *Eur. J. Plant Pathol.* 108:573-583.
- Chen, W. 1999. *Pythium* root rot and feeder root necrosis. In: *Compendium of Corn Diseases*. D. G. White, ed. American Phytopathological Society, St. Paul, MN.
- Cotten, T. K., and Munkvold, G. P. 1998. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in maize stalk residue. *Phytopathology* 88:550-555.
- Dodd, J. L., and White, D. G. 1999. Seed rot, seedling blight, and damping-off. In: *Compendium of Corn Diseases*. D. G. White, ed. American Phytopathological Society, St. Paul, MN.
- Dorrance, A. E., Berry, S. A., Bowen, P., and Lipps, P. E. 2004. Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Online. *Plant Health Progress* doi:10.1094/PHP-2004-0202-01-RS.
- Dufault, N. S., De Wolf, E. D., Lipps, P. E., and Madden, L. V. 2006. Role of temperature and moisture in the production and maturation of *Gibberella zeae* perithecia. *Plant Dis.* 90:637-644.
- Griffith, D. R., Mannering, J. V., and Moldenhauer, W. C. 1977. Conservation tillage in the eastern corn belt. *J. Soil Water Conserv.* 32:20-28.
- Gutierrez Chapin, L. J., Wang, Y., Lutton, E., and McSpadden Gardener, B. B. 2006. Distribution and fungicide sensitivity of fungal pathogens causing anthracnose-like lesions on tomatoes grown in Ohio. *Plant Dis.* 90:397-403.
- Inch, S. A., and Gilbert, J. 2003. Survival of *Gibberella zeae* in *Fusarium*-damaged wheat kernels. *Plant Dis.* 87:282-287.
- Jones, R. K. 1999. Seedling blight development and control in spring wheat damaged by *Fusarium graminearum* group 2. *Plant Dis.* 83:1013-1018.
- Joseph-Horne, T., Wood, P. M., Heppner, C., and Holloman, D. 1999. Involvement of the alternative oxidase in cellular energy production in the wheat 'take all' fungus, *Gaeumannomyces graminis* var. *tritici*. *Pestic. Sci.* 55:367-370.
- Joseph-Horne, T., Wood, P. M., Wood, C. K., Moore, A. L., Headrick, J., and Holloman, D. 1998. Characterization of split respiratory pathway in the wheat "take-all" fungus, *Gaeumannomyces graminis* var. *tritici*. *J. Biol. Chem.* 273:11127-11133.
- Kabeere, F., Hampton, J. G., and Hill, M. J. 1997. Transmission of *Fusarium graminearum* (Schwabe) from maize seeds to seedlings. *Seed Sci. Technol.* 25:245-252.
- Komada, H. 1975. Development of a selective media for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8:114-124.
- Leslie, J. F., Pearson, C. A. S., Nelson, P. E., and Toussoun, T. A. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathology* 80:343-350.
- Leslie, J. F., and Summerell, B. A. 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, IA.
- Lipps, P. E., and Deep, I. W. 1991. Influence of tillage and crop rotation on yield, stalk rot, and recovery of *Fusarium* and *Trichoderma* spp. from corn. *Plant Dis.* 75:828-833.
- Lipps, P. E., Dorrance, A. E., and Mills, D. 2004. Corn Disease Management in Ohio. *Ohio Agric. Res. Dev. Cent. Bull.* 802.
- Mondal, S. N., Bhatia, A., Shilts, T., and Timmer, L. W. 2005. Baseline sensitivities of fungal pathogens of fruit and foliage of citrus to azoxystrobin, pyraclostrobin, and fenbuconazole. *Plant Dis.* 89:1186-1194.
- Mueller, D. S., Hartman, G. L., and Pedersen, W. L. 1999. Development of sclerotia and apothecia of *Sclerotinia sclerotiorum* from infected soybean seed and its control by fungicide seed treatment. *Plant Dis.* 83:1113-1115.
- Munkvold, G. P., and O'Mara, J. K. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by *Fusarium* species. *Plant Dis.* 86:143-149.
- Muyolo, N. G., Lipps, P. E., and Schmitthenner, A. F. 1993. Anastomosis grouping and variation in virulence among isolates of *Rhizoctonia solani* associated with dry bean and soybean in Ohio and Zaire. *Phytopathology* 83:438-444.
- Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
- Nirenburg, H. 1976. Untersuchungen ber die morphologische und biologische differenzierung in der *Fusarium*-Sektion Liseola. *Mitt. Biol. Bundesant. Land-Forstwirtschaft. Berlin-Dahlem* 169:1-117.
- Ocam, C. M., and Komedahl, T. 1994. Rhizosphere competence of *Fusarium* species colonizing corn roots. *Phytopathology* 84:166-172.
- Pioli, R. N., Mozzoni, L., and Morandi, E. N. 2004. First report of pathogenic association between *Fusarium graminearum* and soybean. *Plant Dis.* 88:220.
- Reuveni, M., and Shenglov, D. 2002. Effects of azoxystrobin, difenoconazole, polyoxin B (polar) and trifloxystrobin on germination and growth of *Alternaria alternata* and decay in red delicious apple fruit. *Crop Prot.* 21:951-955.
- Shah, D. A., and Madden, L. V. 2004. Non-parametric analysis of ordinal data in designed factorial experiments. *Phytopathology* 94:33-43.
- Smiley, R. W., and Patterson, L. 1996. Pathogenic fungi associated with *Fusarium* foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Dis.* 80:944-949.
- Steinfeld, U., Sierotzki, H., Parisi, S., Poirey, S., and Gisi, U. 2001. Sensitivity of mitochondrial respiration to different inhibitors in *Venturia inaequalis*. *Pest Manage. Sci.* 57:787-796.
- Van Doren, D. M., Jr., and Triplett, G. B., Jr. 1973. Mulch and tillage relationships in corn culture. *Soil Sci. Soc. Am. Proc.* 37:766-769.
- Vignutelli, A., Hilber-Bodmer, M., and Hilber, U. W. 2002. Genetic analysis of resistance to the phenylpyrrole fludioxonil and the dicarboximide vinclozolin in *Botryotinia fuckeliana* (*Botrytis cinerea*). *Mycol. Res.* 106:329-335.
- Windels, C. E., Kommedahl, T., Stienstra, W. C., and Burnes, P. M. 1988. Occurrence of *Fusarium* species in symptom-free and overwintered cornstalks in Northwestern Minnesota. *Plant Dis.* 72:990-993.
- Zhang, B. Q., and Yang, X. B. 2000. Pathogenicity of *Pythium* populations from corn-soybean rotation fields. *Plant Dis.* 84:94-99.