A COMPARISON OF *FUSARIUM PSEUDOGRAMINEARUM* AND *F. GRAMINEARUM* FROM WHEAT IN AUSTRALIA

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ABSTRACT

In Australia both *Fusarium graminearum* and *F. pseudograminearum* cause head blight and crown rot of wheat and this work compares the aetiology and diversity between the two species. A total of 199 isolates of *F. pseudograminearum* and 118 isolates of *F. graminearum* obtained from wheat grown in Queensland and northern New South Wales and were identified using published species-specific PCR assays. The phenotypic measures used include aggressiveness on wheat, corn, sorghum, canola, barley, rye, oat and triticale, mycotoxins produced in autoclaved-wheat culture, growth rate on potato dextrose agar, spore size and the number of macroconidia produced in culture. To measure aggressiveness, the middle spikelet of wheat, oat, barley, rye and triticale was inoculated at flowering with 10-µL suspension of 10^5 macroconidia/mL. Number of spikelets infected was recorded at 14 days after inoculation. Corn, sorghum and canola were inoculated using sterile toothpicks colonised by the pathogen by growing in potato dextrose broth for 7 days and the length of stem rot was recorded after 14 days. Genotypic relationship between the two species was evaluated using Amplified fragment length polymorphism with five primer pair combinations. Both species produced the same mycotoxins Deoxynivalenol (DON), Zearalenone (ZEA) and nivalenol (NIV). NIV concentration in all samples tested was less than 250 ppb, while DON concentration ranged between 2.5 ppm and 0.71 ppm in *F. pseudograminearum* and 4.4 ppm and 2.4 ppm in *F. graminearum*. ZEA concentration was considerably higher in *F. graminearum* (3.0-11.9 ppm) than in *F. pseudograminearum* (0.9-2.2 ppm). The symptoms and severity of diseases produced on the heads of the cereal crops were similar. Both species caused canker and stem rot in canola, and stalk rot in corn and sorghum. A high level of genotypic diversity was observed within each species. All *F. graminearum* isolates produced homothallic perithecia in culture but only 8% of *F. pseudograminearum* isolates produced heterothallic perithecia in culture. Although there were variations in all the phenotypic and genotypic measures for each species, the level of variation in *F. graminearum* was higher than in *F. pseudograminearum.*

RECOVERY OF *FUSARIUM GRAMINEARUM*, CAUSE OF WHEAT HEAD SCAB, AND DEOXYNIVALENOL FROM INOCULATED LEAVES AT ADULT PLANT STAGE IN THE GREENHOUSE

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ABSTRACT

Fusarium graminearum (teleomorph: *Gibberella zeae*) causes Fusarium head blight (FHB) on wheat and other small grains in North Dakota. The fungus has been recovered frequently from wheat leaf samples collected at any growth stage from various fields, and the leaves are thought to serve as an additional source of inoculum for disease development. However, the pathway of the fungal survival on leaves has not yet been explored. It is also not known if the fungus produces deoxynivalenol (DON) in leaves during colonization process. To answer these questions, leaves of two FHB susceptible wheat genotypes, Glenlea and M-3 (W7976), were inoculated at Feekes scale 11.4 with *F. graminearum* conidial suspension (100, 000 conidia/ml) until run off and kept in a humidity chamber twice for 24-hr, with a 24-hr interval between the two cycles. Thereafter, the plants were kept in a growth chamber set at 24°C day and 20°C night for seven days. The plants were examined daily for any symptom development. Inoculated plants were placed a third time in the humidity chamber for 24 hrs 8 days post inoculation and then they were moved back to the growth chamber. Two days later the leaves were clipped, brought to the laboratory, and observed under a dissecting microscope and a compound microscope for the presence of fungal sporodochia and perithecia. Most of the leaves of both cultivars retained their green color with a little chlorosis, which was more conspicuous while holding leaves toward light. Lower leaves of both cultivars turned mostly chlorotic. The green leaves of both cultivars had several sporodochia, while chlorotic leaves had both sporodochia and some immature perithecia. DON was detected at 4 ppm in inoculated green leaves. The results indicate that the fungus can survive on leaves, produce both spore types (depending on the nature of the leaves (green/chlorotic)), and serve as a source of inoculum. Moreover, the fungus can produce DON in the leaves that could make them unacceptable as forage. This is the first report on the occurrence of DON in intact wheat leaves. This study may help in understanding the fungal pathway from seedling to adult plant stage under field conditions and help in the disease management.

DETERMINATION OF WETNESS DURATION USING RADAR-DERIVED PRECIPITATION ESTIMATES

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ABSTRACT

Fusarium head blight (FHB) of small grains tends to be associated with certain environmental conditions, especially rain-induced wetness periods occurring near anthesis. A Geographic Information System-based model simulation which incorporates 4km resolution weather radar (NEXRAD)-derived precipitation estimates into a crop canopy energy balance-based scheme to estimate wetness duration periods for small grains on the 4 km spatial scale has been developed and tested with field observations. During the past year, an analysis of errors and biases in NEXRAD precipitation estimates (the most critical input data involved in the wetness duration simulation) was completed for the May-September time frame, 1999-2002. Comparisons were made across the state of Michigan between gages in: 1) National Weather Service (NWS) and 2) Michigan Automated Weather Network (MAWN) networks. In terms of rainfall frequency, the NEXRAD estimates were correct in identifying precipitation 89.7% and 89.0% of time on a daily basis for NWS and MAWN networks, respectively, and 95.9% (NWS) and 95.6% (MAWN) of the time on an hourly basis. In terms of differences between estimated and observed precipitation totals, mean differences for daily and hourly periods over the 1999-2002 study period were -0.05mm and 0.13mm and -0.10mm and 0.01mm for NWS and MAWN networks respectively. Validation of the simulated leaf wetness duration in six wheat field sites in Lower Michigan at head height was also carried out resulted during June and July of the 2003 growing season. Collectively across all types of wetness events, the simulation understimated the length of wetness duration. Mean differences and mean absolute differences between simulated and observed leaf wetness across all events were approx. -4.4 hours and 4.5 hours, respectively. The mean absolute difference for precipitation events (those of most significance when monitoring for the incedence of Fusarium) alone was 1.0 hour. Overall, while the results suggest satisfactory performance with wetting events associated with precipitation, the simulation also tended to underestimate wetness duration associated with the formation of dew, especially at the onset of the event.

CALCIUM IONS INCREASE TOXICITY OF DEOXYNIVALENOL TO BARLEY LEAF TISSUES

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ABSTRACT

Deoxynivalenol (DON) had a bleaching effect on detached barley leaf segments (Bushnell et al, Phytopathology 92:S11, 2002). Leaf tissues lost pigmentation after incubation with 30-200 ppm DON for 2-4 days in light. The bleaching was accompanied by disorganization of chloroplasts and other cytoplasmic organelles as viewed by transmission electron microscopy (TEM). However, results were inconsistent; some segments were completely bleached, some developed only white spots or streaks, and others remained entirely green, even at high DON concentrations (90-200 ppm). Here we report that toxicity of DON is increased and tissue responses are more consistent when Ca^{2+} is added. The abaxial epidermis was partially stripped from detached primary barley leaf segments (1.2 cm long) and the segments were then floated with exposed mesophyll in contact with DON solutions, with or without added 10 mM Ca^{2+} (applied as $Ca(NO_3)_2$). Segments were incubated at 25°C in light (250 µmol/m^2/sec). With Ca^{2+}, DON at 10-30 ppm gave white spots or streaks by 24 hr and usually turned entire tissues white by 72 hr. Compared to treatments without Ca^{2+}, the loss of pigment with Ca^{2+} occurred 1-2 days earlier, was more complete in individual segments, and was more consistent among segments. In line with this, amounts of chlorophyll and carotenoid pigments, as measured spectrophotometrically, were reduced more with Ca^{2+} than without. As viewed by TEM, chloroplast degeneration was underway after 18 hr of treatment with 30 ppm DON (with Ca^{2+}) and nearly complete by 24 hr. Increased toxicity could be accounted for by greater concentration of DON within leaf segments treated with DON + Ca^{2+}. Without Ca^{2+}, tissues contained 3 ppm DON; with Ca^{2+} they contained 14 ppm DON (after 48 hr incubation in light on 30 ppm DON). Experiments are in progress to evaluate the effect of Ca^{2+} on DON-treated tissues incubated in the dark. The pronounced increase in toxicity of DON in present experiments suggests that Ca^{2+} concentrations within plant tissues may influence the effect of DON in the development of Fusarium head blight.

EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT AND CROWN ROTOF WHEAT: LESSONS FOR AUSTRALIA

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ABSTRACT

In Australia species of *Fusarium* cause two serious diseases of wheat: Fusarium head blight (FHB) that has emerged as a major problem in recent years, and crown rot (CR), which continues as a chronic problem costing >AUD$56 million every year. Residue-borne *F. pseudograminearum*, *F. graminearum*, F. *culmorum* and *F. avenaceum* have caused recent outbreaks of FHB and many of these cause serious CR. We have adopted a coordinated approach to both diseases because of the linked etiology, biology and epidemiology of *Fusarium spp.* causing CR and FHB. We are studying the interrelationship among *Fusarium* pathogens causing FHB and CR in wheat farming systems using relative abundance; aggressiveness; toxin production; dispersal and effectiveness of inoculum; epidemiology and disease severity. Pathogen isolates collected from field surveys have been identified using species-specific PCR assays and morphology. High throughput bioassays have been developed and/or adopted from other studies to use pathogen aggressiveness as a selection tool to detect small but consistent differences in quantitative resistance for further improvement of host resistance. In Australia, both species and isolates within species differ in aggressiveness and at least 20% of all *F. graminearum* and *F. pseudograminearum* are aggressive to highly aggressive for both FHB and CR, but there are important differences in the form and effectiveness of inoculum. All 17 *Fusarium* species tested caused FHB and all 10 tested caused CR in plant infection assays, with significant ($P < 0.001$) difference in aggressiveness between species and between isolates within species for both diseases. Overall, isolates from stubble and crown were more aggressive for CR whereas isolates from the flag leaf node were more aggressive for FHB. Isolates that were highly aggressive in causing CR were those originating from paddocks with wheat following wheat, while those from fields with wheat following maize or sorghum were highly aggressive for FHB. There is extensive literature on Fusarium affecting wheat and the US wheat and barley scab initiative has helped to collate existing information and to generate new knowledge. Although CR is prevalent in some states of the USA, an overwhelming majority of information on the pathogen and disease epidemiology relates to FHB. A comparison of disease epidemiology and pathogen populations in the two countries is essential to establish if the wealth of research outcomes from the USA is likely to be applicable to the management of CR and FHB in Australia. Through strong and effective collaboration between research teams from the two countries, we hope to share and grow the wealth of knowledge for the management of both diseases.

DEVELOPMENT AND DEPLOYMENT OF THE NEXT GENERATION PREDICTION MODELS FOR FUSARIUM HEAD BLIGHT

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OBJECTIVES

To develop and deliver accurate and timely predictions of Fusarium head blight

INTRODUCTION

Epidemics of Fusarium head blight were common in many states during the 2003 growing season. High levels of disease were reported in throughout the Mid-Atlantic region encompassing NY, PA, MD, VA, and NC. Outbreaks of disease were also reported in OH, IN, and KY. Producers in these areas struggled to find markets for Fusarium damaged grain, and concerns about mycotoxin contamination were common. Providing producers and the wheat industry in the U.S. with accurate and timely forecasts of disease will be important to minimize the impacts of future epidemics. More specifically, disease forecasts can be used to help producers make decisions about the application of a fungicide or biological control, establish harvest priorities, and pursue markets for grain. Grain buyers can use this information to make preparations for mycotoxin testing, grain cleaning, storage, and processing.

Efforts to predict Fusarium head blight is a priority of a cooperative epidemiology effort sponsored by the U.S. Wheat and Barley Scab Initiative. Researchers from this effort have developed two models using 50 cases of hourly weather and disease observations from ND, OH, MO, KS that can be used to provide estimates of the probability of a scab epidemic greater than 10% severity (De Wolf et al. 2003). These models include a pre-flowering model that uses weather variables observed prior to flowering to predict disease, and a post-flowering model that makes predictions using weather variables observed both prior to and during the flowering period. The pre-flowering model has been shown to be 70% accurate and the post-flowering model 83% accurate. One or both these models are currently being used in ND, SD, MI, OH, PA, NY, and IN to provide producers with disease forecasts for Fusarium head blight. However, means of delivery and resources available to provide these disease forecasts vary among these states (Francl 2001; Lipps et al. 2002; Osborne and Jin 2002).

We report here on our recent efforts to improve the accuracy of pre-flowering disease predictions by expanding the amount of weather information considered by these models, and through adjustments in weather variables based on research addressing *Gibberella zeae* perithecial development. We also will introduce a novel source of weather variables that could allow the uniform deployment of Fusarium head blight predictions in 23 states.

MATERIALS AND METHODS

Information used to develop models of this second phase of the disease forecasting effort consisted of observations of disease and crop growth stage within replicated plots. Disease severity was coded as a

binary variable with cases having a disease severity of 10% or greater considered epidemics (1) and cases with lower disease considered to be non-epidemics (0). Each set of observations (case) was associated with hourly observations of weather variables including temperature, relative humidity and rainfall. The cases used to develop these models were collected in seven states representing both spring and winter wheat production systems (Table 1). The total data set consisted in 119 cases, and this information was partitioned into a model development (n=89) and validation (n=30) data sets.

Table 1. Information from multiple states and locations representing both spring and winter wheat production regions were used to develop prediction models for Fusarium head blight.

State	Locations	Cases
North Dakota	6	33
Ohio	3	48
Missouri	3	11
Indiana	1	11
Pennsylvania	2	8
South Dakota	1	6
Kansas	1	2
Total	**17**	**119**

The hourly weather observations were used to construct variables representing potential time periods critical for the reproduction of the fungus or infection of the host. More specifically, variables considered were summarized for 14 or 7 days prior to, or 7, 10 or 14 days after the anthesis date identified for each case. The temperature variables were selected to represent recent research on the reproduction of the fungus, which indicates that perithecial development of *G. zeae* is limited by temperatures less than 9°C as apposed to 15°C as was previously reported (Dufualt et al 2003, Tchsanz et al 1976). A class variable designating each cases as either a winter or spring wheat was also considered in the analysis.

Variables useful in predicting Fusarium head blight epidemics were identified using best- subsets regression procedures. The identified variables were used to construct interaction terms (multiplication of two or three variables), and together these variables and interaction terms were used to develop logistic regression models for classifying cases as epidemics or non-epidemic (low disease years). This modeling effort focused on developing on two groups of models, pre-flowering models and post-flowering models. The pre-flowering models used only variables available prior to flowering and the post-flowering models allowed for combination of variables available prior to and during the flowering period. The prediction accuracy (percentage of correctly classified cases) for each of model was evaluated with the cases of the model development and validation data sets. Models with reasonable prediction accuracy were selected for further evaluation.

RESULTS AND DISCUSSION

Variables identified by the best-subsets procedure included variables that summarized weather conditions 7 or14 days prior and 7 days after flowering. Variables selected also represented the adjusted temperature range for perithecia development and the class variable designating a case as from winter or spring wheat production region (Table 2). Prediction accuracy of models that used only variables available prior to flowering ranged from 80 to 82% for cases used to develop the models, and 80 to 87% for cases used in

model validation. The prediction accuracy of models using variables available prior to and during crop flowering ranged from 78 to 82% for the model development data set, and consistently classified 87% of the validation cases.

Selection of the final model to deploy as part of an updated disease forecasting system will depend on ongoing analysis of model errors and final model validation with cases from the 2003 growing season. The models produced in this analysis improve the accuracy of pre-flowering predictions from 70% to near 80%. This improvement in accuracy should allow for more accurate disease forecasts at the time of flowering when fungicide applications are most effective (McMullen et al. 2000).

Table 2. Prediction accuracy of logistic regression models used to classify Fusarium head blight epidemics.

			Accuracy (%)		
Model	**Type**	**Variables**	**Development**	**Validation**	**Overall**
A	Pre	T9307,T914,TRH93014, (WC*TM7)	80.9	86.7	82.4
B	Pre	WC,RH9014,T914, RH9014*T914),	82.0	80.0	81.5
C	Pre	WC,RH9014,T914,TRH9014	80.9	80.0	80.7
D	Post	WC,RH9014,T914,TRH9014, (RH9014*T914*PRHM7)	82.0	86.7	83.2
E	Post	WC,RH9014,T914,TRH9014, PRHM7	83.1	80.0	82.4
F	Post	T9307,(RH9014*T914), (RH9014*PRHM7), (WC*T914)	77.5	86.7	79.8

Type: Pre-Flowering or Post-Flowering Prediction
Variables: WC = Class variable indicating spring or winter wheat; T914 = Duration (h) that temperature is greater than 9 C for 14 days prior to flowering; T9307 = Duration (h) that temperature is between 9 and 30°C for 7 days prior to flowering; TM7 = Mean temperature for 7 days prior to flowering; RH9014 = Duration (h) that relative humidity is greater than 90% for 14 days prior to flowering; TRH9014 = Duration (h) that both temperature is between 9 and 30°C and relative humidity is greater than 90% for 14 days prior to flowering; PRHM7 = Mean relative humidity for 7 days after the flowering begins.
Accuracy: Percentage of correctly classified epidemics and non-epidemics for cases used to develop (n=89) and validate (n=30) the models. Overall accuracy is the weighted average of development and validations cases.

We are currently working to deploy the models resulting from this analysis during the 2004 growing season in 23 states that have been impacted by scab epidemics. This experimental system will use weather variables collected from hourly reporting stations across the region and from the analysis of the Rapid Update Cycle provided by the National Weather Service. The Rapid Update Cycle (RUC20) provides hourly observations of temperature and dewpoint temperature at a 20 km grid throughout the U.S. using multiple sources of atmospheric observations. Verification for the accuracy of variables generated by the RUC20 is

currently underway. In the near future these sources of weather variables could allow predictions for Fusarium head blight at 20 km spatial resolution for all 23 states.

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FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION IN WHEAT INOCULATED AT DEVELOPMENTAL STAGES FROM FLOWERING THROUGH GRAIN MATURATION

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OBJECTIVE

Determine the relative vulnerability of wheat to Fusarium head blight and dexynivalenol contamination when infection occurs at various developmental stages from mid-flowering through kernel maturation.

INTRODUCTION

Fusarium head blight (FHB) is one of the most severe diseases of wheat affecting grain yeld and quality. The most important mycotoxin produced by *Gibberella zeae* is deoxynivalenol (DON), which is harmful to humans and livestock (Parry et al., 1995; McMullen, 1997). Contamination of wheat with DON at levels exceeding 2 ppm results in rejection of sale or severe price dockage by millers and other grain buyers.

It has been stated that, under moist conditions, the fungus can infect wheat spikelets anytime from anthesis to soft dough stages (Parry et al., 1995; McMullen et al, 1997). Yet, controversy still persists over the precise phenological window of vulnerability to infection, and especially that which results in significant accumulation of DON. Some authors demonstrated that infection occurred principally at anthesis and that anthers were a main infection site (Atanasoff, 1920; Pearce et al., 1976). However, others demonstrated an extended window of infection with a peak after flowering (Andersen, 1948; Schroeder and Christensen, 1963). DON accumulation in kernels is influenced by many factors such as strain of the pathogen, aggressiveness, temperature, moisture and host resistance (Hart et al, 1984; Wang and Miller, 1988; Mirocha et al, 1989). We investigated the role that host developmental stage at the time of infection plays in the final level of DON contamination in harvested wheat grains.

MATERIAL AND METHODS

Greenhouse experiments: Seeds of cv. Norm were sown in soil substrate within pots (2.5 L). During early flowering, groups of 6 pots were established according to phenological similarity. In each pot, 7 to 10 spikes (main tillers) were left and late tillers were consistently eliminated until maturity.

Treatments consisted of spraying a macroconidial suspension of *G. zeae* (isolate GZ014 adjusted to 10^5 spores/ml) on the spikes, at different wheat stages, 5 to 6 days apart, as follows: 1) mid-flowering; 2) kernel watery ripe; 3) kernel early milk; 4) kernel late milk; 5) kernel soft dough and 6) kernel early hard dough. The check treatment consisted of a group of spikes inoculated with water. After inoculation, plants were moved into a mist chamber for 48 hours under continuous moisture with temperature in the range of 21-24º C. After the incubation period, plants were moved back to the greenhouse until maturity. FHB incidence, percentage of spikes blighted per pot, and FHB severity, percentage of blighted spikelets per blighted

spike, were recorded when symptoms appeared, generally on the fifth day after inoculation. At maturity, kernels were harvested and evaluated for: *Fusarium* damaged kernels (FDK); 100-kernel weight; kernel infection on selective medium and DON concentration, using an Elisa Kit - DonFast Ridascreen. The experiment was repeated twice.

RESULTS AND DISCUSSION

Norm was susceptible to FHB from flowering through all stages of grain development. At the later stage, i.e., early hard dough, visual incidence and severity could not be evaluated due to natural spike senescence. FHB incidence ranged from 90-100% and typical symptoms were mostly observed 3 to 4 days after inoculation, except for the inoculation at flowering, when symptoms took 7 to 10 days to be noticed. FHB severity was higher in spikes inoculated after flowering, though severity increased until maturity (data not shown). Average percentage of relative severity from flowering to soft dough was 0, 31.3, 40.6, 67 and 84.2, respectively. FDK ranged from 94-100% between flowering and milk stage inoculations, decreasing at the later stages, but still showing 23% of damaged kernels at the early hard dough stage (Fig. 1). *G. zeae* was detected at high incidence on kernels following inoculation at all stages. Peak kernel infection occurred at the kernel late milk and soft dough stages. Average percentage values from flowering to hard dough stage was: 52, 73.6, 93.3, 99, and 70.3, respectively.

DON levels ranged from 1.2 to 98 ppm. The highest levels were detected in kernels inoculated at watery ripe stage, which was two times higher than DON levels resulting from inoculation at flowering or at late milk stages. Considerable (13.3 ppm) toxin levels were detected in grains following inoculation at soft dough, and trace levels were detected in grains inoculated at early hard dough (Fig. 1). Kernel weight was greatly reduced by inoculations from flowering to milk stages. Inoculations at dough stages produced kernels with weight similar to the nontreated check. (Fig. 1). Significant negative correlation was observed between kernel weight and DON (r^2=-0.94) and kernel weight and FDK (r^2= -0.70). Significant positive correlation was observed between DON and FDK (r^2= 0.57). Other correlations were not significant ($P<0.05$).

It was clear that Norm is a highly suscetible variety with a wide window of infection. According to Andersen (1948), wheat heads were also most susceptible to infection at post-flowering stages, contrary to the results of others who have reported that the peak of infection occurs during flowering (Atanasoff, 1920), possibly due to the stimulatory effect of anthers (Strange, 1974). Our results showed that kernels are likely to be infected whenever there is a conducive environment at very late stages of grain development, which is in agreement with a field study carried out by Fernando et al. (1997). Those authors moved plants at different stages from the greenhouse to field conditions and observed that the peak of kernel infection, detected on selective media, occurred from flowering to milk stages and that considerable levels of kernel infection occurred at dough stages. In our study, *G. zeae* was detected at high incidence in kernels inoculated at dough stages. This was not correlated positively with damage in kernels or DON.

Hart et al. (1984) found that production of DON in wheat inoculated in the greenhouse depended on the duration of head wetness, and occurred independently of the stages of kernel development after the kernels were filled. The authors stated that it was clear that DON could be produced in wheat when there was adequate moisture for fungal growth, even at later stages of kernel development. Our findings here are in agreement with those, though we have observed a discernable peak in DON production in kernels resulting from inoculations at milk stages, even in comparison to inoculation at mid-flowering. A steep decrease in

DON accumulation was observed in kernels inoculated later, with only trace amounts of toxin detected in kernels inoculated at dough stages.

In several recent years in New York state, USA (Bergstrom, unpublished), we have observed an apparent uncoupling of DON contamination in soft winter wheat from the occurrence of FHB symptoms and grain weight reduction. That is, plump, high-yielding wheat has been contaminated with DON above acceptable levels. A possible explanation supported by the current study as well as the findings of Hart et al. (1984) is that DON may be produced from infections late in grain development that do not produce dramatic reductions in grain weight or significant FHB symptoms. This complicates the already challenging task of controlling FHB and toxin contamination. Fungicide applications and FHB risk assessments today are focused almost entirely on infection during a narrow window around crop flowering. If in fact that window of vulnerability, especially for DON contamination, extends through much of grain development, then we need to consider integrated strategies that will protect wheat spikes from infection for several weeks rather than several days after flowering.

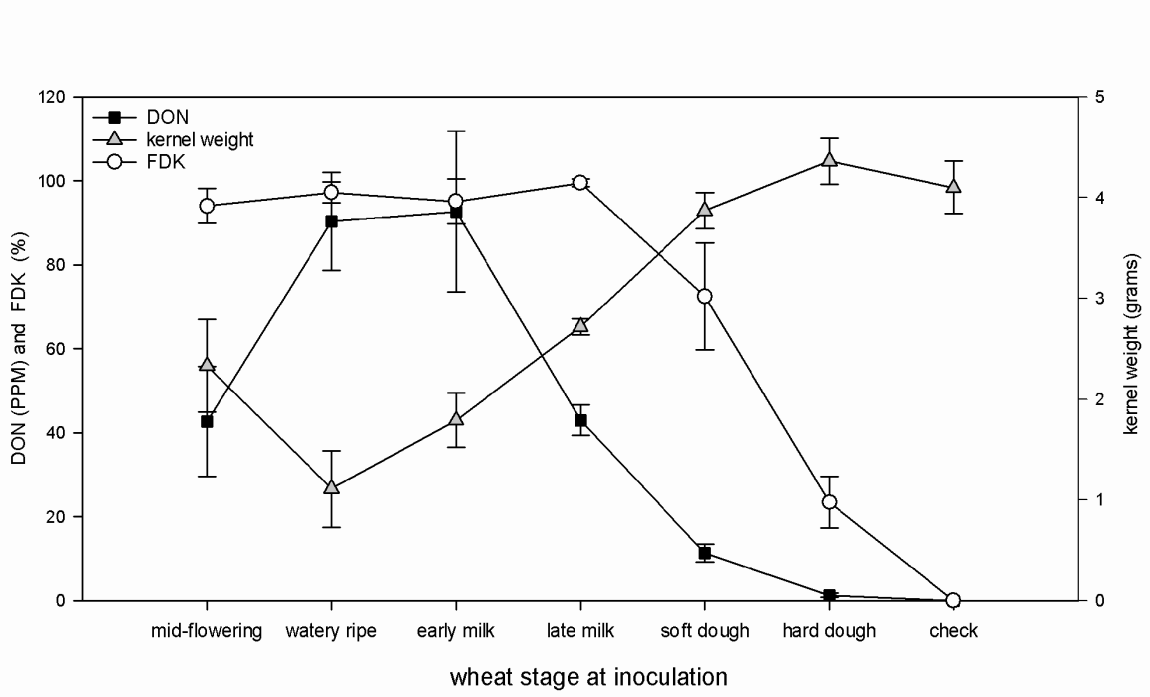

Fig. 1. Effect of wheat growth stage at time of inoculation with *Gibberella zeae* on Fusarium head blight symptoms and quality parameters in mature grain. Vertical bars are the standard deviation. Sample sizes are N=6 (DON) and N=12 (Kernel weight and FDK).

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OXYLIPIN-MEDIATED SIGNALING EVENTS CONNECTING MYCOTOXIN BIOSYNTHESIS AND SPORULATION IN *ASPERGILLUS* AND *FUSARIUM SPP.*

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OBJECTIVE

To identify *Fusarium* dioxygenases and determine their role in spore production.

INTRODUCTION

Fusarium head blight (scab) is one of the most devastating diseases of wheat and barley. It is caused by a number of mycotoxin producing *Fusarium spp.* including *F. graminearum, F. culmorum*, and *F. sporotrichioides.* The latter two *spp.* infect primarily by asexual spores (conidia) whereas *F. graminearum,* the principle scab causing fungus infects host plants with both sexual (ascospores) and asexual spores. Impediments to spore production would be useful in controlling this disease. Biochemical and genetic studies suggest that oxylipins, oxygenated derivatives of unsaturated fatty acids, are conserved signaling and structural molecules modulating fungal asexual and sexual spore development.

In 1987, Champe et al reported the detection of a secreted substance in *A. nidulans*, called psi factor (for precocious sexual inducer), that induced premature cleistothecia formation and sexual sporulation and blocked conidiation. Extensive chemical studies of the psi factor resulted in the identification of linoleic and oleic acid derived oxylipins. We now know that psi factor is a mixture of hydroxylated oleic (18:1), linoleic (18:2) and linolenic acid (18:3) derivatives (termed psiAα,β & γ, psiBα,β & γ and psiCα,β & γ) (2-4) likely produced by all filamentous fungi.

Most recently, we have been able to clone three genes (e.g. *ppoA, ppoB* and *ppoC* for psi producing oxygenase) encoding dioxygeanses that are likely to be responsible for psi production in *Aspergillus nidulans* (7). The amino acid sequence of the encoding proteins shows very high similarity to that of the psi producing protein Lds from the fungus *Gaeumannomyces graminis* (5). A putative ortholog has also been described in *Ustilago maydis* where it is found to be expressed in teliospores (6). Our goal was to identify these genes in *F. graminearum* and start to characterize their role in sporulation. As described below, these genes are not only important for sporulation but also mycotoxin production in both *Aspergillus* and *Fusarium* species.

MATERIALS AND METHODS

Sequence data from a *F. verticilliodes* EST indicated it to be a likely *ppo* gene (e value ca. -75 to *A. nidulans ppoA*). We amplified this DNA sequence (which we call *Fvppo1*) from *F. verticilliodes* genomic DNA, sequenced *Fvppo1* to confirm identity, and then used *Fvppo1* to probe *F. verticilliodes, F. graminearum* and *F. sporotrichiodes* cosmid libraries. Each *Fusarium* species contained several strongly hybridizing cosmids. Subcloning and sequencing of these cosmids yielded putative *ppo* genes. Subsequent

to this, BLAST analysis of the newly released *F. graminearum* genome (http://www-genome.wi.mit.edu/annotation/fungi/) revealed the presence of all three *ppo* genes called *Fgppo1*, *Fgppo2* and *Fgppo3* (Table 1).

A *Fvppo1* disruption vector was created in which the hygromycin resistance gene, *hygB*, was ligated between *Fvppo1* flanking DNA (e.g. ca. 1 kb of flank 5' and 3' to the *Fvppo1* ORF). This vector was then used to transform *F. sporotrichioides* to hygromycin resistance.

RESULTS AND DISCUSSION

An examination of a *ppoA* deletion strain of *A. nidulans* shows it to be defective in both spore and ST production. Deletion of *ppoA* significantly reduced the level of psiB1á and increased the ratio of asexual to sexual spore numbers four-fold (7). In contrast, forced expression of *ppoA* resulted in elevated levels of psiB1á and decreased the ratio of asexual to sexual spore numbers six-fold (7). Additionally, the *ppoA* deletion strain showed reduced ST synthesis (Tsitsigiannis and Keller, data not shown).

To determine if *ppo* genes could be playing a similar role in *Fusarium spp.*, we first identified putative *ppo* genes based on identity to *Asperillus ppo* genes. Three putative genes were found in *F. graminearum.* Transcript analysis of these three genes is shown in Figure

Next we tried to disrupt a *ppo* gene in three *Fusarium* spp. (*F. graminearum, F. verticilliodes* and *F. sporotrichiodes*) using a *Fvppo1* disruption vector. We used the same vector as DNA sequence between the three spp. is conserved and we thought it possible we could disrupt the *ppo* gene in all three spp. using one vector. Examination of 200 transformants of *F. verticillioides* and 60 transformants of *F. graminearum* did not reveal any *ppo* disruptants. Disruption was only successful for *F. sporotrichiodes* where two *ppo* deletion mutants were obtained. Examination of these *Δppo* strains of *F. sporotrichioides* indicate that T-2 toxin gene expression is greatly reduced (Figure 2) and T-2 toxin production has been inhibited (Plattner, Devi and Keller, data not shown). In addition, asexual spore production is severely reduced compared to that of wild type and oxylipin content altered (Table 2).

Taken together, these data suggest that oxylipin signaling affects both sporulation and secondary metabolism. Based on extensive studies of oxylipin signaling in mammals, we predict that oxylipins generated from the *ppo* gene products act as ligands initiating several signal transduction cascades governing global developmental pathways.

We also suggest that the linkage of sporulation and metabolite production is not spurious as other signaling pathways (e.g. a G protein pathway) has also been found to link sporulation and mycotoxin production in *Aspergillus* and *Fusarium* (1 and references therein). The reasons for this co-regulation of sporulation and metabolite production are most likely to be discovered at the ecological and organismal level. It is tempting to speculate that the simultaneous regulation of both processes is associated with protective properties (e.g. allelopathic or anti-herbivoric chemicals, UV damage mitigation) of secondary metabolites in a sporulating colony.

ACKNOWLEDGEMENTS

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Homology of Fusarium dioxygenases to linoleate diol synthase (*lds*) gene of *Gaeumannomyces. graminis*.

		E value	% identity	% similarity
F. graminearum	Ppo1	0.0	42	56
	Ppo2	1×10^{-98}	36	52
	Ppo3	1×10^{-158}	40	54
F. verticillioides	Ppo1	1×10^{-158}	39	53
F. sporotrichioides	Ppo1	1×10^{-157}	40	54

Table 2. Spore production and oxylipin content of wild type and *Δppo1* strains of *F. sporotrichioides.*

	Conidia/µl[1]	8-HOE[2]	8-HODE
Wild type	6.6×10^{4}	8.3	41.0
Δppo1	3.2×10^{4}	1.9	1.9

[1]The number of spores are statistically different ($P < 0.05$) between wild type and *Δppo1* with *Δppo1* consistently producing ca. 1/2 the amount of spores as wild type.

[2]8-HOE = 8 hydroxyoleic acid or psiBß? 8-HODE = 8 hydroxylinoleic acid or psiBα in µg/g mycelium (dry weight). Both 8-HOE and 8-HODE values where significantly reduced in the *Δppo1* strain compared to wild type ($P < 0.05$).

Data was replicated three times.

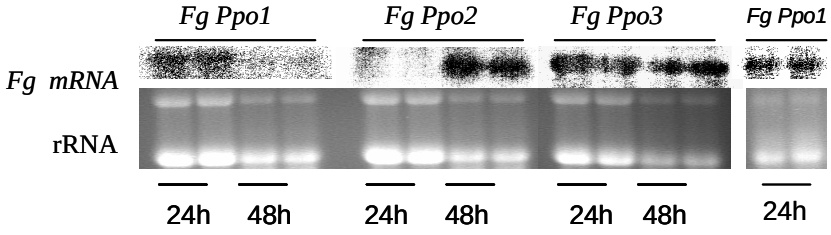

Figure 1. mRNA analysis of *ppo* genes in wild-type strains of *F. graminearum* and *F. sporotrichioides.*

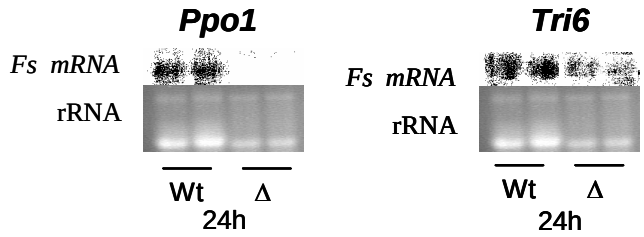

Figure 2. mRNA analysis of *ppo1* and *tri6* in wild-type and *Δppo1* strains of *F. sporotrichioides.* Note that deletion of *ppo1* results in a decrease in *tri6* expression. *tri6* encodes an enzymatic gene required for the production of the trichothecene T2 toxin in *F. sporotrichiodes.*

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RAIN SPLASH DISPERSAL OF *GIBBERELLA ZEAE* SPORES IN A WHEAT CANOPY

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ABSTRACT

To examine the role of rain splash in the dispersal of *Gibberella zeae* (anamorph: *Fusarium graminearum*), the cause of Fusarium head blight of wheat, splashed water was collected during rain events at three heights (0, 30, and 100 cm) above the soil surface over 3 years. Samplers were placed in a reduced tillage wheat field with corn residue and in a breeding nursery. The splash samplers consisted of sheltered funnels and flasks. Splashed rain was collected for three rain events in 2001, seven in 2002, and 10 in 2003. To determine spore deposition levels, for each rain episode, 1 ml of rain splash water was transferred to replicated petri plates with Komada's selective medium, and colony forming units were counted after an incubation period. Based on the flux density of splashed water, and the spores per ml of water, spore flux density (spores per square centimeter per hour) was determined for each rain event. The intensity of splashed rain (mm/h) was highest at 100 cm, indicating that substantial splashing of incident rain occurs from the upper wheat canopy (heads and flag leaves). Spores were detected in every sampled rain event at all heights, with slightly fewer spores at 100 cm compared to the other two heights. There was a strong linear relation, on a log-log scale, between spore flux density and both incident rain intensity and splash rain intensity. Therefore, in addition to aerial dispersal of spores by wind, rain splash dispersal contributes to the movement of inoculum within wheat canopies and may contribute to Fusarium head blight epidemics.

REACTION OF PRIMARY LEAVES OF 26 WHEAT GENOTYPES INOCULATED WITH MACROCONIDIA OF *FUSARIUM GRAMINEARUM* AT THE SEEDLING STAGE AND ASSESSED FOR LESION LENGTH AND DEOXYNIVALENOL ACCUMULATION AT 96 H POST-INOCULATION

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ABSTRACT

Lesion reaction and deoxynivalenol accumulation was investigated in primary leaves of seedlings of 26 wheat genotypes following inoculation with *Fusarium graminearum*. Our objective was to develop a model system to investigate how *F. graminearum* infects and colonizes tissues other than those found in spikes. We developed an inoculum consisting of macroconidia of *F. graminearum* suspended in a weak solution of water-agar (0.3% w/v) containing the surfactant Tween-20 (2 ml/l). The surfactant facilitated adhesion of the inoculum to the leaf cuticle and the weak agar suspension provided an increased viscosity so that the droplets of inoculum would not run or fall off. Water-agar was utilized so that the addition of nutrients for spore germination and fungal growth would be minimal. Primary leaves of seedlings were inoculated 14 days after planting. Seedlings were planted at 4-5 plants per conetainer (4 x 20 cm, dia. x length) containing a soil-less potting mix. The entries were replicated (7 reps, 28 leaves) and the experiment was repeated once. Leaves were inoculated by carefully placing a 10-µl droplet on the center of the leaf, between leaf tip and ligule, of the abaxial leaf surface using a micropipette. Droplets of inoculum on leaf surfaces were allowed to dry for 1-2 hours before moving inoculated plants to a dew chamber providing ca. 100% relative humidity. Plants were maintained in the dew chamber for 72 h then removed to greenhouse benches for another 24 h. Lesions on the inoculated leaves were measured at 96 h post-inoculation. Primary leaves of seedlings of each replicate were first removed then lesions were measured as the length of the longest necrotic lesion dimension in the longitudinal orientation of each leaf. Leaves of seedlings from each conetainer (replicate) of the 26 genotypes were bulked and placed in 1-dram vials and frozen at –20º or -80º C. Leaves were later extracted for analyses and quantification of deoxynivalenol and 15-acetyldeoxynivalenol. In the first experiment we observed significant differences ($P < 0.001$) among the 26 wheat genotypes for both lesion reaction (mean = 3.3 mm, range = 0-27.0 mm) and for accumulation of deoxynivalenol (mean = 0.2 ppm, range = 0-0.92 ppm). We observed large lesions on the primary leaves of Alsen (8.9 mm long), a cultivar known to be resistant to spike and grain colonization by *F. graminearum* in the field, but did not detect any deoxynivalenol accumulation (0 ppm). Frontana was unusual in that it was the only genotype we did not observe any lesion reaction (0 mm) however toxin accumulation (0.53 ppm) was detected in the inoculated but non-symptomatic leaves. The inoculation technique described may be useful to molecular investigations of gene expression and in studies of tissue colonization and Fusarium head blight epidemiology.

DETECTION OF DISTINCT SUBPOPULATIONS OF *FUSARIUM GRAMINEARUM* LINEAGE 7 IN THE U.S.

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ABSTRACT

A collection of the cereal head blight pathogen *Fusarium graminearum* from nine U.S. states, representing 86 fields in 53 counties, was characterized using ten single-copy RFLP probes, a telomeric probe and RFLP probes diagnostic for species and lineage. In addition, isolates were assigned to one of three profiles of trichothecene metabolites (chemotypes) using a PCR-based approach. All 708 isolates determined to be *F. graminearum* were confirmed as lineage 7. The telomeric probe was used for clone determination, leaving 587 isolates for subsequent data analyses. Most lineage 7 isolates (94.6%) from the U.S. were of 15-acetyl deoxynivalenol (15ADON) chemotype. The 3-acetyl deoxynivalenol (3ADON) chemotype was found at 5% and was only identified in samples from North Dakota and Minnesota. The nivalenol chemotype was infrequent at 0.4%. Gene flow analysis demonstrates that the 15ADON population in the U.S. is genetically isolated from the 3ADON population (Nm = 0.5). In comparison, a representative collection consisting of 19 isolates of lineage 7 from Italy was genetically similar to the 3ADON population from the U.S. (Nm > 2), though the Italian collection consisted of all three chemotypes. These results would indicate that lineage 7 consists of at least two distinct subpopulations.

ANALYSIS OF GENE EXPRESSION IN *FUSARIUM GRAMINEARUM* DURING INFECTION ON WHEAT

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ABSTRACT

Fusarium graminearum is a species complex containing at least nine biogeographically structured lineages. With an aim to elucidate gene function related to pathogenicity, we evaluated the abilities of different strains of *F. graminearum* to spread in their hosts and are currently conducting genomic analyses of these interactions. Our previous studies on 31 representative strains from these lineages have shown that they can differ significantly in their aggressiveness on wheat and also in the type and amount of mycotoxin they produce including deoxynivalenol, nivalenol and others. Based on these differences, we selected two strains with high (PH-1; NRRL 31084) and low (NRRL 28303) virulence for genomic studies. Two cDNA libraries were created by suppression subtractive hybridization to compare mRNA populations from wheat heads inoculated with the above mentioned strains and to identify genes specific to each interaction. Upon examination of 1339 EST sequences from these libraries, marked differences in overall gene expression were observed. However, the percentage of fungal sequences found was quite low. Therefore, to further characterize fungal genes expressed during the disease interaction, another subtractive library was constructed using wheat inoculated with NRRL 31084 and mock inoculated wheat heads. Nearly 25% of the 1236 EST sequences examined from this library were of fungal origin as determined by matches to the *F. graminearum* genome sequence. Comparisons of ESTs were also made to databases of other fungi for which whole genome sequences are available including *Magnaporthe grisea* and *Neurospora crassa*. Based on such comparisons and predicted function of genes corresponding to ESTs, candidate sequences potentially involved in pathogenicity have been identified and are being targeted for gene disruption.

THE WHOLE GENOME SEQUENCE OF *FUSARIUM GRAMINEARUM*, LINEAGE 7

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ABSTRACT

We have generated a draft sequence assembly of the *F. graminearum* genome that is available on the web for download and query. The sequence is of high quality with the entire 36 Mb assembly consisting of just 511 contigs (> 2 kb) contained within 43 supercontigs (scaffolds). The second genome release (October 2003) contains automated annotation, preliminary genome analysis and integration with the genetic map. Using organism-specific parameters for gene prediction, 11,640 protein-coding genes have been identified, representing over 1,500 more genes than predicted by the same method for the non-pathogenic filamentous fungi, *Neurospora crassa* and *Aspergillus nidulans*. A genetic map has been constructed that anchors 99.5% of the sequence assembly. Details of the automated annotation, efforts toward manual annotation and coordination of functional analysis of the genome will be discussed.

BIOASSAY VS. CONVENTIONAL CHARACTERIZATION OF FHB RESISTANCE IN NING 7840

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INTRODUCTION

Type II resistance to FHB is the primary focus of many U.S. wheat breeding programs (Rudd *et al.*, 2001). The most common greenhouse method for screening Type II resistance is single floret inoculation followed by counting the number of visually "scabby" spikelets at various days post inoculation (dpi) (Schroeder & Christensen, 1963). Although the number or proportion of scabby spikelets is a convenient method for scoring genotypes for resistance, researchers have questioned the ability of this method to accurately reflect the amount of disease present on a wheat spike (TeKrony *et al.*, 2000; Edge *et al.*, 2001). In this study we examined Type II resistance in FHB resistant line 'Ning 7840' and susceptible 'Norm' in the spikelets and rachis using visual and bioassay techniques.

MATERIALS AND METHODS

Planting: Seeds of the cultivars Ning 7840 and Norm were imbibed at room temperature for 24 hours, and subsequently vernalized (~2°C) in the dark for 5 weeks. After vernalization, germinated seeds were kept at room temperature in the dark for 48 hours before transplanting in the greenhouse at a density of four seedlings per pot. Rows of pots were alternated by genotype to reduce the effects of environmental gradients in the greenhouse.

Inoculation: Inoculum of *Fusarium graminearum* (Schwabe) isolate PH-1 (isolated from MI by Dr. L. Patrick Hart) was produced in Carboxymethyl Cellulose (CMC) liquid medium (Cappellini & Peterson, 1965). The CMC medium was removed through centrifugation and the spore concentration was adjusted to 1.4 × 1066 spores/ml (equivalent to10,000 spores/7µl) by the addition of sterile water. Just prior to anthesis (anthers slightly yellow to very yellow) forty spikes of each genotype were pipette inoculated with 7µl of spore suspension into one basal floret of one central spikelet of each head. Four spikes were randomly selected as non-inoculated controls. Immediately after inoculation, plants were misted for 5 seconds every 5 minutes for 72 hours in a growth chamber (~ 22°C/72°F). Seven and 14 days post inoculation (dpi), 20 inoculated and 2 control heads of each genotype were randomly selected and harvested for disease evaluation. Disease was evaluated using 3 primary methods:

1. <u>Number of Scabby Spikelets (SS)</u>: The number of Scabby Spikelets (SS) was counted based on discolored tissue (due to chlorosis, necrosis, or brownish/reddish discoloration typical of the disease) on one or more glumes, lemma, or palea of any of the florets of the spikelet. Only a portion of a spikelet needed to be symptomatic for the spikelet to be counted as scabby. Both the total SS and the SS up (towards the terminal spikelet) and down (towards the peduncle) from the inoculated spikelet were recorded.

2. Visually Infected Rachis Sections (VIRS): Spikelets were removed from the rachis and the rachis was visually inspected for symptoms of disease. The node subtending the originally inoculated spikelet was termed the 'node inoculated' and was considered a single rachis section. Rachis sections were defined for the remainder of the rachis depending on their location with respect to the 'node inoculated'. *Above* the 'node inoculated' sections were defined as starting just *above* one node and ending just *above* the adjacent node. *Below* the "node inoculated" sections were defined as starting just *below* one node and ending just *below* the adjacent node. In this way, the number of rachis sections infected on a spike was counted, and this count was termed the Visually Infected Rachis Sections (VIRS). Both the total VIRS and the VIRS up (towards terminal spikelet) and down (towards peduncle) from the node inoculated were recorded.

3. Bioassay Infected Rachis Sections (BIRS): The rachis was surface sterilized by soaking in 20% bleach + 0.1% Tween 20 for 2 minutes, then rinsed in sterile water. The rachis was cut into sections (as defined in method 2 above) using a sterile scalpel. Rachis sections from a single spike were plated sequentially in a circular pattern on a large petri dish (150 × 15mm) containing PDA. The number of sections from which *F. graminearum* grew into the media were counted and termed the Bioassay Infected Rachis Sections (BIRS) for that spike. Both the total BIRS and the BIRS up from (towards the terminal spikelet) and down from (towards the peduncle) the node inoculated were recorded.

***Replication*:** The entire experiment was replicated three times over a period of several months.

***Excluded Data*:** BIRS data for replication 1 at 7 and 14dpi and replication 2 at 7dpi was not used because surface sterilization of plant tissue was not effective, resulting in contamination. In addition, twenty-eight spikes (out of 240 inoculated) were not included in SS, VIRS or BIRS data analysis set because of escape, injury, missing data, strange visual symptoms resembling glume blotch (caused by *Septoria nodorum*), or a suspected second infection point.

***Transformation of Data*:** A square-root plus one transformation was used for data analyses. Back-transformation was used to report the estimated means.

RESULTS AND DISCUSSION

Total Spread:

***Within a Genotype* (Fig. 1A):** The mean number of Scabby Spikelets (SS) was significantly less (alpha 0.05) than Visually Infected Rachis Sections (VIRS) and Bioassay Infected Rachis Sections (BIRS) for both Ning 7840 and Norm at 7 and14 days post inoculation (dpi). In contrast, VIRS was not significantly different from BIRS for either genotype at either 7 or 14dpi. These data are consistent with other researchers that have shown that the fungus spread in the rachis is more extensive than in the spikelets (Pugh *et al.*, 1933; Edge *et al.*, 2001; TeKrony *et al.*, 2000). In addition, the greater spread of symptoms in the rachis versus the spikelets suggests that different genetic mechanisms may be responsible for these two measurements, as has been proposed by Yu 1990 (see Bai and Shaner, 1996).

***Between Genotypes* (Fig. 1B):** The mean SS, VIRS and BIRS of Ning 7840 was significantly less than the mean total SS, VIRS and BIRS (respectively) of Norm at both 7 and 14dpi (alpha 0.05).

***Between 7 and 14dpi* (Fig. 1A and 1B):** Comparison between 7 and 14dpi for SS, VIRS and BIRS of Ning 7840 and Norm revealed that Ning7840 SS was not significantly different (p-value = 0.35) between

the two time points, whereas all other measurements for Ning7840 and Norm were significantly different (alpha 0.05). In addition, Ning7840 SS was approximately 53% of the values of VIRS and BIRS at 7dpi, while it was only 37% and 39% of the values of VIRS and BIRS values (respectively) at 14dpi. These data reveal that although Ning7840 shows reduced SS, VIRS and BIRS in comparison to Norm (Fig. 1B), Ning 7840 may be more effective in resisting the spread of scabby spikelets than in resisting the spread of visually and bioassay infected rachis section.

Bimodal Spread of BIRS in Ning7840: Histograms of the percent of the total number of observed spikes at each level of SS, VIRS, and BIRS (Fig. 2) revealed that 34% of Ning 7840 spikes did not show spread of the fungus (BIRS) beyond the initially inoculated node at both 7 and 14dpi. The spread of *F. graminearum* in the rachis of Ning 7840 appeared to have a bimodal trend: either showing minimal or extensive spread of the fungus. For Ning 7840 BIRS at 14dpi approximately 44% of the spikes showed infection in 2 or fewer rachis sections, whereas approximately 56% showed infection in 9 to 14 rachis sections. Norm, in contrast, did not have any spikes in which the fungus was restricted to the initially inoculated node at either 7 or 14dpi. For Norm BIRS at 14dpi 100% of spikes showed at least 6 infected rachis sections, and approximately 84% of spikes showed infection in 9 to 14 rachis sections. Histopathological examinations of Sumai 3 showed that barriers formed in some xylem cells, which were eventually overcome by the fungus (Ribichich *et al.* 2000). In addition, Ning7840 is thought to have the same major QTL for resistance as Sumai 3 (Bai *et al.* 2003). The presence of these structural barriers, which may be overcome, may explain our bimodal results in Ning 7840, where the fungus never moved beyond the initially inoculated node for several spikes, while in others it moved extensively in the rachis but did not appear to invade the adjoining spikelets.

Spread Up and Down from Inoculated Node:

Within a Genotype **(Fig. 1C):** The number of Scabby Spikelets (SS) up (towards the terminal spikelet) from the node inoculated was not significantly different from SS down (towards the peduncle) from the node inoculated except for Norm at 14dpi, in which SS up was significantly less than down. In contrast, visually infected rachis sections (VIRS) and bioassay infected rachis sections (BIRS) up from the node inoculated were significantly less than down for Ning7840 at both 7 and 14dpi, and for Norm at 14dpi. For Norm at 7dpi, none of the measurements (SS, VIRS and BIRS) up were significantly different from the respective measurements down, although all SS, VIRS and BIRS up were significantly less than the respective measurements down at 14dpi. Overall SS, VIRS and BIRS up represented 30-49% of the total spread from the node inoculated, though it was often significantly less (alpha 0.05) than the measurements down. This data is in agreement with other studies that found that the fungus spreads primarily down the rachis from the point of inoculation (TeKrony *et al.*, 2000), although it does reveal that there is also a substantial amount of spread up.

Between Genotypes **(Fig. 1D):** Ning7840 SS, VIRS and BIRS up from the inoculated node were significantly less than Norm SS, VIRS and BIRS (respectively) for 7 and 14dpi. Ning 7840 SS, VIRS and BIRS down from the inoculated node were significantly less than Norm SS, VIRS and BIRS (respectively) for all cases except SS at 7dpi (for which there was no significant difference).

Bimodal Spread of BIRS in Ning7840: The bimodal spread observed in the total BIRS of Ning 7840 (see above) was reflected in BIRS up, but was easily seen in BIRS down (data not shown).

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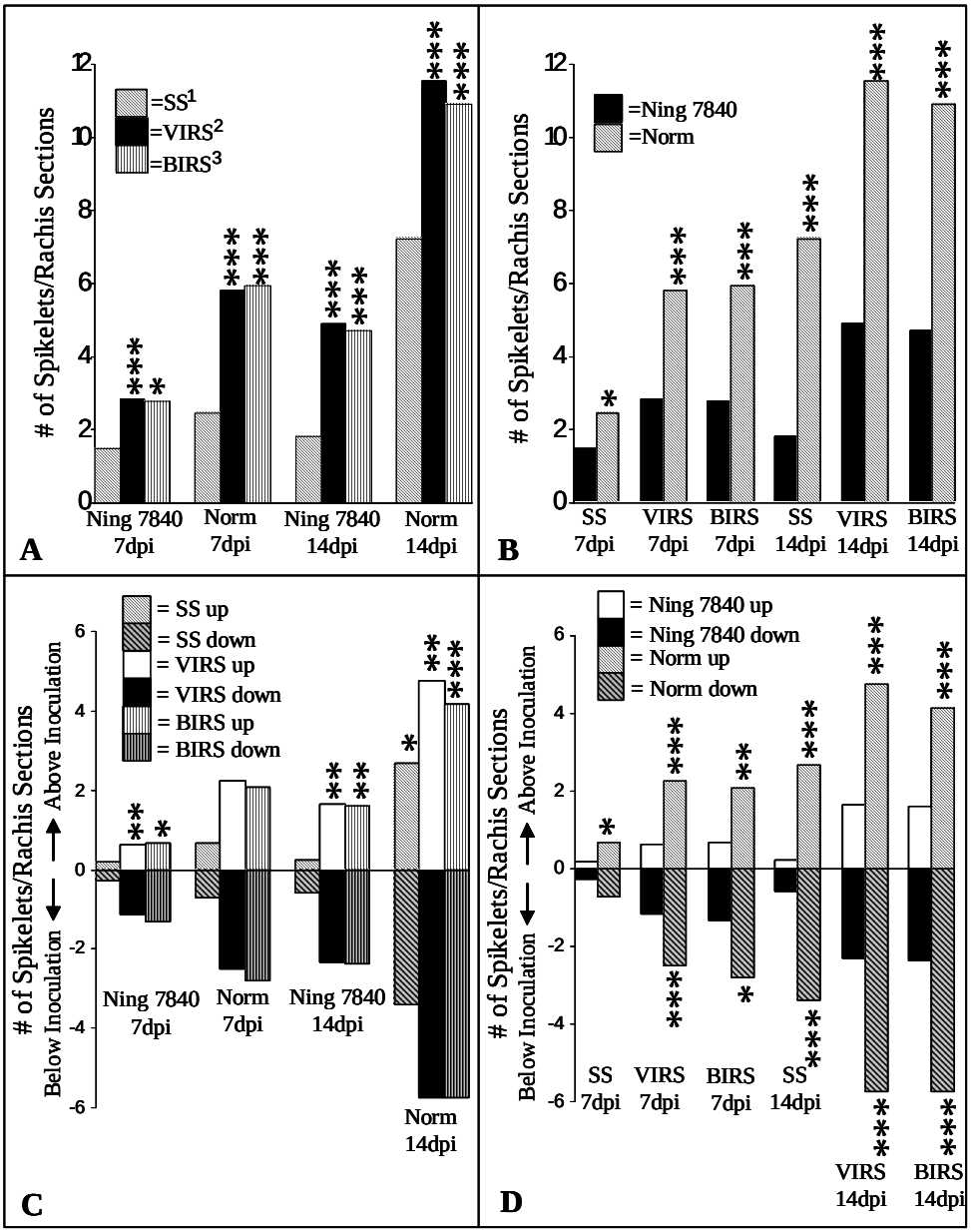

Fig. 1. Effect of Genotype, time of assay, and method of assay on the number of infected spikelets or rachis sections in a resistant (Ning 7840) and susceptible (Norm) wheat cultivar. A. Comparisons between the number of scabby spikelets (SS) and the number of infected rachis sections determined either visually (VIRS) or by bioassay (BIRS) within genotypes at 7 and 14 dpi. Asterisks above a column indicate a significant difference between that column's mean and the # of scabby spikelets for that genotype/dpi combination. **B.** Comparisons between Ning 7840 and Norm for three measures of disease spread at 7 and 14 dpi. Asterisks above a column indicate a significant difference between Ning7840 and Norm for that measurement/dpi combination. **C.** Comparisons between upward and downward spread from the inoculated node for three measures of disease spread in Ning 7840 and Norm. Asterisks above a column indicate a significant difference between the upward and downward spread for that measurement/genotype/dpi combination. The "0" on the Y axis represents the inoculated node. Spread up from the inoculated node is represented by numbers >0, and down from the node inoculated is represented by numbers <0. **D.** Comparisons between Ning 7840 and Norm for upward and downward spread from the inoculated node for three measures of disease. Asterisks indicate a significant difference between Ning7840 and Norm for the upward (asterisks above upper bars), or downward (asterisks below lower bars) spread for that measurement/dpi combination. The "0" on the Y axis represents the inoculated node. Spread up from the inoculated node is represented by numbers >0, and down from the inoculated node is represented by numbers <0. *p< 0.05, ** p < 0.01, ***p< 0.001.
1. SS = Number of Scabby Spikelets. 2. VIRS = Visually Infected Rachis Sections. 3. BIRS = Bioassay Infected Rachis Sections.

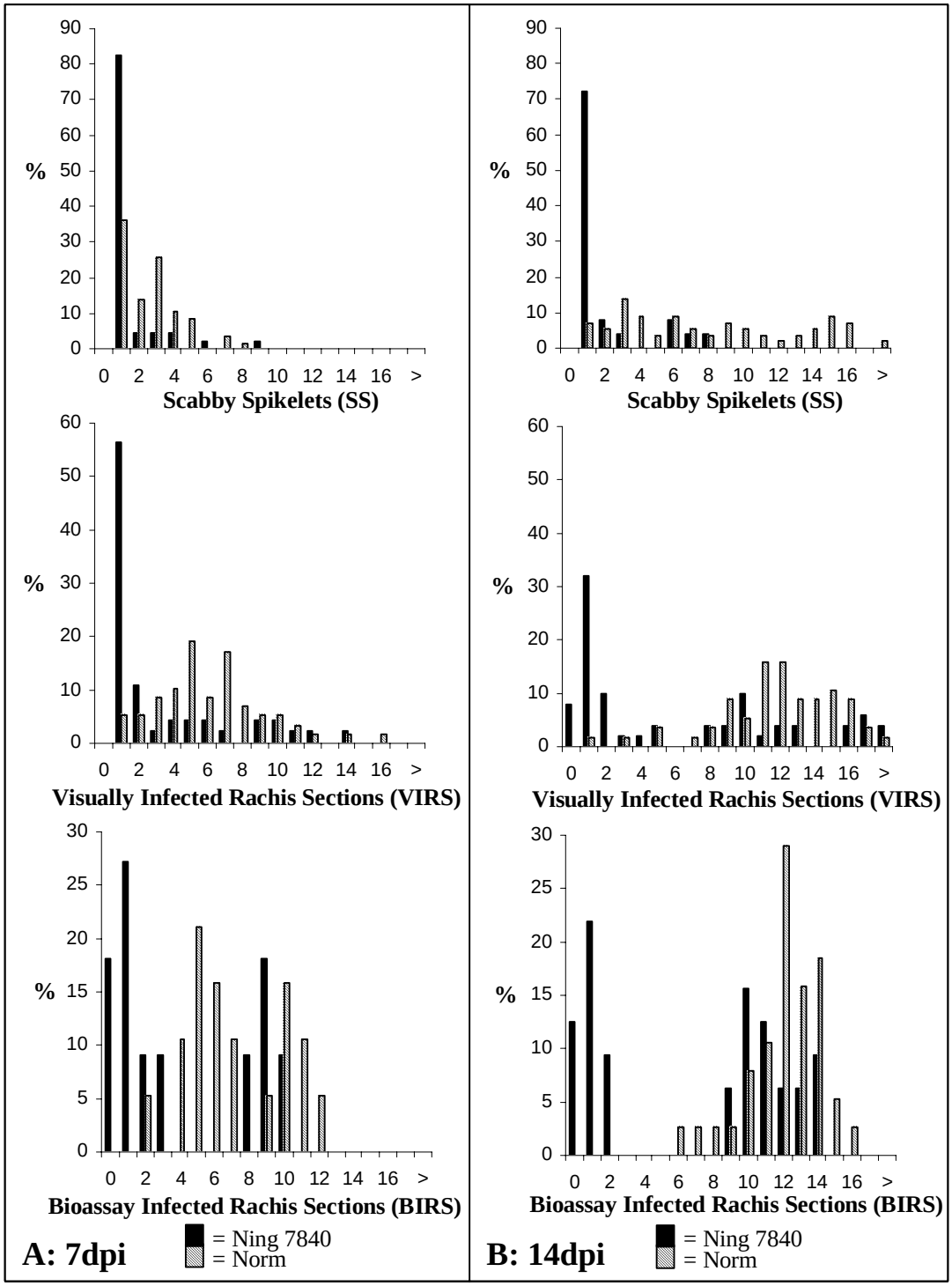

Fig. 2. Relative frequency distributions of disease as determined by three measures in Ning 7840 and Norm at 7 dpi (A), and 14 dpi (B). The Y axis represents the percent of observed spikes with a given X axis category. Note that the scaling of the Y axis values varies with measure of disease.

FUSARIUM HEAD SCAB RISK FORECASTING FOR OHIO, 2002-2003

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ABSTRACT

During the 2002 and 2003 wheat growing season, models were used to predict the risk of Fusarium head scab in Ohio. This was the second and third years for testing these models in the state. Head scab risk assessment probabilities were derived from logistic models previously developed from hourly weather, crop growth and disease observations from 50 location-years representing three wheat production regions in the US. Hourly weather data from weather stations were used to determine duration of weather events for the pre- and post-anthesis time periods examined by the models. Disease risk probabilities were calculated using logistic equations determined by two models representing the critical weather conditions during the time period 7 days prior to anthesis (Model I) and the time period of 10 days post anthesis (Model II). During both years, weather conditions in early April were relatively dry and warm providing conditions for rapid and early development of the crop. Anthesis dates for wheat fields from south to north in the state varied by more than four weeks (10 May to 9 June 2002 and 9 May to 4 June 2003) due to cool weather that slowed plant development in May. Precipitation events became more frequent throughout May across the state with most locations reporting up to 32 and 37 hours of measurable precipitation during the 7 days prior to anthesis for 2002 and 2003, respectively. However, average daily temperatures for most locations in the state were generally below 15 C when most of the wheat was in anthesis. Scab risk probabilities were calculated for early, mid and late anthesis dates for each weather station location. Calculated risk probabilities ranged from 0.00 to 0.81 for Model I and from 0.02 to 0.69 for Model II during 2002, and from 0.00 to 0.82 for Model 1 and from 0.03 to 0.24 for Model II in 2003. Of 42 location-anthesis date scab-risk probabilities calculated during 2002, Model I predicted 31 location-anthesis dates with low to moderately low risk and Model II predicted 40 location-anthesis dates with low or moderately low risk. Of 21 scab risk probabilities calculated during 2003, Model I predicted 17 location-anthesis dates with low to moderately low risk and Model II predicted 21 location-anthesis dates with low to moderately low risk. Based on these results, the head scab risk prediction was reported to be low to moderately low for the majority of locations in the state both years. Head scab risk predictions were posted on the Ohio State University Ohio Field Crop Disease web page (www.oardc.ohio-state.edu/ohiofieldcropdisease/) during the critical time of disease development through harvest. Approximately 14 to 18 days after anthesis, 159 fields in 2002 and 148 fields in 2003 in 30 counties were surveyed for scab incidence by the OSU Extension Agents. From 1 to 10 fields were surveyed per county. Disease surveys indicated the mean incidence of head scab was 4.1% with a range of 0% to 49% in 2002 and 8.9% with a range of 0% to 73% in 2003. Results of the Scab Risk Assessment Models indicated that they generally predicted the risk of scab adequately for the majority of locations in the state both years.

GLOBAL GENETIC DIVERSITY OF *FUSARIUM GRAMINEARUM* CLADE SPECIES AND THEIR MYCOTOXIN POTENTIAL

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ABSTRACT

Although the primary etiological agent of FHB, *Fusarium graminearum,* has been regarded as a single, panmictic species worldwide, phylogenetic analyses of DNA sequences from 11 nuclear genes totaling 13.6 kb [i.e., genealogical concordance phylogenetic species recognition (GCPSR)] have shown that this morphospecies actually consists of 9 phylogenetically distinct and biogeographically structured species (hereafter referred to as the *Fg* clade) [PNAS 97:7905-7910 (2000) and PNAS 99:9278-9283(2002)]. GCPSR is based on the fact that population-splitting events associated with speciation eliminate shared neutral polymorphism over time, resulting in descendant species with reciprocally monophyletic genealogies of orthologs. Given their importance to world agriculture, species rank is formally proposed for the eight unnamed cryptic species within the *Fg* clade using fixed nucleotide characters and conidial characters. In addition to the unexpectedly high level of species diversity within the *Fg* clade, the virulence-associated trichothecene mycotoxin genes are under a novel form of balancing selection resulting in the maintenance of B-trichothecene chemotype polymorphism through multiple speciation events, which may have important consequences for the fitness and aggressiveness of FHB pathogens on particular hosts or in particular environments. Taken together, these studies suggest that the combined species and mycotoxin diversity of FHB pathogens is remarkably high. However, it appears that only a fraction of this diversity is currently represented within North America. Therefore, the introduction of novel FHB pathogens or chemotypes via global trade in agricultural products has the potential to exacerbate the FHB problem in the U.S. We have developed protocols for the multiplex amplification of two sets of chemotype-specific primers, previously designed from genes within the trichothecene gene cluster (TRI3 and TRI12). Using these tests, chemotype diversity has been assessed in a collection of isolates from the U.S., China and Brazil. Chemotype frequencies were more balanced within Brazil (15ADON, 3ADON, NIV) and China (3ADON, NIV) compared with the U.S. [predominantly 15ADON, see Gale at al. poster], although the 15ADON chemotype was completely absent from the four Chinese populations surveyed to date. The development of robust molecular tools for FHB species identification and chemotype determination will significantly improve disease surveillance and global monitoring efforts, and will make available for the first time detailed information on the geographic and host distributions of FHB pathogens and their trichothecene chemotypes, enhancing current knowledge of the ecology, epidemiology and population dynamics of these mycotoxigenic cereal pathogens.

EPIDEMIOLOGICAL STUDIES ON FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTH DAKOTA FOR 2003

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INTRODUCTION AND OBJECTIVES

It has been observed that FHB occurs at epidemic levels when warm, humid conditions and frequent precipitation have occurred at anthesis (Bai and Shaner, 1994; McMullen et al., 1997; Parry et al., 1995). By investigating the relationship of FHB incidence and severity to environmental conditions, a better characterization of the disease can be made. Environmentally-based forecasting systems have been shown to be effective in predicting epidemic levels of FHB in field situations (De Wolf et al, 2003) using temperature, precipitation and relative humidity parameters, however the accuracy of these modeling systems is considered to be only moderate. Through the course of collecting disease and environmental data over numerous environments, it has been observed that field disease can be highly variable under environments falling near the prediction threshold for the models mentioned above. It was hypothesized that in those instances when environment is not entirely conducive to disease development, inoculum level may be more predictive of final disease than environment on its' own.

South Dakota State University is part of a multi-state collaborative project studying the epidemiology of Fusarium head blight (FHB) on wheat under different environments throughout the upper mid-west. The ultimate goal is to refine a disease risk advisory/forecast system, and to elucidate principle components of the FHB disease cycle. In 2003, a project was established to examine the influence of varying inoculum load on field disease. The primary objectives include: 1) establishment of three distinct inoculum (spore) loads through varying the amount of corn stalk residue on the soil surface beneath experimental plots; 2) to determine the effects of high, medium and low inoculum loads and weather on final disease and mycotoxin levels in grain; and 3) to continue to collect and analyze environmental data in conjunction with inoculum and disease monitoring for use in developing and evaluating FHB forecasting models.

MATERIALS AND METHODS

Field plots of spring wheat (*Triticum aestivum* L.) were established near Brookings, SD in a randomized complete block split-plot design. Whole-plots were 6.10m by 15.24m (20ft by 50ft) and consisted of corn stalk residues at levels of: 1) zero (0%), 2) moderate (15%), and 3) heavy (80%) ground cover as measured by the line-transect method. Sub-plots consisted of 2 planting dates (14 Apr and 23 Apr), with a second split for cultivar ("Norm" and "Alsen") resulting in experimental units ('plots') of 3.05m by 6.10m (10ft by 20ft). Whole-plots (residue treatments) were buffered on all sides by 9.14m (30ft) of "Reeder", a tall, late flowering spring wheat variety.

Wheat development was recorded weekly until boot stage, then daily thereafter. Final disease levels were assessing as incidence and severity on 100 heads per 'plot'. Severity was assessed based on percentage of the spike area blighted (Stack and McMullen, 1995). All plots were evaluated at late dough stage (Feekes 11.2) for disease development. Test weights, yield, deoxynivalenol (DON) concentration, and percent scabby kernels were assessed for each 'plot'.

Weather and microenvironment data were continuously collected using a datalogger (Campbell Scientific Inc. model CR10X) and various instruments. Leaf wetness sensors (Campbell Scientific Inc. model 237) were used to estimate the duration of leaf wetness within the canopy. Additional sensors were constructed and deployed to detect moisture at the soil surface (Osborne and Jin, 2000).

Daily airborne inoculum levels were monitored during the sampling period using a Burkhard Cyclone Sampler (Burkhard Manufacturing) placed within the border area of the field. A wash of the cyclone unit was performed daily to ensure uniform sampling. The sample and wash were plated on Komada's medium, selective for *Fusaium* (Komada, 1975). Counts were reported as colony forming units (CFU) per day. Inoculum within 'plots' was enumerated by sampling and washing spikes using protocols described by Francl et al. (1999). On each day, five primary spikes per replicate were collected and placed in a flask with 50ml of sterile deionized water, shaken vigorously for 60 seconds to dislodge spores, then discarded. A 0.5ml aliquot of the wash was then spread-plated onto each of three plates of Komada's medium. Plates were then incubated 5-8 days. Colonies of *F. graminearum* were counted after incubation. Colonies were reported as CFU per spike per day.

Data from the 2003 FHB monitoring plots will be entered into two FHB risk assessment/disease forecast models made available by Ohio State University (Ohio I and Ohio II; De Wolf, et al, 2003). Ohio model I is used to predict risk of a FHB epidemic based on temperature and precipitation variables prior to anthesis. Ohio model II is intended to predict disease risk based on temperature and humidity before and after flowering begins. Model I is intended to predict epidemics before infection, while Model II is intended to estimate disease risk after infection may have occurred.

RESULTS AND DISCUSSION

The 2003 field season was highly favorable for spring wheat yields in much of South Dakota though FHB levels were moderate to low across the region. Temperatures were warm during much of the flowering period, and were considered to be within the FHB-favorable range. Rainfall was limiting during susceptible periods for both planting dates. Only three significant precipitation events (>3mm) fell during the three-week monitoring period corresponding to flowering-to-grain fill periods for both planting dates. Disease levels (incidence and severity) are given in Fig. 1. Values represent low levels of incidence and severity for both varieties. For planting date 2, there is a trend of increased incidence with increased residue cover, which suggests higher levels of spore inoculum present in the higher residue plots during susceptible periods. The severity values show no clear differences in relation to residue cover, as would be expected with low incidence and the dry environment. Figure 2, results of the inoculum bio-assay (head washing), indicate differences in inoculum concentration on spikes corresponding to levels of residue cover for both of the planting dates. Inoculum levels are considered to be favorable for high levels of disease development, therefore it is assumed that some other factor was limiting (presumably precipitation).

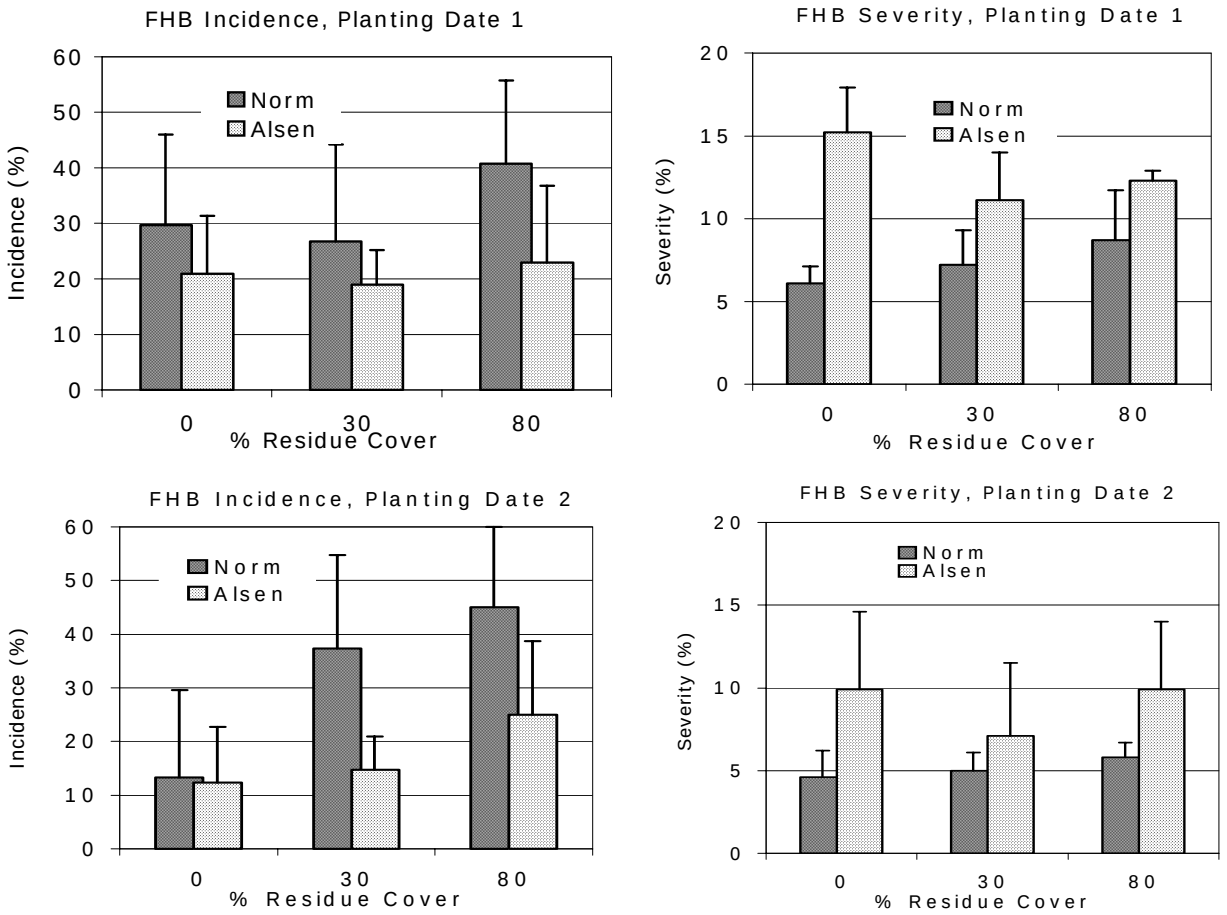

FIGURE 1. Field FHB incidence and severity.

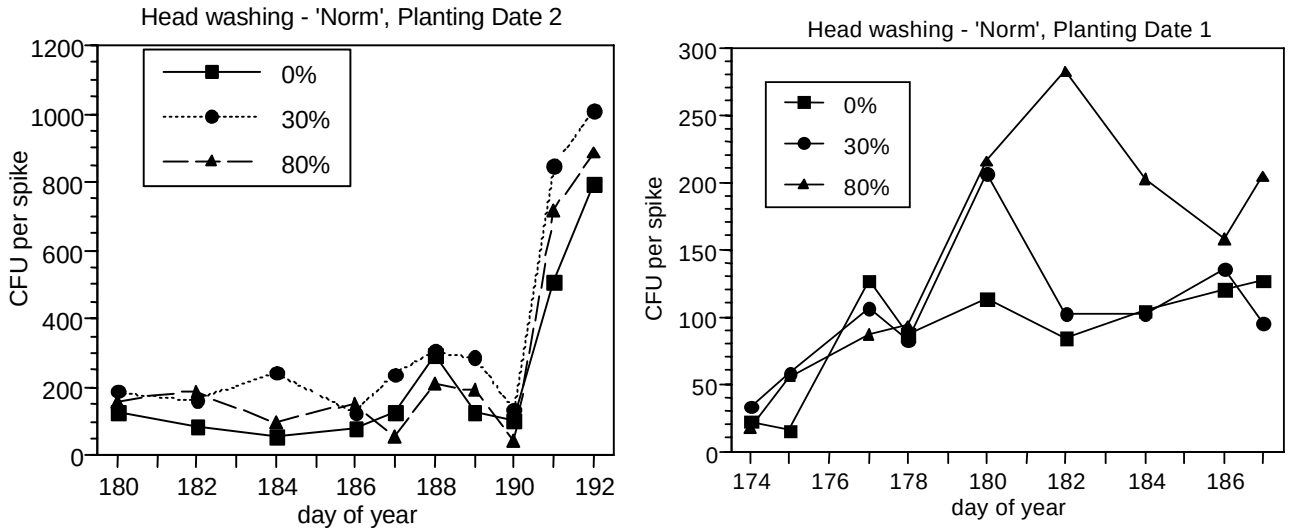

FIGURE 2. Inoculum washed from spikes

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FHB RISK ADVISORY FOR SPRING WHEAT IN SOUTH DAKOTA, 2003

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ABSTRACT

In 2003, the small grains pathology project at South Dakota State University continued to provide a web-delivered, weather-based risk advisory for Fusarium head blight (FHB) in northeastern South Dakota. An twelve-county area comprising the majority of the spring wheat production region in the state was selected for intensive inoculum, disease and environmental monitoring. This area was selected for a FHB risk advisory to be issued on a county by county basis. Advisory information was posted to the internet every one to two days during peak susceptibility periods (flowering) detailing potential risk of disease to wheat crops in each of the 12 counties. Experimental risk assessment models (Ohio I and Ohio II) were utilized to provide risk probability based on a few selected environmental parameters. Model output was considered as part of the overall risk assessment upon which advisories were based. A 'high-risk' advisory was issued for all 12 counties at some point during the three weeks of intensive monitoring (June 16 through July 9). Two counties (Marshall and Roberts) were under 'high-risk' advisory for the entire three weeks. FHB scouting across the region showed disease index levels ranging from low (<less than 8%), to very high (>20%) in parts of Marshall and Roberts counties.

SPATIAL RELATION OF DON CONTAMINATION TO CORN RESIDUES IN SPRING WHEAT

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ABSTRACT

In 2003, as part of a collaborative study investigating the effect of variable inoculum levels on field levels of Fusarium head blight (FHB), plots were established near Brookings, SD to monitor inoculum and disease in spring wheat. Plots were treated by spreading corn stalk residue to achieve three distinct coverage levels (0%, 30% and 80%), thereby attempting to establish low, medium, and high levels of airborne inoculum for FHB. The plots were intentionally isolated with 30' buffer zones surrounding the residue-treated areas. At harvest, these buffers zones and the plots themselves were sampled for *Fusarium* damaged kernels (FDK) and DON contamination. The samples were collected at 5' intervals between and surrounding residue treated plots. The objective of the sampling was to assess the impact of the corn residue levels on DON contamination in grain grown outside of the residue area.

DEVELOPMENT OF FUSARIUM HEAD BLIGHT OF WINTER WHEAT IN OHIO AS INFLUENCED BY PLANTING DATE, CULTIVAR MATURITY, AND INOCULUM LEVEL

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OBJECTIVES

1) Determine the effects of different cultural practices on the development of Fusarium head blight of winter wheat, and 2) determine the relationship among disease intensity, residue level, and inoculum level.

INTRODUCTION

Fusarium head blight (FHB), caused primarily by *Gibberella zeae*, is one of the most devastating diseases of wheat and barley in the North America (McMullen et al., 1997). No single management strategy has been effective against this disease. An integrated approach using cultural practices to reduce inoculum levels and to escape disease-favorable periods, cultivars with partial resistance to FHB, and timely application of fungicides through accurate disease prediction and risk assessment may be the most effective. Weather-driven risk assessment models for FHB have been developed and are currently being validated in several states (De Wolf et al., 2003, Lipps and Mills, 2003). In order to incorporate variables related to cultural practices and inoculum levels into these models, a thorough understanding of the influence of these factors on disease development under different conditions is necessary.

MATERIALS AND METHODS

Three soft red winter wheat cultivars ('Patterson', 'Elkhart', and 'Hopewell') that differ in heading dates were planted on different dates during the 2000/2001, 2001/2002, and 2002/2003 growing seasons at the Ohio Agricultural Research and Development Center, near Wooster. In 2000/2001 and 2001/2002, a split-plot design was used, with planting date, and cultivar being the whole- and sub-plot factors, respectively. In 2002/2003, a split-split-plot design was used. Density of corn residue, planting date, and cultivar were the whole-plot, sub-plot, and sub-sub-plot factors, respectively. Whole plots treatments were established by spreading different densities of corn residue (0, 15, and 80%) over the soil surface in early spring of 2003. Residue levels were determined by the line-transect method. In each growing season, there were three replicate blocks of each treatment combination. Strips of Freedom, a cultivar with moderate scab resistance, were used to separate adjacent blocks and whole plots within each block.

Burkard cyclone spore samplers were used to monitor daily numbers of airborne spores of *F. graminearum* from Feekes growth stage 10 through 11.2. During the same period, wheat heads were collected and assayed directly for spores of *F. graminearum* using head washing.

Beginning at Feekes 10.5.4, incidence and severity of FHB were assessed three times per week in each of the smallest experimental units. Each head within a 1-ft length of row at 10 arbitrarily selected sites within each plot was assessed for percentage of affected spikelets. Diseases incidence was scored as the percentage of diseased

heads, while disease severity was recorded as the average percentage of diseased spikelets per head (= "index").

FHB incidence and severity data were analyzed using Proc Mixed (SAS, Cary, NC) to assess the main and interaction effects of planting date and cultivar on disease development.

RESULTS AND DISCUSSION

In general, late planting of mid-season cultivar, Elkhart, resulted in the highest disease intensity (Table 1). This is probably because the period of greatest crop susceptibility coincided with periods of FHB-favorable weather conditions. In 2001, planting date had no significant effect on FHB development on cultivars Hopewell and Patterson. In both 2001 and 2002, within a given planting date, Elkhart and Patterson, respectively, were the most and least affected by FHB.

In 2003, disease development at 80% residue was comparable with development at 15 and 0% (Figure 1A). This may be due in part to the fact that very similar levels of inoculum were recorded from wheat heads sampled from each residue plot (Figure 1B). In addition, the influence of surface residue on inoculum levels and disease development may be dependent on the weather. Further investigation of this factor over multiple years and locations may provide better insight into its effect of FHB development.

No clear association was observed between levels of FHB inoculum in the air and on wheat heads (Figure 2). Peaks in the level of airborne spores trapped using the Burkard sampler corresponded only to subtle increases in the number of spores recovered from wheat heads. In all three growing seasons, there was a marked association between rainfall amounts and number of CFU recovered from wheat heads via head washes. No overall association was observed between CFU recovered through Burkard spore sampling and rain events. In many cases, peaks in rainfall amounts coincided with reductions in the number of spores sampled from the air and increases in the number of spores sampled from wheat heads. De Wolf et al. (2001) also observed an association between rainfall and *G. zeae* inoculum on wheat heads in North Dakota. In addition to potentially washing spores out of the air, rain may be contributing to increases in inoculum levels on the heads by splashing spores from other sources. Partial results of research on splash dispersal of *G. zeae* support this hypothesis (El-Allaf et al 2003). Further investigation of the relationship among inoculum levels (in the air, on residue and on wheat head), disease intensity, and weather conditions may provide a clearer understanding of the relative importance of airborne and residue-borne inoculum for the development of FHB.

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Table 1. Comparison of the effect of planting date within each cultivar and cultivar within each planting on the final incidence and severity of Fusarium head blight of winter wheat in Wooster, Ohio in 2001 and 2002

Cultivar	Incidence			Severity		
2001						
	18 Sept.	2 Oct.	16 Oct.	18 Sept.	2 Oct.	16 Oct.
Elkhart	72.7 *A b*	77.4 *A a b*	81.6 *A a*	39.6 B b	41.4 A b	52.9 A a
Hopewell	69.1 *A a*	72.3 *A a*	75.5 *A a*	40.7 A a	40.6 A a	44.1 B a
Patterson	50.4 *B b*	56.8 *B a*	58.3 *C a*	30.4 C a	31.0 B a	32.2 C a
2002						
	18 Sept.	1 Oct.	23 Oct.	18 Sept.	1 Oct.	23 Oct.
Elkhart	35.4 *A c*	54.7 *A b*	61.3 *A a*	24.4 A b	27.0 A b	36.5 A a
Hopewell	30.0 *B c*	39.0 *B b*	47.0 *B a*	15.7 B c	19.3 B b	23.2 B a
Patterson	24.0 *C c*	31.2 *C b*	34.9 *C a*	12.6 B c	15.5 C b	18.1 C a

Means followed by the same uppercase letter in each column and by the same lowercase letter within each row are not significantly different at $P \leq 0.05$ by the LSD-test.

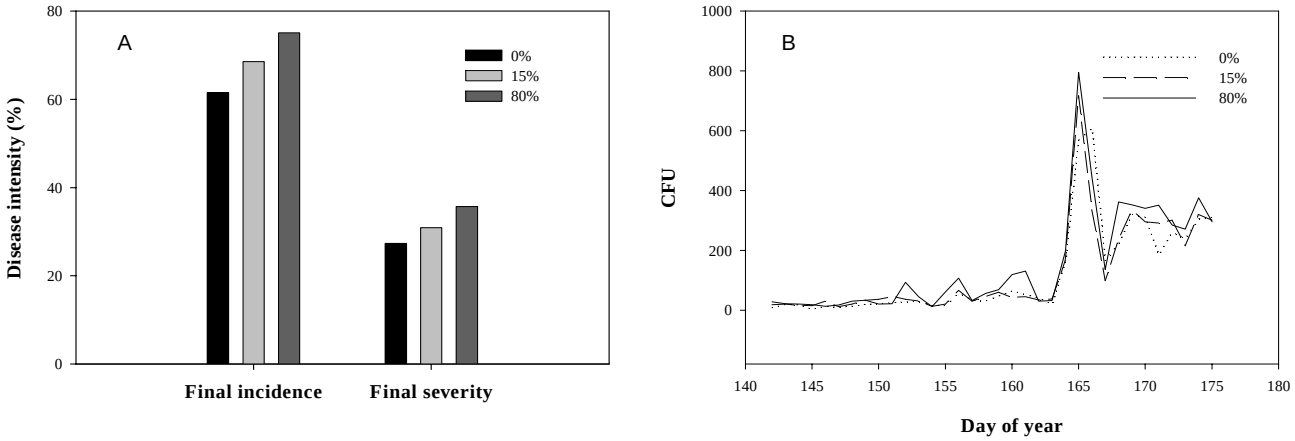

Figure 1 - Effects of surface residue on FHB intensity (A) and inoculum levels on wheat heads (B) in Wooster, Ohio. Final disease assessment was done on 26 June 2003. Head wash assays were used to determine inoculum levels

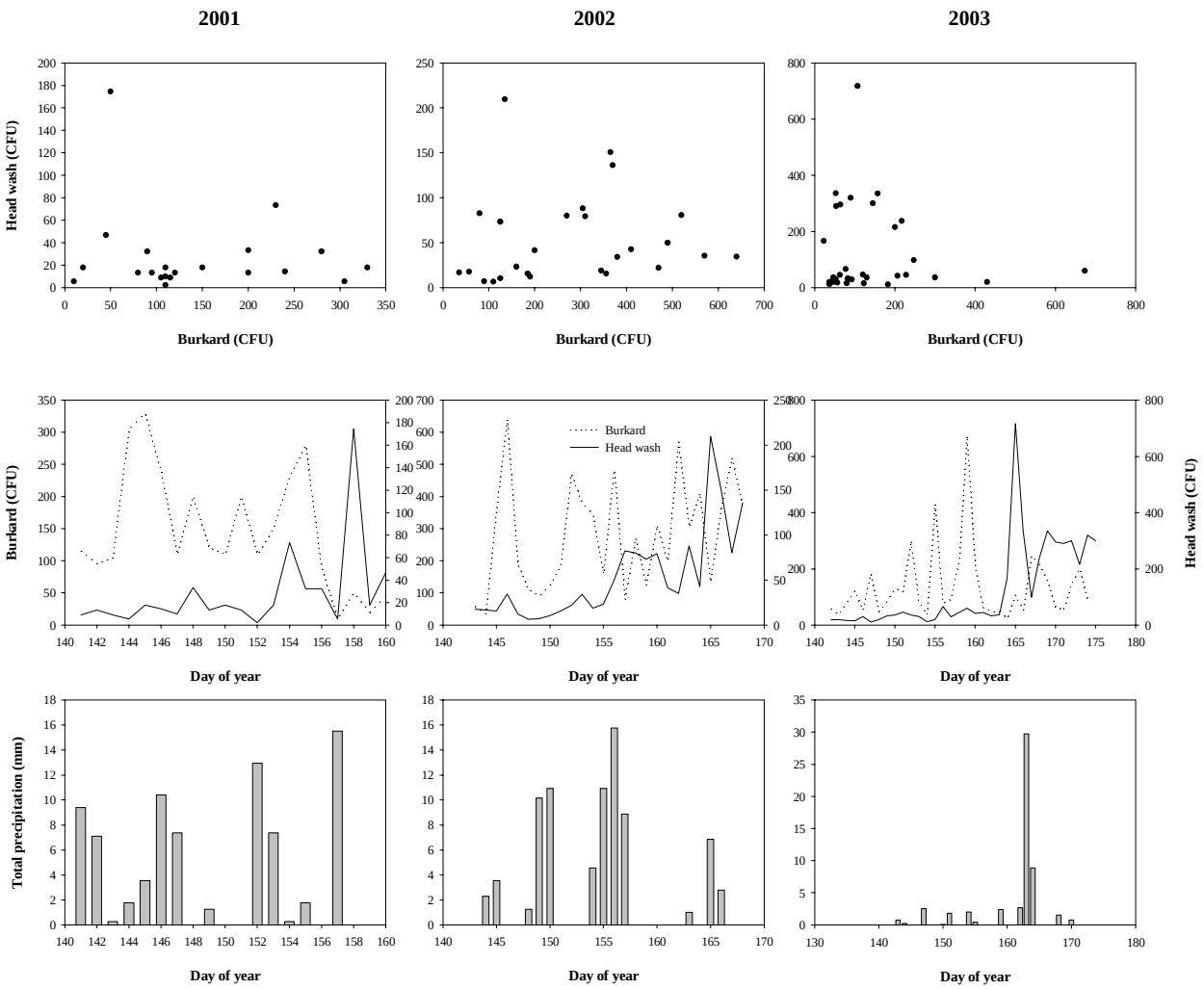

Figure 2 - Relationship among daily inoculum levels of *G. zeae* in the air sampled using Burkard spore traps, on wheat head assayed via head washes, and total daily precipitation during the 2001, 2002, and 2003 winter wheat growing seasons in Wooster, Ohio.

SPLASH DISPERSAL OF SPORES OF *FUSARIUM GRAMINEARUM* USING A SINGLE-DROP GENERATOR

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ABSTRACT

Splash dispersal of *Fusarium graminearum*, causal agent of head blight of wheat, was studied using a single drop generator. Hypodermic needles were used to generate drops 2.7, 3.3, and 3.7 mm in diameter, falling from heights of 50, 75, 100, and 125 cm. A macroconidial suspension of *F. graminearum*, at a concentration of 3×10^4 conidia ml^{-1}, and wheat leaves sprayed with a suspension of 8×10^4 spores ml^{-1} were used as targets. Leaves of wheat cultivar Norm were cut into 3-cm pieces, affixed to microscope slides, and the spore suspension applied in five uniform passes at a rate of 0.23 ml/sec. Three milliliters of the 3×10^4 spores ml^{-1} spore suspension was placed into a small watch glass, forming a 2-mm-deep film. The source materials were placed at the point of drop impact and 50 drops falling at a rate of 130 drops per minute from each fall height/drop diameter combination were allowed to hit the targets. PVC tubes were used to form tunnels along the path of the falling drop to minimize the effects of the wind on the impact position. Five sets of petri plates containing Komada's selective media were placed at 10, 20, and 30 cm from the point of impact to collect splashed droplets. Each set of plates was placed at a different angle from the source. After the splash droplets were collected, 1 ml of sterile distilled water amended with Tween 20 was applied to each plate to enhance the spread and germination of spores in the sampler. Plates were incubated at room temperature under a 14-h photoperiod for 48 h. The number of colony forming units (CFU) was then counted in each plate. There was greater variability in spore dispersal from wheat leaves than from spore suspensions, however, for both targets, the majority of spores were collected between 10 and 20 cm from the point of impact. Drops falling from 125 and 100 cm resulted in greater spore dispersal and more spores collected 30 cm from the source than drops falling from 75 and 50 cm. In general, for a given fall height, more spores were dispersed by 3.7- and 3.3-mm drops than by 2.7-mm drops. For both targets, positive relationships were found between CFUs and functions of the impacting drop velocity and diameter. These relationships were stronger for spore suspensions than wheat leaves. The relationship among the characteristics of different sources of inoculum, physical properties of incident drops, and number of spores dispersed may be used estimate the spread of *F. graminearum* within the wheat canopy and to model rain-splash dissemination of Fusarium head blight.

PATHOGENIC SPECIES, GEOGRAPHIC DISTRIBUTION, AND SEVERITY OF FUSARIUM HEAD BLIGHT ON BARLEY IN THE CENTRAL HIGHLANDS OF MEXICO

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OBJECTIVES

The present study was carried out to: 1) determine the geographical distribution and severity of FHB in the field in the Central Highlands of Mexico; 2) identify *Fusarium* species causing FHB on barley in the field; and 3) confirm the pathogenicity of identified species.

INTRODUCTION

In Mexico, malting barley (*Hordeum vulgare* L.) is grown on approximately 301,000 hectares. The main malting barley producing area of Mexico is the Central Highlands, which accounts for nearly 50% of Mexico's total barley area. Since 1998 there has been an increase of Fusarium head blight (FHB) in barley produced in the Highlands (7,11). Although few studies of FHB on barley have been conducted in Mexico (9,10), field pathogenicity was not confirmed for each species of *Fusarium*. Lack of knowledge of this disease is impeding effective FHB control and putting barley grain produced in Mexico at a competitive disadvantage on the international market.

MATERIALS AND METHODS

Geographical distribution and evaluation of FHB severity on barley heads. Field samples were collected in the 2001 and 2002 growing seasons. Heads showing FHB symptoms were collected at the grain filling stage. The methodology made it possible to collect the greatest possible number of *Fusarium* species present in the field, and to identify other fungal species involved in the symptoms of FHB. Samples taken from the whole area each growing season included a total of 600 heads, which were then evaluated to determine disease severity, isolate species of *Fusarium* and other fungi, and estimate the percent frequency for each isolated species. The percent severity for each sample was the sum of infected grains in the entire sample in relation to the total number of grains in each sample (8). The percent frequency for each species was the number of isolates of the species in relation to the total number of *Fusarium spp.* isolates.

Species identification. The isolates were made in potato dextrose agar and carnation leaf agar (4). Monoconidial cultures were made for each isolate (2,3,8,13). Pathology tests were also conducted in the 2002 season using the Neergard technique (12), as modified by CIMMYT's Seed Health Unit. Monoconidial cultures of different *Fusarium* colonies were obtained. Monoconidial colonies of *Fusarium spp.* obtained from blotters (2002 sampling) and from direct seed isolates (2001 sampling) were identified

according to Booth's classification (2), aided by Burgess et al. (3) and Nelson et al. (13). Identification of *Fusarium* species was confirmed via RFLPs in the Laboratory of Dr. R. De La Torre-Almaraz of the UNAM at Iztacala, State of Mexico (unpublished).

Pathogenicity tests. Pathogenicity tests were conducted in the 2001 growing season at CIMMYT's Experiment Station in Atizapán, State of Mexico (19.10°N, 99.51°W), on 10 species (Table 1) inoculated in the malting barley cultivar Esmeralda harvested in the Central Highlands of Mexico in the 2001 growing season. Isolates were increased in liquid mung bean medium (1), at a concentration of $50x10^3$ conidia per ml (6). Twenty spikelets per experimental plot were inoculated at flowering using the cotton technique (1,5). Sprinkler irrigation was not applied, as daily precipitation favored disease development. Pathogenicity was evaluated 20 days after inoculation.

RESULTS AND DISCUSSION

Species associated with FHB in barley. All symptoms found were related to the presence of *Fusarium spp.*; especially significant was the observation of partial discoloration with dark margins, which indicates the presence of *F. poae*. *Fusarium graminearum* typically causes dark brown coloration in the grain, but this symptom is also strongly associated with *Epicoccum spp.*, *Alternaria spp.*, and *Bipolaris sorokiniana.* Identification of *Fusarium* species was confirmed by RFLPs (unpublished). One outstanding finding is the ubiquitous presence of *Bipolaris sorokiniana*, detected in 100% of the samples in 2002, and of other fungal species, mainly saprophytes or weak parasites such as *Alternaria spp.* and *Epicoccum spp.* (Table 1).

The present study showed that *F. avenaceum* and *F. graminearum* were the main causal pathogens of FHB, given that both were isolated in 96.7% of the sites and showed high frequency levels (Table 1). It is important to note the presence of *F. sambucinum* (although we did not test its pathogenicity), which had not been reported previously (6).

Pathogenicity of individual species. The range of symptoms described in the literature was found in the field, on inoculated heads, and in kernels adjacent to the point of infection. Symptoms at the inoculation points were clearly differentiated, given that moisture and temperature conditions at the test site (Atizapán, State of Mexico) favored disease development. Salmon pink colored mycelial growth on the kernel surface appeared on most of the inoculated heads, in addition to symptoms typical of *F. poae* (14). Re-isolations from the inoculated kernels agree with descriptions of the inoculated species, which confirms their pathogenicity in the field.

Geographic distribution and disease severity. FHB was present throughout the sampled zone, as evidenced by the fact that pathogenic species of *Fusarium* were isolated from 100% of samples showing symptoms. Final disease severity average for both samplings was 6.21%. Disease severity was similar in both years of sampling. Frequency distribution of FHB was higher with *F. avenaceum*, *F. graminearum*, and *F. tricinctum*. Disease severity in general was low; however, the disease causes major economic losses as a result of yield reductions and poor industrial quality due to toxin-contaminated grain (14).

Table 1. Species associated with symptoms of Fusarium head blight (FHB) in barley in the Central Highlands of Mexico, 2001-2002.

Species	Pathogenicity 2001	Sites where FHB was present (%)	Percent frequency	
			2002	2001
Fusarium avenaceum	P	96.7	25.5	30.0
F. graminearum	P	96.7	23.5	20.0
F. tricinctum	P	26.7	6.5	11.0
F. subglutinans	P	43.3	9.0	10.0
F. poae	P	10.0	2.0	5.0
Microdochium nivale	P	13.3	4.5	8.0
F. lateritium	P	13.3	1.0	9.0
F. heterosporum	P	13.3	0.0	5.0
F. equiseti	Nt	33.3	5.0	0.0
F. culmorum	Nt	23.3	7.0	0.0
M. dimerum	Nt	36.7	12.0	0.0
F. sambucinum	Nt	16.7	4.0	0.0
F. merismoides	Np	10.0	0.0	1.0
F. stilboides	Np	10.0	0.0	1.0

Species	Sites where fungi were present (%)
Fusarium spp. and Microdochium spp.	100.0
Bipolaris sorokiniana	100.0
Alternaria spp.	96.6
Epicoccum nigrum	96.6
Trichothecium roseum	66.7
Gonatobotrys spp.	56.6
Penicillium spp.	30.0
Phoma spp.	20.0
Cladosporium spp.	16.6
Cephalosporium acremonium	6.7
Aspergillus flavus	6.7
Acremoniella spp.	3.3

P = Pathogenic, Nt = Not tested; Np = Non pathogenic

CONCLUSIONS

- FHB incidence in commercial barley fields in the Central Highlands is high, as *Fusarium spp.* were detected in 100% of sampling sites in the region.

- Among species isolated in 2001 sample, pathogenicity was confirmed for *F. avenaceum, F. graminearum, F. tricinctum, F. subglutinans, F. poae, M. nivale, F. lateritium,* and *F. heterosporum*.

- Frequency distribution of FHB was higher with *F. avenaceum, F. graminearum*, and *F. tricinctum*. The average disease severity for two years of sampling was 5.7%, within a 4.4 to 8.4% range.

Failure to take measures to control and manage the disease could result in economic losses due to grain yield reductions and the presence of toxins in the grain. This might also have consequences for barley producers and the brewing industry in Mexico.

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TOXINS AND DAMAGE INDUCED BY SEVEN SPECIES OF FUSARIUM HEAD BLIGHT ON BARLEY IN THE CENTRAL HIGHLANDS OF MEXICO

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OBJECTIVES

The present study was carried out to: 1) Evaluate the expression in severity of Fusarium head blight (FHB) caused by *Fusarium avenaceum, F. graminearum, F. tricinctum, F. subglutinans, F. poae, Microdochium nivale, F. lateritium* and *F. heterosporum* 2) Estimate its aggressiveness through its effects on yield components and 3) Determine the production of trichothecenes of each isolate for the different species in the environment of Atizapán, State of Mexico.

INTRODUCTION

Since 1998 FHB has increased, affecting barley (*Hordeum vulgare* L.) in the Highlands of Mexico, where the disease causes losses in both yield and grain quality (8). Among the species causing FHB, *F. avenaceum, F. graminearum* and *F. culmorum* have been reported to be the main toxigenic species, although other less aggressive or opportunistic species are also reported as toxins producers (3). There are diverse reports that indicate the difference in aggressiveness among species as well as among isolates of a single species (10,11).

The mycotoxins most frequently associated with fusariosis caused by *F. graminearum* are deoxynivalenol (DON) and nivalenol (NIV) (3,15) and *F. avenaceum* is reported to be a producer of DON in liquid cultures (1). The capacity of DON production has been related to the aggressiveness of *Fusarium*, which is strongly related to the atmospheric conditions (10) and with the system of cultivation (15).

MATERIALS AND METHODS

Establishment of field test. The evaluation of damages induced by the isolates of the different species of *Fusarium* were carried in the experimental field of Atizapán, State of Mexico (CIMMYT, Int.) (19.10° N, 99.51° W, 2640 masl) during the 2002 growing season. The treatments were established under a random design, with two replications per isolate. The experimental unit consisted of two 1.5 m long rows, which was considered a replication. Planting was done manually using seed of the Esmeralda variety.

Isolates used and method of inoculation. An evaluation was made of 11 monoconidial isolates of the species obtained from the samplings carried out in the Highlands of Mexico during the 2001growing season in barley of the Esmeralda variety. The pathogenicity of the isolates of this species was previously deter-

mined (14). The increase of the inoculum was carried out in liquid mung bean medium (*Vigna radiata*) (2,5). Twenty heads per experimental unit were labeled at the same flowering stage (7,9), and then inoculated by aspersion of conidial solution (50×10^3 per ml) with a manual 1-liter bottle atomizer (7). In order to avoid the spread of conidia, a screen was placed between plots at the moment of inoculation. The application of artificial irrigation was unnecessary, as daily precipitation favored the development of the disease.

Evaluations. Severity evaluation of the 20 labeled and inoculated heads was carried out 7, 14, 13 and 28 days after inoculation (dai), and consisted in counting the infected grains in relation to the total number of grains in each head, thus obtaining a percentage of severity in each reading. Hectolitric weight and thousand kernel weight (TKW) was measured after harvesting.

Confirmation of species. Ten inoculated kernels at each replication were taken to a laboratory to obtain monoconidial isolates and confirmation of the inoculated species (5, 12).

Quantification of toxins. Kernels harvested from each replication were used for the quantification of trichothecenes (DON and NIV). Kernels were ground (commercial Braun mill) and processed with the Romer Labs. Inc. technique (DonFluoroQuantTM method #FQD1NC, version 95.9).

Data analysis. Data obtained of severity, hectolitric weight and TKW was analyzed using the SAS statistical program (version 8.0, SAS Institute Inc., Cary, NC, USA). The PROC GLM procedure was used for the variance analysis, and means were subjected to comparison by Least Significant Difference (LSD) with a= 0.05.

RESULTS AND DISCUSSION

The experiment showed that isolates 1 and 10 (*F. avenaceum* and *F. graminearum*) were the most aggressive. These species came from the same region (Apan, Hidalgo), considered the main zone of barley reception and commercialization, which leads to the supposition that a greater genetic recombination exists in this zone. Same results were found in previous studies (11,18). The sexual and/or asexual recombination and the alternation between the saprophytic and parasitic phases could play an important role in the variations of the populations (11,18), whereas virulence and aggressiveness are influenced by the atmospheric conditions and the cultivation systems, among other factors (10).

Evaluated isolates of *F. avenaceum* from Apan, Hgo. and Calpulalpan, Tlax., showed different values of final severity, although they did not present significant differences, which indicates a high risk (17), taking into consideration that this species is the principal cause of the fusariosis in barley in the Highlands of Mexico (14).

Greater damage was observed from isolate 1 (*F. avenaceum*) than from isolates 10 and 11 (*F. graminearum*), contrary to what was expected. However, the results should be considered with discretion, given that *F. graminearum* is more aggressive in humid areas (16). Strong interactions between the pathogen, the variety and the atmospheric conditions in the expression of aggressiveness were also observed (10).

Table 1. Evaluation of severity for type II resistance in barley heads of the Esmeralda variety inoculated with cotton containing isolates of pathogenic species of *Fusarium* spp. in Atizapán, State of Mexico, 2002.

Isolated	Origen	Severity percentage		
		7 dai**	14dai	21dai
1. *F. Avenaceum*	*Apan, Hgo.*	3.68 a*	5.31 a	9.60 a
2. *F. Avenaceum*	*Calpulalpan, Tlax.*	2.87 a	3.75 a	6.15 a
3. *F. lateritium*	*Zapata,Hgo.*	2.87 a	4.26 a	7.45 a
4. *F. subglutinans*	*Benito Juárez, Tlax.*	2.50 a	4.08 a	6.80 a
5. *F. subglutinans*	*Apan, Hgo.*	3.12 a	3.93 a	6.60 a
6. *F. trincinctum*	*Almoloya, Hgo.*	3.57 a	4.84 a	6.90 a
7. *F. trincinctum*	*Calpulalpan, Tlax.*	2.97 a	4.22 a	7.50 a
8. *F. heterosporum*	*Zapata, Hgo.*	2.85 a	4.43 a	8.05 a
9. *F. Poae*	*Zaragoza, Tlax.*	3.13 a	3.84 a	7.30 a
10. *F. graminearum*	*Apan, Hgo.*	3.06 a	5.93 a	8.50 a
11. *F. graminearum*	*CIMMYT*	2.18 a	3.25 a	5.75 a
Coeficiente de variación		21.98	23.62	16.53

*means with the same letter are not significantly different (DMS, α= 0.05);
** dai, days after inoculation

Table 2. Evaluation of the effect on final severity, hectolitric weight, thousand kernel weight and production of trichothecenes in barley heads of 'Esmeralda' artificially inoculated with isolates of different species of *Fusarium* sp. in Atizapán, State of Mexico. 2002.

Isolate	Origen	Final severity (%)	Hectolitric weight (g/l)	Weight of 1000 grains (g)	Toxins (ppm)**
1. F. avenaceum	*Apan, Hgo.*	9.60 a*	548.25 a	34.49 a	0.00
2. F. avenaceum	*Calpulalpan, Tlax.*	6.15 a	555.07 a	35.51 a	0.00-0.06
3. F. lateritium	*Zapata, Hgo.*	7.45 a	582.83 a	34.79 a	0.00-0.02
4. F. subglutinans	*Benito Juárez, Tlax.*	6.80 a	535.56 a	35.21 a	0.00-0.05
5. F. subglutinans	*Apan, Hgo.*	6.60 a	531.02 a	33.30 a	0.08-0.20
6. F. trincinctum	*Almoloya, Hgo.*	6.90 a	587.68 a	31.64 a	0.00
7. F. trincinctum	*Calpulalpan, Tlax.*	7.50 a	575.28 a	33.18 a	0.00
8. F. heterosporum	*Zapata, Hgo.*	8.05 a	570.12 a	35.70 a	0.03-0.22
9. F. Poae	*Zaragoza, Tlax.*	7.30 a	597.98 a	35.81 a	0.00-0.11
10. F. Graminearum	*Apan, Hgo.*	8.50 a	568.96 a	34.93 a	0.92-2.70
11. F. Graminearum	*CIMMYT*	5.75 a	557.32 a	33.63 a	0.21-0.42
Coeficiente de variación		16.53	4.71	67.56	

*means with the same letter are not significantly different (DMS, α= 0.05)
** deoxynivalenol + nivalenol

F. poae presents high TKW, giving this isolate the highest value in hectolitric weight, for which combined with the data of final severity, it can be concluded that *F. poae* presented the least effects on 'Esmeralda' under the conditions in which the test was developed. On the other hand, *F. subglutinans* presented the greatest effects on hectolitric weight, but not on TKW, where although no statistical differences were shown, *F. tricinctum* presented the greatest reduction in yield. Furthermore, it is important to observe that the isolates of *F. avenaceum* and those of *F. graminearum*, although presenting the greatest final severity, did not reduce yield in relation to the rest of the isolates evaluated.

The production of trichothecenes (DON and NIV), it can be observed that *F. avenaceum* resulted positive to the detection of these compounds, results that have been observed in others studies (1,6). To this respect, studies carried out detected DON in heads inoculated with *F. avenaceum* (15), however, these studies point out that the levels were low, which could be attributed to the fact that some colonies of *F. graminearum* were found in these heads, some contamination could have occurred in the present test, however the results should be considered for confirmation in future investigations.

In the heads inoculated with *F. poae*, the presence of trichothecenes was detected, which coincides with diverse reports (3,4,15), whereas the heads inoculated with *F. lateritium* present low levels of these compounds in the present study, despite the fact that there are not many reports of the production of trichothecenes in this species (4,6). Both isolates of *F. subglutinans* resulted positive to the detection of trichothecenes, although in low levels, thus it is assumed that what was indicated by the case of *F. avenaceum*. Therefore, these results, as well as those obtained with *F. avenaceum*, should be considered as a possibility to be confirmed in future studies.

The results of the present investigation indicate that trichothecenes were not detected in the heads inoculated with the isolates of *F. tricinctum*, although in the case of *F. heterosporum*, low levels of these compounds were present. It is widely known that *F. graminearum* is the principal species that produces trichothecenes (3,4,15), and in the present study the isolates of *F. graminearum* presented the highest levels of trichothecenes of the isolates evaluated.

The literature published on *F. graminearum* suggests that toxins may serve as factors of aggressiveness and virulence of the pathogen (5). It is important to keep this consideration in mind, as the results show that isolates of *F. graminearum*-Apan presented greater severity and a higher concentration of trichothecenes than isolate *F. graminearum*-CIMMYT. However, no differences were observed in the effect on yield, which suggests that the aggressiveness is related to the production of toxins, a situation that has been widely documented (10,13).

The results obtained in the present investigation show that the isolates of the species which cause fusariosis in barley in the Highlands of Mexico are producers of trichothecenes (DON and NIV), demonstrating in a general way that there are no direct relationships between DON concentration with the effect on yield and the aggressiveness. However, it is demonstrated that diverse species produce trichothecenes and cause damage in the yield of the Esmeralda variety, which represents more than 70% of the surface sown with barley in the Highlands of Mexico.

The above, added to the general ignorance of the toxigenic capacity of the Fusarium species in the Esmeralda variety, and under the conditions of the Highlands, needs the programming and execution of research projects that contemplate aspects of ecology, biology, damages and production of toxins of the different species which cause fusariosis in barley.

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INCIDENCE OF *FUSARIUM GRAMINEARUM* IN KERNELS OF WHEAT AND BARLEY CULTIVARS AT FOUR LOCATIONS IN MINNESOTA

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ABSTRACT

Harvested grain of the wheat and barley entries in the 2003 Red River Valley On-Farm Yield Trials grown at Fergus Falls, Humboldt, Oklee and Strathcona MN, were assayed for colonization by Fusarium head blight pathogens. Each trial site consisted of 7.6 m x 1.98 m plots of each of the sixteen wheat and eight barley entries arranged in a randomized complete block design with two replications. Plots were subject to natural infection by Fusarium head blight. Kernels (100 per plot), arbitrarily selected from the harvested grain, were surface sterilized, plated onto Komada's medium (selective for *Fusarium spp.*) and incubated at 20-24ºC under fluorescent lights (12:12, light:dark) for ca. 12 days. *Fusarium* species isolated were identified using standard taxonomic procedures. Regardless of trial location and cultivars, *Fusarium graminearum* was the *Fusarium* species most frequently isolated from kernels (wheat, 9.2%; barley, 11.3%), followed by *F. avenaceum* (wheat, 2%; barley, 4.3%), *F. sporotrichioides* (wheat, 0.8%; barley, 2.4%), and *F. poae* (wheat, 0.7%; barley, 0.9%). In wheat, the highest incidence of *F. graminearum* colonized kernels was found at Strathcona (11.7%) and Humboldt (11.6%). In barley the highest incidence of *F. graminearum* colonized kernels was found at Oklee (14.4%) and Fergus Falls (14.3%). Ranking of wheat cultivars for kernel colonization by *F. graminearum* was significantly (P = 0.01) affected by the interaction of cultivar by location. Overall, the wheat cultivars Alsen (6.3%) and Hanna (3.4%) had lower levels of *F. graminearum* infection than Reeder (12.8%), Oxen (12.5%), Mercury (12.3%), Norpro (10.9%), Parshall (10.7%), MN97803A (9.8%) and Walworth (9.6%) which were significantly more highly colonized ($LSD_{(P = 0.05)}$ = 3.2). Ranking of barley cultivars was not affected by the interaction of cultivar by location (P = 0.11). The six-rowed barley lines MN110 (17.2%) and Drummond (13.2%) had the highest levels of kernel colonization by *F. graminearum*. Robust (9.6%) and the two-rowed barley Conlon (5.8%) had the lowest incidence of *F. graminearum* of the eight barley cultivars examined ($LSD_{(P = 0.05)}$ = 4.1). These data suggest that the kernels of wheat and barley cultivars are colonized differentially by *Fusarium* species depending on their resistance. Other factors such as the environment during the window of infection may also affect kernel colonization.

PREVIOUS CROP AFFECTING SOIL POPULATIONS OF FUSARIUM HEAD BLIGHT PATHOGENS IN MINNESOTA

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OBJECTIVES

To examine the effect of previous crop on soil populations of *F. graminearum* and other cereal pathogenic Fusaria.

INTRODUCTION

Fusarium head blight of wheat and barley, caused primarily by *Fusarium graminearum*, has become a major problem in the Upper Midwest of the United States. In an effort to control FHB, studies have been undertaken to understand the epidemiology of FHB and implement control practices including host resistance, chemical, biological and cultural control practices (Dill-Macky and Jones, 2000; Sutton, 1982). Although FHB epidemics are known to be associated with the inoculum present in host residues (Dill-Macky and Jones, 2000; Sutton, 1982), it is possible that *F. graminearum* in soil is also a source of inoculum. Knowledge of factors affecting soil populations of *F. graminearum* may help in the management of this devastating disease, however an understanding of *F. graminearum* populations in soils is lacking. The objective of this study was to examine soil populations of *F. graminearum*, and other cereal pathogenic Fusaria, in wheat fields as affected by the preceding crop.

MATERIALS AND METHODS

Fifty wheat fields in five counties (Becker, Clay, Mahnomen, Norman and Polk) in Minnesota, and one field in North Dakota (Trail) were surveyed for soil populations of *F. graminearum* and other Fusaria pathogenic to wheat. Information on the preceding crop for each field was obtained from county agents or property owners. In each field, five soil samples (26 g) were collected from the surface (0-2 cm depth) one meter apart along each of two five-meter transects, located at least 30 m from the edge of the field. Soil samples from each transect were air dried for four days at 20-24ºC, and sieved through a battery of sieves (250, 500,1000 and 2000 microns). Based on preliminary experiments, six milligrams of the finest soil particles (<250 microns) from each sample were dispersed onto each of five Petri plates containing Komada's medium (selective for Fusarium species) (Fig. 1A). Plates were incubated at 20-24ºC under cool white and UVA (1:1) fluorescent lights (12 hr photoperiod) for 14 days. *Fusarium* colonies were identified according to Burgess *et al.* (1994). Statistical analysis of soil populations of Fusaria as affected by the previous crop was analyzed using SAS PROC GLM.

RESULTS

Recovery of *F. graminearum* was significantly (P = 0.01) higher from soil particles <250 micron than from soil larger particles (251-499 microns). *F. graminearum* was present in all soil samples tested and was the most frequently isolated *Fusarium* species (78%) among the FHB pathogens recovered (Fig. 1). Other FHB pathogens were isolated at lower frequencies. Populations of *F. graminearum* in soil samples ranged

from 18 CFU/g to 1435 CFU/g dry soil. Population ranges for each species, as affected by rotational crop in 2001, is presented in Table 1. Populations of *F. graminearum* in soil after 2002 wheat crops were significantly (P = 0.01) affected by the immediate previous crop. Although we sampled only one field where wheat followed corn, the highest *F. graminearum* population (1159 CFU/g) (Table 2) was found in this particular field. Populations of *F. graminearum* in the soils of wheat crops following soybeans (356 CFU/g) and sugarbeets (341 CFU/g) were significantly less than in wheat after wheat (642 CFU/g) and wheat after dry beans (497 CFU/g) (Table 2). Populations of *F. culmorum*, *F. sporotrichioides* and *F. avenaceum* were also high in the soil sampled from wheat planted after corn (Table 2).

DISCUSSION

Our data shows that *F. graminearum* can readily be isolated from fine soil particles (<250 microns). It also indicates that soil may be a more important source of inoculum in FHB epidemics than previously thought. Recent epidemics of FHB in wheat and barley crops may have enabled *F. graminearum* populations to build up in the soils. In previous studies, *F. graminearum* was isolated from only 30% of soil samples from corn fields in non-epidemic years (Windels and Kommedahl, 1984) or soil populations found were low (17–24 CFU/g soil) (Salas, 1991). While we only sampled the soil of one field where wheat followed corn, it is not surprising that *F. graminearum* population was high in this particular field as other workers have reported similar results in relation to *F. graminearum* in association with residues of wheat crops following corn (Dill-Macky and Jones, 2000; Sutton, 1982; Windels and Kommedahl, 1984). In contrast, soil populations of *F. graminearum* in wheat after dry beans or soybeans were lower than in wheat after corn or wheat. A lower incidence of FHB has been reported in wheat following soybeans (Dill-Macky and Jones, 2000). Although the relationship between FHB and soil populations of *F. graminearum* has not previously been reported, it appears that monitoring soil populations of *F. graminearum* may be helpful in identifying the most effective crop rotation to help manage FHB epidemics.

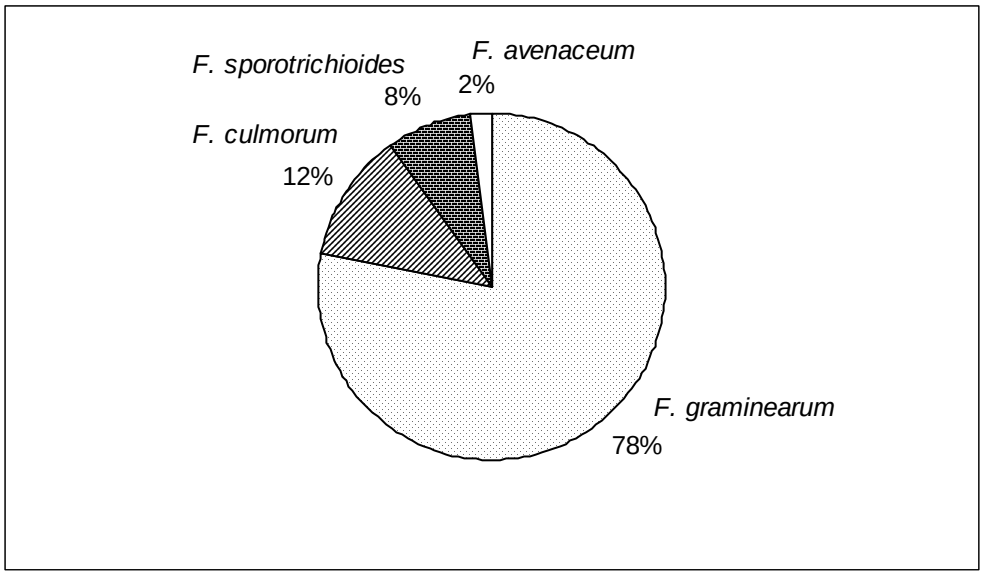

Figure 1. Frequency of recovery of *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, and *F. avenaceum* among pathogenic Fusaria recovered from fifty field soils in Minnesota and one field in North Dakota in 2002.

Table 1. Range of soil populations of *F. graminearum* and other cereal pathogenic Fusaria in soil sampled following the 2002 wheat crop as affected by previous crop.

Previous crop (2001)	Fields sampled (No.)	Colony forming units (CFU)/g dry soil)			
		Fusarium graminearum	*Fusarium sporotrichioides*	*Fusarium culmorum*	*Fusarium avenaceum*
Corn	1	1159	91	168	21
Wheat	5	474 - 1074	38 - 71	63 - 138	10 – 22
Dry bean	3	421 - 628	37 - 84	62 - 103	7 – 20
Soybean	32	49 - 943	6 - 139	7 - 188	1 – 35
Sugarbeet	10	96 - 639	9 - 57	17 - 98	1 – 21

Table 2. Effect of previous crop on soil populations of *F. graminearum*, *F. sporotrichioides*, *F. culmorum*, and *F. avenaceum*. Values given are the mean of the number of field samples examined for each previous crop treatment.

Previous crop (2001)	Fields sampled (No.)	Colony forming units (CFU)/g dry soil)			
		Fusarium graminearum	*Fusarium sporotrichioides*	*Fusarium culmorum*	*Fusarium avenaceum*
Corn	1	1159	91	168	21
Wheat	5	642	52	89	14
Dry bean	3	497	54	79	13
Soybean	32	356	37	56	10
Sugarbeet	10	341	37	55	9
LSD $_{P = 0.05}$		237.3	24.8	33.5	8.2

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PREDICTING DEOXYNIVALENOL IN WHEAT FOR ONTARIO

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ABSTRACT

Eight years of data were used to develop DONcast– a model to predict deoxynivalenol (DON) in mature wheat grain for fungicide-spray decisions at heading. A website was launched in 2000 for providing DON predictions to agri-business through the Ontario Weather Network (OWN) (http://www.ownweb.ca). The model was adapted for Uruguay, where severe *Fusarium* epidemics have resulted in DON concentrations of up to 5 ppm in baked goods. For predictions in 2004, DONcast will have evolved using an array of weather and agronomic data from over 630 private farms across Ontario and Uruguay. In addition to daily rainfall and temperature data, DONcast for 2004 will include relative humidity (RH) >80% at 11:00 between 3 to 10 d after heading for more accurate decisions of whether or not to apply a fungicide at heading. For the first time, the model will also be extended to include rain and RH between 20 and 36 d after heading (near harvest). Using actual weather and agronomic variables specific to individual farm fields, the overall model explains 75% of the variation of DON using data from 600 farm fields from 1996 to 2003. DON concentrations of less than 1.0 ppm were predicted correctly on 88% of the fields at heading. In other fields where DON concentrations exceeded 1.0 ppm, the model predicted correctly on 72% of the fields at heading.

AIRBORNE PROPAGULES OF *GIBBERELLA ZEAE*: TECHNIQUES FOR MONITORING SPORE RELEASE AND VIABILITY

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ABSTRACT

We report a series of techniques for monitoring spore release and viability of propagules of *Gibberella zeae*. A series of five identical wind tunnels were constructed to monitor the spore release of *G. zeae* under a variety of environmental conditions. The tunnels were operated at temperatures within a range of 10 to 30°C, and at varying degrees of air pressure. In preliminary experiments, ascospore discharge and distant movement in turbulent air currents occurred between 10 and 30°C, with peak discharge and movement at 25°C. To estimate propagule survival in air, viable propagules of *G. zeae* (ascospores and macroconidia) were applied to natural and artificial (plastic) wheat heads and placed in natural environments for varying durations of time. Spores were washed off treated heads, and quantified by plating out washes on selective medium and observing the number of resultant colony forming units. We documented significant ascospore and macroconidia viability on both natural and artificial wheat heads following up to three days of exposure to natural environments.

IDENTIFYING VIRULENCE FACTORS IN *FUSARIUM GRAMINEARUM* USING FORWARD AND REVERSE GENETIC APPROACHES

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ABSTRACT

Fusarium head blight or scab caused by *Fusarium graminearum* is a destructive disease on wheat and barley. Infested cereals are reduced in yield and contaminated with harmful mycotoxins. A molecular approach to the study of *F. graminearum* is critical because there are no effective fungicides and highly resistant plant varieties for controlling scab. Our goal is to determine molecular mechanisms of fungal pathogenesis in *F. graminearum*. In the past few years, we have generated over 9,000 REMI (restriction-enzyme mediated integration) transformants and identified 14 mutants defective in plant infection. One objective of this research is to generate randomly tagged mutant populations and characterize the genes disrupted in these REMI mutants that are reduced in virulence or are nonpathogenic. Genes that have been recovered to date include the HMG-CoA reductase and cystathionine beta-lyase genes. Further characterization of these genes will be discussed. We also have generated over 10,000 ESTs and 10x coverage of *F. graminearum* genome sequence in collaboration with the Whitehead Genome Research Institute at MIT. As a pilot test for large scale functional analyses in *F. graminearum*, over 15 candidate genes have been selected for targeted gene disruption or replacement. These genes are either homologous to known fungal virulence factors or predicted to be involved in various fungal developmental processes or secondary metabolism, such as polyketide synthases and signaling components. Phenotypes of mutants deleted of specific genes will be presented. Overall, both reverse and forward genetic approaches were found to be useful for identifying genes important for *F. graminearum* pathogenesis. The creation of a mutant population and functional analysis approaches developed in these studies will be useful resources for pursuing systematic characterization of *F. graminearum*-wheat interactions at the genome level.

REMI MUTAGENESIS IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium graminearum is an important pathogen of small grains and maize in many areas of the world. Infected grains are often contaminated with mycotoxins harmful to humans and animals. To better understand the molecular mechanism of plant infection and virulence of *F. graminearum*, we used the REMI (Restriction-Enzyme Mediated Integration) approach to generate random targeted mutants. Over 9,000 hygromycin-resistant transformants have been generated in the wild-type strain PH-1. Genes disrupted in two of these REMI mutants were recovered by plasmid rescue. In mutant 222, the transforming vector was integrated at the 268 amino acid of the hydroxymethylglutaryl CoA reductase gene (*HMR1*) that is essential for lipid biosynthesis. Disruption of *HMR1* significantly reduced the growth rate and aerial hyphal development in *F. graminearum*. Mutant 222 was non-pathogenic on flowering wheat heads and produced hyperbranching hyphae that are wider in diameter than that of the wild type strain PH-1. In mutant M8, the plasmid was integrated in the promoter region (110 bp upstream) of the cystathionine beta-lyase gene (*CBL1*). Mutant M8 had normal growth rate but produced rare aerial hyphae. Its virulence was significantly reduced. Gene replacement mutants deleted of *CBL1* had phenotypes identical to that of REMI mutant M8. Further characterization of the *HMR1* and *CBL1* genes will be presented.

RELATION BETWEEN HEAD BLIGHT AND GRAIN QUALITY IN THE INDIANA FHB EPIDEMIC OF 2003

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OBJECTIVES

To compare head blight severity, visible damage to grain, rate of infection of sound grain, and contamination by deoxynivalenol in wheat grown under a range of FHB epidemic intensities.

INTRODUCTION

Fusarium head blight (FHB) reduces yield of wheat. Various fungicide trials have demonstrated a negative correlation between FHB index and yield (Kawamoto et al. 2003, Lipps et al. 2003, McMullen et al. 2003). Of concern also is the quality of the grain, particularly the content of dexoynivalenol (DON). High levels of DON further reduce the value of the crop for the farmer, and pose problems for millers and bakers. In 2003, there were reports of high levels of DON in grain from southern Illinois, southern Indiana, and western Kentucky that did not appear to be badly damaged from scab, had good test weight, and came from fields with good yield. We observed that FHB ranged from severe in southern Indiana to almost absent in the north. Cultivar trials in several locations throughout the state allowed us to quantify head blight severity and various expressions of disease in the harvested grain. We used these data to examine relationships among these traits.

MATERIALS AND METHODS

Soft red winter wheat cultivar trials were conducted at research farms in different regions of Indiana. These included the Agronomy Center for Research and Education in west central Indiana (WC), Davis-Purdue Agricultural Center in east-central Indiana (EC), the Southeast Purdue Agricultural Center (SE), and the Southwest Purdue Agricultural Center (SW). Altogether there were 29 cultivars in the tests, but only 17 were common to all tests. At each location cultivars were sown in a randomized complete block design with 4 replications. Sowing dates were at the recommended time for each location and ranged from 27 Sep in the north to 15 Oct in the south.

To assess head blight, we counted the blighted heads in ten 1-ft lengths of row when symptoms were clearly visible and before natural ripening. We also recorded the total number of heads in several 1-ft lengths of row in order to express head blight incidence as a percentage. We rated severity of head blight as the percentage of spikelets blighted on those heads that showed any blight symptom. From this, the FHB index (product of incidence and severity, expressed as percent) could be calculated.

We counted the *Fusarium*-damaged kernels (FDK) in a sample of 100 kernels from each plot. We also plated, on Komada's medium, 25 kernels that showed no evidence of scab from each plot. The kernels were surface-sterilized in 5% bleach (0.2625% sodium hypochlorite) for 2 min and rinsed for 1 min in sterile water before being plated. The plates were incubated at 25°C and 12-h photoperiod for 5 d, after which

kernels contaminated with *Fusarium* were counted. This variable is identified as FCG (*Fusarium*-contaminated grain) and is expressed as a percentage. Finally, a 100-g sample of kernels from each plot was ground in a blender, and the coarse flour was sent to Dr. Pat Hart at Michigan State University for DON analysis.

RESULTS

Head blight was severe in the two southern Indiana sites (SW and SE), moderate at the WC site, present only on one very susceptible cultivar at the EC, and nonexistent at a site in northwest Indiana. Analysis of variance revealed that the effects of location, cultivar, and the location x cultivar interaction were highly significant for FHB index, FDK, FCG, and DON level.

To examine the correlation between head blight and variables associated with grain, we used the FHB index, because this is an overall estimate of head blight intensity that combines incidence and severity. For the three sites where head blight could be rated, there was a low correlation between FHB index and FDK or DON (Fig. 1). When the FHB index was above16%, there were no low values of FDK or DON, but for lower FHB indexes there was a wide range of FDK (Fig. 1A) and DON (Fig. 1B). For samples over the full range of FHB index values some DON levels were greater than 5 ppm. Correlation between FDK and DON was moderately high (Fig. 2A), as was the correlation between FDK and FGC (not shown, r = 0.75).

In every sample, we found infection of apparently sound kernels by *F. graminearum*. Levels of infection ranged from 5 to 69%. The correlation between FCG and FDK was moderately high (r = 0.76). The correlation between DON and FCG was 0.66, lower than that between DON and FDK (Fig 2A). We calculated a total percentage of *Fusarium* contamination (TFCG) by combining FDK and FCC (TFCG = [{100-FDK} × FCG/100] + FDK). The correlation between DON and TFCG was 0.72. Even though all of these correlations were significant, there was a considerable scatter of points, and the use of regression to predict kernel contamination or DON content from FHB index or FDK would not be reliable. Even for the relation between DON and FDK, which had the strongest correlation, there was a considerable range in DON levels over the range of 3% to 15% FDK. Only when FDK was 1% or less was DON below 2 ppm (Fig. 2A).

DISCUSSION

We took the opportunity provided by natural epidemics of various intensities to investigate the relation between head blight intensity in the field and grain quality. We found only modest correlations between FHB index and various grain traits associated with infection by *F. graminearum*. Above an FHB index of 16%, the frequency of FDK or level of DON in the harvested grain was consistently high. We do not know to what extent this threshold would vary from year to year. Unfortunately, many samples below this threshold also had high frequencies of FDK or high levels of DON (see particularly the boxed points in Fig. 1B). Thus, a low FHB index in the field does not necessarily mean a crop of high quality grain. This reflects what millers buying wheat from southern Illinois and Indiana, or western Kentucky, experienced this year.

It might be expected that the frequency of FDK in a grain sample would be a better predictor of DON than head blight symptoms in the field. Of all the correlations between various disease variables and DON, that between DON and FDK was the highest. DON levels below 2 ppm were confined to samples that had less

than 6% FDK. However, among samples of grain with less than 6% FDK, about half had DON levels of 2 ppm or greater.

The data we examined are all from natural epidemics of FHB. The data are from 17 cultivars, some of which have been bred for a degree of resistance to FHB. However, the outliers were not represented by any particular group of cultivars. For example, each of the boxed points in Fig 2B is a different cultivar. Our correlations between DON and field severity or damaged kernels are similar to those reported for field studies in which plants were inoculated and in misted or bagged to promote disease development (Bai et al. 2001, Liu et al. 1997, Mesterhazy et al. 1999).

Weather based risk assessment models have been developed (De Wolf et al. 2003) and are being refined. These models focus on head blight severity in the field. Results of this study indicate that additional effort should also be devoted to prediction of DON (Hooker et al. 2002). In the meantime, when current models used in the U.S. give even a modest chance of severe head blight, tests for DON should be performed on a sufficient sample of fields or grain lots to indicate whether a problem with grain quality exists.

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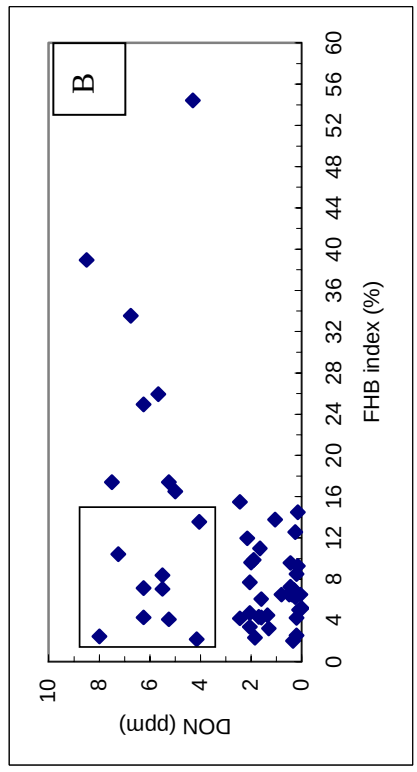

Fig. 1. Relation between frequency of *Fusarium*-damaged kernels (FDK) or DON and FHB index for 17 wheat cultivars grown at 4 locations in Indiana in 2003. For the graph of DON data, one outlier is not depicted because its DON level was much higher than any other entry (DON = 12.5 ppm, FHB index = 15.5%)

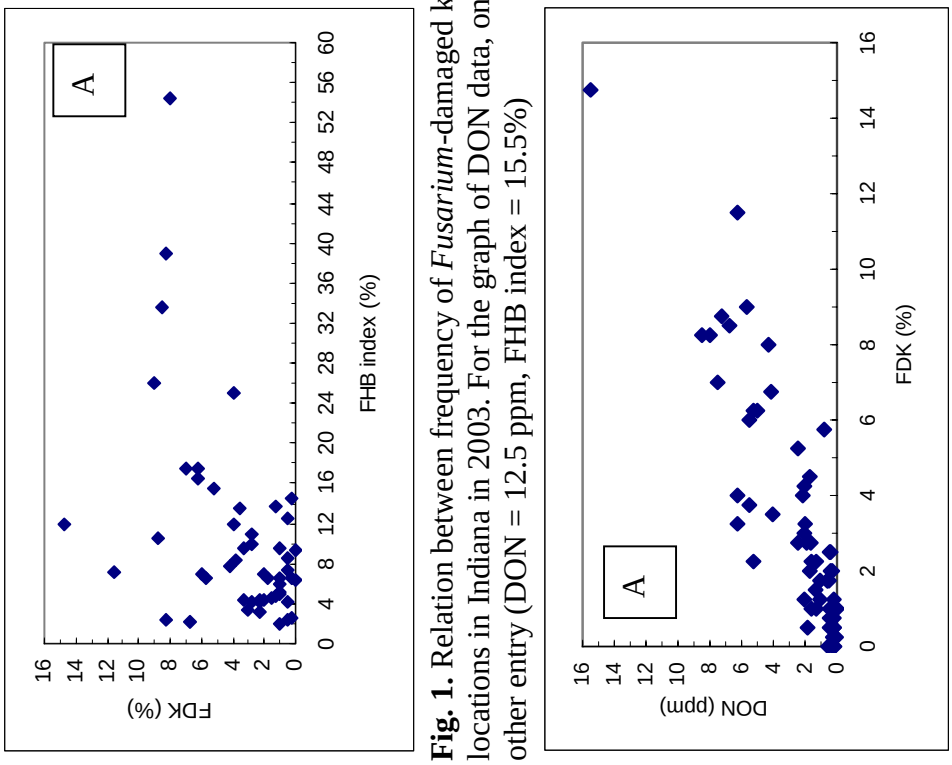

Fig. 2. Relation between DON and frequency of *Fusarium*-damaged kernels (FDK) or Fusarium-contaminated kernels (FCG) for 17 wheat cultivars grown at 4 locations in Indiana in 2003.

SEXUAL DEVELOPMENT AND FUNCTION IN *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) produces its sexual spores (ascospores) in sacs called asci. The asci are produced in ephemeral perithecia which are produced on the surface of field debris and which, when mature, fire their spores into the air. We have been investigating formation of perithecia and the mechanism of forcible ascospore discharge through a variety of molecular, histological and physiological techniques. We will present the results of these studies. Among the findings will be results of microarray analyses to identify genes expressed during perithecium maturation, evidence that accumulation of mannitol and potassium ions is important to generation of the turgor pressure for discharge of these spores, and identification of tissue specificity during colonization of wheat. The recent availability of a genomic sequence for *F. graminearum* has greatly facilitated the study of perithecium development and function, and host colonization. Some results facilitated by the availability of the genome sequence will be presented.

EFFECT OF HARVESTING TIME ON INCIDENCE OF SEEDBORNE *FUSARIUM SPP.* IN SPRING WHEAT IN EASTERN ONTARIO

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ABSTRACT

In the last two decades, there has been an increase in the incidence of fusarium head blight (FHB) in spring wheat in eastern Ontario. As a result, seed harvested from the region often contaminated by *Fusarium spp.* and mycotoxins, making wheat unacceptable for milling. This study was undertaken to examine the effect of five harvesting times on incidence of seedborne *Fusarium spp.* using three spring wheat cultivars grown at two locations in Ontario in 1999 and 2000. Twelve *Fusarium spp.* were isolated from 3,831 of the 24,000 seeds which were surface disinfected and plated onto modified potato dextrose agar. *Fusarium sporotrichioides, F. graminearum, F. poae, F. equiseti*, and *F. avenaceum*, were the most frequently detected species and were isolated from 6.8, 3.7, 2.8, 1.8, and 0.6% of the seeds, respectively. The remaining species, *F. acuminatum, F. crookwellense, F. culmorum, F. oxysporum, F. sambucinum, F. solani*, and *F. tricinctum*, collectively infected only 0.3% of the seeds. The incidence of *F. graminearum, F. sporotrichioides,* and total *Fusarium spp.* increased about two fold, from 1.7, 3.9, and 9.5% in seed harvested very early to 5.5, 8.7, and 19.8%, respectively after delayed harvest. Also, *F. poae* had significantly lower incidence at very early and early harvest times compared to normal or later harvest dates. Incidence of the other *Fusarium spp.* was relatively low and not affected by harvesting time. Cultivar, location, and year variation in the incidence of *Fusarium spp.* were observed and likely related to the different levels of varietal resistance to these pathogens, inoculum present, and weather conditions before and during harvesting times.

PATHOGENICITY OF *FUSARIUM* SPECIES CAUSING HEAD BLIGHT ON BARLEY

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ABSTRACT

The pathogenicity of eight *Fusarium spp.* causing fusarium head blight (FHB) in barley was studied under controlled conditions. Six barley lines varying in resistance to FHB were artificially inoculated with six isolates each of *F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. equiseti, F. graminearum F. poae,* and *F. sporotrichioides* at the late-flowering stage. Symptoms of FHB were rated as disease severity on a 0-9 scale, 4, 7, 14, 21, and 28 days after inoculation, and as percentage of infected spikelets (IS) after 21 days. All species caused visible infections in the barley lines, but only *F. crookwellense, F. culmorum,* and *F. graminearum* resulted in severe disease development (>60% IS) and were considered highly pathogenic. *F. avenaceum* had IS of 48.3%, which was significantly lower than those of the three highly pathogenic species, being moderately pathogenic; and, the remaining species had <20% IS, being weakly pathogenic. There were significant differences ($P < 0.05$) in aggressiveness among isolates within species and in susceptibility among barley lines, suggesting that screening for resistance to FHB requires the use of aggressive isolates or a mixture of several isolates. This is also the first report showing that *F. crookwellense* is highly pathogenic and *F. avenaceum* is moderately pathogenic in barley.

POPULATION STRUCTURE OF *GIBBERELLA ZEAE* (*FUSARIUM GRAMINEARUM*) CAUSING FUSARIUM HEAD BLIGHT OF WHEAT IN MEXICO

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ABSTRACT

We have characterized 217 strains of *G. zeae* isolated from wheat in two locations in Mexico collected during growing seasons in 2000 and 2001. AFLP data from 215 isolates is consistent with *G. zeae* phylogenetic lineage 3. Of the remaining two isolates, one clusters closely with phylogenetic lineage 7, and the other does not cluster closely with representatives of the described phylogenetic lineages. We are assessing phylogenetic affiliations of representative lineage 3 isolates, and of the two other isolates relative to the described phylogenetic lineages by comparisons of DNA sequence data from the *benA*, *red*, and *tef* loci. Within these two populations, AFLP diversity is high (>100 AFLP genotypes among 215 lineage 3 isolates), and linkage disequilibrium is low, suggesting that sexual recombination has occurred. The allelic divergence between populations is also low ($G_{ST} < 0.045$), suggesting that there has been extensive genetic exchange between populations. From these data we conclude that populations of *G. zeae* causing Fusarium Head Blight (FHB) of wheat in Mexico differ from those in the United States, which include only isolates of phylogenetic lineage 7.