EFFECTS OF RAINFALL AND TEMPERATURE ON PRODUCTION OF PERITHECIA BY *GIBBERELLA ZEAE* IN FIELD DEBRIS IN MICHIGAN

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OBJECTIVES

As *Gibberella zeae* infects during crop flowering, knowledge of the timing of formation of perithecia in the field is important in designing novel control methods. To evaluate the timing of perithecium formation, we collected wheat and corn stubble from commercial fields year-round from 1997 to 2000. We analyzed the timing of perithecium formation relative to local temperature and rainfall.

INTRODUCTION

Gibberella zeae produces Fusarium Head Blight (FHB) on wheat, barley and corn. FHB is considered to be primarily a monocyclic disease due to the small window of susceptibility of the affected crops. *G. zeae* produces two types of spores: the sexual ascospores and the asexual conidia. Ascospores are known to forcibly discharge from perithecia forming on crop debris and become airborne. Ascospores serve as one of the main sources of inoculum of head blight; the role of the conidia in infection is unclear (Fernando *et al.*, 1997; Parry *et al.*, 1995; Sutton and Proctor, 1982). Airborne spores land on flowers, germinate and penetrate the glume stomata (Pritsch et al., 2000), and spread throughout the wheat head resulting in chlorosis of infected kernels (Parry *et al.*, 1995; Sutton, 1982). Favorable conditions for disease outbreaks coincide with extended periods of high relative humidity (RH) and warm temperatures (24 - 29°C). (McMullen *et al.*, 1997a).

Perithecium formation in *G. zeae* and other fungi favor surfaces that are exposed to direct light (El-Gholl *et al.*, 1979; Halama and Lacoste, 1992; Khonga and Sutton, 1988; Reis, 1990b). Near ultra-violet (UV) light and RH may be significant factors in triggering perithecium development in *G. zeae* (Paulitz, 1996). The perithecia of *G. zeae* are ephemeral. Under laboratory conditions, a perithecium forms and discharges its ascospores within two weeks of induction (a solution of Tween 80 is added to the surface of hyphal cultures to stimulate perithecium formation) (Klittich and Leslie, 1988; Trail and Common, 2000). Therefore, presence of perithecia closely coincides with the presence of ascospore inoculum.

MATERIALS AND METHODS

Field collection. Between June 1997 and March 2000, thirteen commercial fields that had experienced previous outbreaks of FHB were surveyed in Ingham County, Michigan. The traditional order of crop rotation is corn, soybean, and wheat, however rotation patterns often varied. Fields containing wheat or corn stubble from the previous year's harvest were sampled. A decreasing number of wheat fields were sampled in 1999 and 2000 due to wheat being dropped from the crop rotation on many farms.

Debris samples that had visible signs of fungal fruiting structures were collected monthly unless impeded by snow cover. During the period of wheat flowering, sampling was increased to two-week intervals. Eight locations were sampled along transects in each field in a diamond pattern (two locations on each side of the diamond). The length of transects were scaled to the acreage of each field. Debris at each location was examined for the presence of fungal fruiting bodies. Samples within a 25-foot radius of each location were examined and four pieces of debris (at least 3 inches long) exhibiting symptoms of fungal infestation were collected from each location. Samples were stored in plastic bags at –20° C prior to analysis. Daily weather data, from the nearest National Weather Service (NWS) station at the Lansing Capitol Airport, was obtained from the National Oceanic and Atmospheric Administration National Climatic Data Center (NOAA-NCDC) database (NOAA-NNDC, 2000). All fields were within a 60-mile radius of the NWS station.

Identification. Each corn or wheat stubble sample was microscopically examined at 70X magnification for the presence or absence of perithecia. All pieces of debris were examined, the presence of one or more perithecia positively identified as *G. zeae* was designated as a positive occurrence for that location and sample. Species identification was confirmed by perithecium wall structure, color and ascospore morphology at 400X as described by Nelson *et al*. (1983).

Data Analysis. Data was expressed as a proportion of samples with *G. zeae* perithecia per total samples collected. The proportion of samples with perithecia was evaluated against calendar days. Wheat stubble samples were not included in analyses due to the low number of perithecia in these samples.

The distribution of proportion data was normalized with the arcsine square root transformation. All analyses were performed using PROC REG of SAS version 7.0 (SAS Institute, Cary, N.C.). Single factor regression analysis was used to evaluate the effect of rainfall and temperature. A stepwise regression analysis for all variables was used to determine if combined variables increased correlation. In all cases the y-intercept of the regression was forced through 0. Variables used are defined in Table 1.

Table 1. Variables for regression analysis.

Designation	Time period
T4	Average daily temperature 1 – 7 days preceding sampling
T7	Average daily temperature 4 – 10 days preceding sampling
T14	Average daily temperature 11 – 17 days preceding sampling
R4	Average daily rainfall 1 – 7 days preceding sampling
R7	Average daily rainfall 4 – 10 days preceding
R14	Average daily rainfall 11 – 17 days preceding sampling

RESULTS AND DISCUSSION

Of the 2186 samples collected, 272 were found to have perithecia of *G. zeae* (Table 2). Over the three years sampled, the highest proportion of samples with perithecia were found during the summer months. There was a peak in the proportion of samples containing perithecia in May of 1997 and 1998, just prior to wheat flowering. In 1999, the peak occurred in July after heavy rainfall in late June and early July (approximately 7 in). Rainfall in May (near the time of wheat flowering) was minimal (approximately 1.7 in.) and may have delayed perithecium formation until conditions became more favorable in June and July. Although it is likely moisture also plays a role in perithecium formation (Paulitz, 1996), the rainfall data used in this study was collected 25 – 60 miles away from the field sites and may not adequately represent field conditions.

Approximately 83% of the total samples with perithecia were found on corn debris. Thus, corn stubble was the predominant substrate for perithecium formation of *G. zeae*. However, the disease outbreak of FHB in wheat fields was minimal in 1998 and 1999 due to hot, dry conditions during the spring (Hart, 1998; 1999). Weather conditions that decreased colonization of the wheat plants would have resulted in lower numbers of perithecia on wheat stubble.

Both T4 and T14 (Table 1) were highly correlated with the proportion of mature perithecia with each temperature variable explaining approximately 50% of the variance (Table 3). However, combining T4 and T14 into a single model did not improve the overall correlation due to the fact that the two appear to covary. Surprisingly, none of the relative humidity variables were significantly correlated with perithecial maturation. Thus, multiple regression involving temperature and relative humidity did not improve the regression coefficient beyond that explained by T14 alone. Temperatures below 9°C appeared to inhibit perithecium formation (Table 3). If data points of T14 below 9°C are removed from the analysis, the R^2

value of T14 increases to 0.73. These data closely parallel the two-week cycle of development and maturation of perithecia in the lab (Trail *et al.*, 1998). The data did not show a limiting high temperature for perithecium formation.

Table 2. Corn and wheat debris containing *G. zeae* perithecia.

Dates of collection	Average no. of fields	No. corn fields[1]	No. wheat fields	Total no. corn samples	Total no. wheat samples	No. corn samples w/ perithecia	No. wheat samples w/ perithecia
Spring 1997 – Fall 1997	10	8	2	320	80	31	1
Fall 1997-Fall 1998	11	7	4	368	312	40	24
Fall 1998-Fall 1999	9	7	2	684	200	161	6
Fall 1999-Winter 2000	8	5	2	160	62	9	0
TOTAL	**38**	**27**	**10**	**1532**	**654**	**241**	**31**

1. Corn and wheat fields are defined here as those fields containing corn or wheat debris. Depending on the time of year the debris was collected, these fields may have been planted in another crop. At each fall harvest, the fields were redefined according to the fresh debris.

In summary, our results indicate that perithecium formation is limited by average daily temperatures below 9° C, and that corn stubble may be the predominant substrate for perithecium formation in *G. zeae* in Michigan. Perithecium formation may also be linked to day length and solarization (El-Gholl *et al.*, 1979; Halama and Lacoste, 1992). Further studies that monitor on-site weather conditions and controls for factors such as plant variety, pesticide and fertilizer applications, and initial inoculum are needed to construct a predictive model for *G. zeae* perithecium development.

Table 3. Effect of temperature on development of perithecia

Condition at days prior to collection	F value	P > F	Adjusted R^2
T4	32.34	0.0001	0.5281
T14	30.53	0.0001	0.5133
T14 (9° C threshold)	50.87	0.0001	0.7348

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EFFECT OF FUSARIUM INFECTION DURING WHEAT SEED DEVELOPMENT ON THE PRODUCTION OF DON AND SEED QUALITY

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OBJECTIVES

1) Determine the time of infection of *Fusarium graminearum* during wheat seed development and its effect on the production of DON and seed quality.

2) Investigate effect of disease tolerance and susceptibility on severity of seed infection

INTRODUCTION

Head scab caused by Fusarium graminaerum (Schwabe) has caused significant losses in the soft red winter wheat crop in Kentucky and in small grain crops in many regions of North America. Damage from head scab results in reductions in seed quality, emergence, and yield of wheat. In addition, losses in food-grain quality are caused by production of fungal mycotoxins, specifically vomitoxin (deoxynivalenol = DON). Relatively little information is available regarding when peak infection occurs during seed development and maturation and how these infection levels relate to the production of DON and the eventual seed germination and vigor. Considering that the seed is the delivery system for improvements in germplasm and the source for regeneration of new cultivars, it provides a vital link between the FHB research initiative and the farmer. This study will determine the effect of fungal infection during wheat seed development and maturation on the production of DON and on seed germination and vigor. This could have direct application regarding the timing of harvest for both seed and grain producers to achieve maximum seed and grain quality, as well as providing preliminary information on genetic tolerance to seed infection during seed development.

MATERIALS AND METHODS

Field plot establishment and environment

Replicated plots of four soft red winter wheat cultivars, two susceptible (Roane, Pioneer 2552) and two tolerant (Coker 9474, Pioneer 25R18) were established following corn in a chisel plowed and disked seedbed on Spindletop Farm in Lexington, KY in October of production year1999-2000. This study was conducted as part of the uniform southern scab nursery. The corn seed inoculation procedure was modeled after the method of Paulitz (1996) and inoculum was distributed among field plots on April 24. An irrigation schedule initiated on April 28, 2000 continued throughout seed development to stimulate FHB epidemic conditions. Air and canopy temperature were recorded, as well as temperature in the

developing heads of two cultivars as described in Panozzo et al., (1999). Plots were mist-irrigated twice daily until May 26. At anthesis (Feekes 10.2), spikes in each replication of each cultivar with anthers extruded in mid-spikelet were identified. At ten days after anthesis (DAA) seventy-five previously marked spikes were harvested, with harvesting continuing at four-day intervals through harvest maturity (HM, ~14 % seed moisture, fwb).

Seed Development

Fresh weight, dry weight and seed moisture were determined at each harvest for all varieties. In addition, seeds were also assigned a numerical rating as an indicator of disease severity and classified as normal=3, slightly shriveled or discolored=2, and white tombstone=1.

Seed Assessment

Floral structures (glumes, lemma, palea, caryopsis) of Pioneer 2552 were evaluated for infection at each harvest date. Ten consecutive spikelets from each spike were numbered, and the basal glume and floret in each of the ten spikelets was removed for evaluation. The ten fresh, complete florets were separated into glume, lemma, palea, and caryopsis, surface sterilized, plated on Komada medium, and evaluated for *Fusarium spp*. infection approximately fourteen days later. The remaining three varieties were evaluated for seed infection only.

Seed quality

Seed from twenty-five spikes of each harvest was submitted to laboratory of L. P. Hart, Michigan State University for analysis of deoxynivalenol (DON) as described previously (Hart and Brazelton, 1983). Standard germination, accelerated aging germination, a stress vigor test, and the conductivity test for membrane integrity were conducted according to the Association of Official Seed Analysts (AOSA, 1999) guidelines.

RESULTS AND DISCUSSION

Physiological maturity (PM, maximum seed dry weight) occurred between 40-45% seed moisture (dwb) for all varieties. Roane and P25R18 reached PM 17 and 6 days respectively before the highest *Fusarium spp*. seed infection levels (Figure 1). Similar trends were shown for P2552 and Coker 9474, with PM at 51 and 30 DAA (data not shown). Peak infection occurred between 22-28% seed moisture in three of four varieties. The average infection by *Fusarium spp.* in floral parts from seven harvests of P2552 ranged from 25% on Jun. 8 to over 90% on Jun. 16 (Fig. 2). Seed infection followed similar trends, ranging from 20-68%, and was significantly lower than other floral structures at harvests four and five.

The cultivar, P2552 was most susceptible to infection (19-67%), but this susceptibility had little impact on measures of seed quality (Table 1). Germinability of seeds from all harvests and all varieties was generally high. Weak correlations between both laboratory quality tests (standard germination (SG) and accelerated aging vigor (AA)) and seed infection percentage were observed for all varieties. Accelerated aging germination was higher than SG,

which would indicate the fungus was killed during aging at 41°C. A moderate relationship was observed for Roane and P2552 when visually assigned seed infection was correlated with actual seed infection by *Fusarium spp*.(data not shown).

Individual head and whole plant canopy temperature data taken from Jun. 9 to Jun. 26 (Figure 3) show similar minimum and maximum temperatures during cooler, rainy conditions. However, in warmer and drier conditions, temperature differences in the head exceeded those in the canopy by 3-4°C. Temperature and irrigation provided a favorable environment for infection, but no significant disease pressure was observed in any variety until early to mid June (Figure4). Significantly higher levels of seed infection were observed in P2552 for the final 3 harvests, while Roane and P25R18, exhibited intermediate seed infection levels.

The retention of high seed quality in our study with significant seed infection late in the field season suggests many of the infections were late and mostly superficial, leaving a somewhat depleted seed, but a viable embryo. The absence of significant disease pressure at flowering and throughout early development can be attributed to late placement of inoculum, delaying ascospore maturation, and resulting in minimal infection at anthesis. This study will be repeated for the 2000-2001 production year using the same experimental procedures. Inoculum will be placed in field plots earlier to stimulate more severe disease conditions in early reproductive development.

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Figure 2. Infection of floral components over 7 harvests in Pioneer 2552.

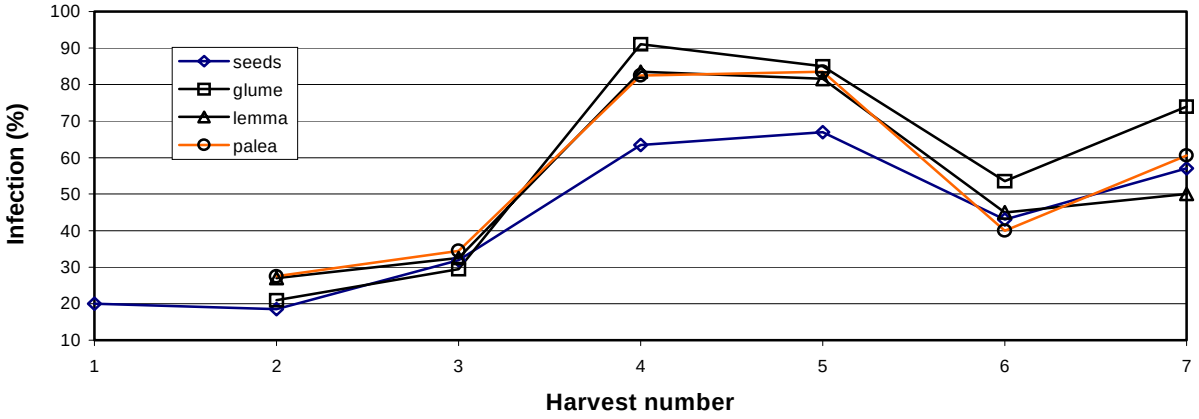
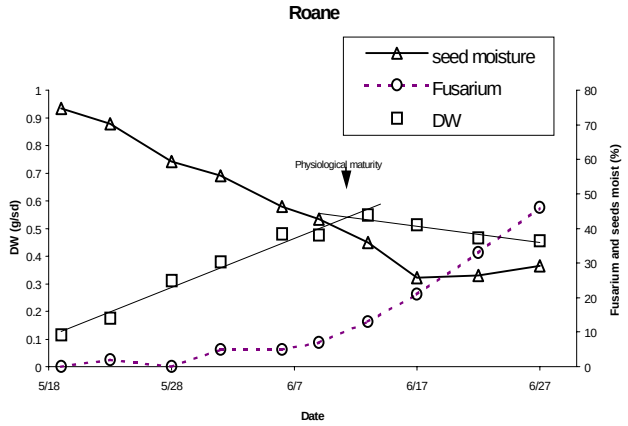


Table 1. Seed development and quality characteristics for Pioneer 2552

Date	Visual seed FHB rating score	Dry wt. (g/sd)	Seed moist. (%)	Fusarium (%) 100 sd	SG (%) 100 sd	AA (%)100 sd
8-Jun	2.6	0.641	47.5	20	91	91
12-Jun	2.7	0.79	43.1	19	91	94
16-Jun	2.3	0.779	37.9	32	88	97

Figure 1. Changes in dry weight, seed moisture and Fusarium spp. infection during seed development in two wheat varieties



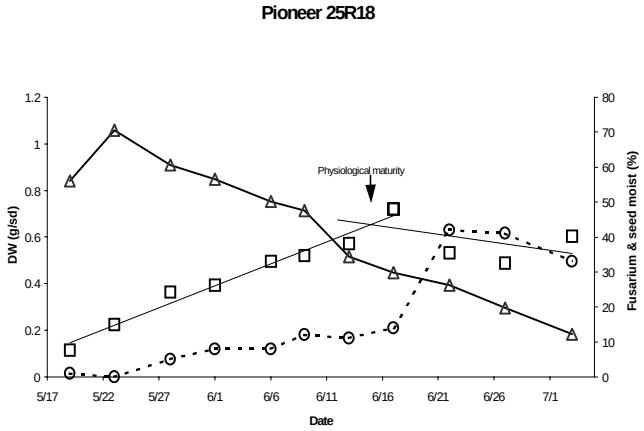


Figure 3.. Head and canopy temperature comparison in Coker 9474

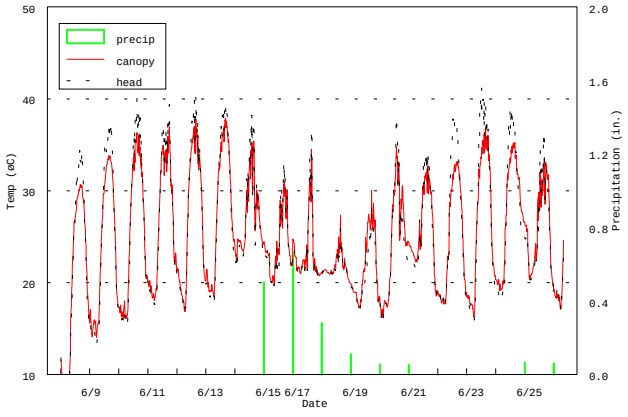
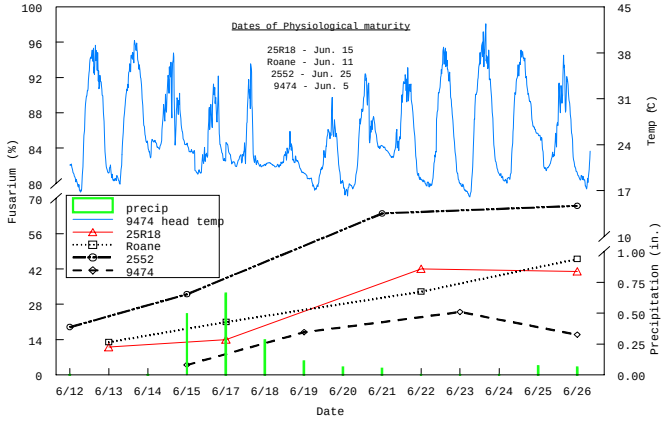


Figure 4. Relationship of temperature and precipitation to *Fusarium spp.* seed infection in four wheat varieties during seed maturation



ARE *GIBBERELLA ZEAE* SEXUAL SPORES THE CRITICAL INOCULUM FOR WHEAT HEAD BLIGHT?

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) causes scab (blight) in wheat and barley, and ear rot in corn. Since 1991, epidemics of *Gibberella* head blight have struck the Midwestern states with disastrous effects on wheat and barley growers. The fungus decreases yields and also contaminates grain with trichothecene mycotoxins that are harmful to human and animal health. To understand and control head scab, the factors and conditions that lead to epidemics must be identified. We propose that the sexual spores of *Gibberella zeae* play a important role in head blight epidemics. We will test this hypothesis by deleting critical genes required for sexual spore development (ascospores) and examine the resulting strains under field conditions for their effect on disease progression on wheat. If ascospores are the major inoculum source, than we predict that exposure of wheat to a MAT-null strain will result in significantly less disease than exposure to a wild-type strain.

DEVELOPMENT OF *FUSARIUM GRAMINEARUM* IN DETACHED SEGMENTS OF BARLEY LEAVES

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ABSTRACT

With the objective of understanding pathogenesis in tissues invaded by *Fusarium graminearum*, we are using a transformed strain of the fungus containing a constitutively-expressed gene for green fluorescent protein (GFP) in a model detached leaf system. Segments 2 cm long are cut from seedling leaves of Robust barley and placed on agar containing 60ppm benzimidazole. The segments are inoculated through a cut end with mycelium growing from 5x5mm squares of dialysis membrane. The fungus is viewed by epifluorescence microscopy in living tissues of intact segments or from sections cut from the segments either free hand with a razor blade or with a Vibratome. With these techniques, the fungus is readily visible on the leaf surface, beneath the cuticle, and beneath the epidermis in intercellular spaces of the mesophyll. In 12 separate experiments, we followed the development of the fungus for 4 days as it progressed along the segments from the inoculated cut end. By 3 days, surface hyphae advanced 2.4 mm from the cut end; intercellular hyphae, 1.6 mm; and intracellular hyphae, 0.6 mm. At 3 days, chlorosis extended 0.9 mm from the cut end. The intercellular hyphae extended well into living tissues, 1-2 days ahead of chlorosis and intracellular hyphae, confirming that the fungus initially establishes a biotrophic relationship with living leaf tissues. However, the GFP-labeled fungus became difficult to see in chlorotic tissues because these tissues became highly autofluorescent. We have not been able to visualize how the fungus enters host cells to become intracellular or determine whether the host cells are alive at the time of entry. To help see the fungus in chlorotic tissue, we have sectioned resin-embedded leaf segments and stained the sections with methylene blue/azure I, followed by basic fuchsin. The fungus stains lavender and is visible in either longitudinal or cross sections. Using both fresh and resin-embedded tissue, we will further investigate the transition from intercellular to intracellular growth. The techniques are also being used to investigate infection processes and pathogenesis in barley florets.

VARIATION IN *FUSARIUM GRAMINEARUM* ISOLATES FROM NEPAL ASSOCIATED WITH THEIR HOST OF ORIGIN

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ABSTRACT

A collection of group II *Fusarium graminearum* isolates obtained from maize, wheat, and rice from different locations in Nepal was identified using a combination of morphological and molecular criteria. The variation within this collection was analyzed using RAPD markers, IGS RFLP and PCR polymorphisms. The isolates divided into two groups, designated A and B, by RAPD analysis. Isolates in group A yielded four different PCR polymorphic markers but all of the isolates in group B yielded a single polymorphic marker. The IGS RFLP analysis was consistent with the division of the isolates into the two groups. Isolates from wheat and rice were more frequently placed in group A, with isolates from maize more evenly distributed between the two groups. Results indicate that host preference might be a factor in the division of the isolates, although the year of isolation may have had an influence as well. No geographical factors or agricultural practices could be identified that could account for the observed variation.

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PREDICTION OF FUSARIUM HEAD BLIGHT EPIDEMICS

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OBJECTIVES

Develop risk assessment models for wheat Fusarium head blight

INTRODUCTION

Nearly a century ago, the United States experienced a series of severe Fusarium head blight epidemics (Adams 1921, Atanasoff 1920, Pugh et al. 1933). Plant pathologists noted the severity of these epidemics and observed the apparent relationship between extended periods of wet weather and disease severity. For many years, Fusarium head blight was not reported to be a major problem in the U.S.; however, during the past decade many wheat production regions have experienced a series of severe epidemics that have resulted in tremendous losses to producers (McMullen et al. 1997). A disease forecasting system that could provide a reliable and timely prognosis of disease is highly desirable.

Prediction of Fusarium head blight epidemics has been attempted in Argentina where researchers used information collected over a number of years in small plots to identify weather variables correlated with disease levels and developed linear equations to predict the severity of disease (Moschini and Fortugno 1996). In North America, Francl et al. (1999) emphasized the importance of both inoculum and environment to epidemic development. De Wolf et al. (2000), used weather and disease information collected in Ohio from 1982 through 1999 to identify critical environmental periods and develop risk assessment models. We report here on the expansion of this modeling effort to include data from other wheat production regions, and discuss progress in the development of risk assessment models for wheat Fusarium head blight.

MATERIALS AND METHODS

Information was collected from records maintained at Ohio, North Dakota, Missouri, and Kansas. Weather variables included hourly temperature (EC), relative humidity (%) and precipitation (mm) observations, and researchers provided the corresponding mean disease levels from each location. The total data set consisted of 50 location-years (number of locations x the number years), and represented two wheat classes and three distinctly different environments in which wheat is grown in the U.S (Table 1).

Hourly weather data were used to create a data set of various representations of temperature, relative humidity and precipitation information from each year. Representations of weather included averages, minimums, maximums, and durations of favorable temperature, relative humidity and rainfall. Variables that combined temperature and RH into single variable were also evaluated. Each variable was summarized for two presumed critical time

periods. The first, 7 days prior to crop flowering, and the second, 10 days after initial flowering. All variables were rescaled between zero and one to facilitate the calculations of interaction terms later in the analysis. Disease intensity level or yield loss estimation (Kansas data only) was coded as a binary variable in which a location-year with >10% field severity or >15% yield loss was considered to be a major epidemic (assigned the value of 1). Location-years with less than this level of disease severity or yield loss criterion were concluded to be no or minor epidemics (assigned the value of 0).

Correlation analysis was used to identify representations of weather variables potentially associated with epidemic status. Variables with a Kendall correlation coefficient of <0.23 were dropped from the modeling process unless they were deemed to contain valuable information not already represented by other variables with greater correlation coefficients. This process reduced the number of potential independent variables from 49 to 25.

Variables identified in correlation analysis were then used to develop logistic regression models for classifying the epidemics in the location-years. Variable selection was done using a stepwise regression procedure. The stepwise procedure identified three variables useful for the prediction of epidemics. Two of the variables were summaries of the environment 7 days prior to crop anthesis, and included the duration (hours) of precipitation (DPPT7) and the duration of temperature within the temperature range of 15 to 30EC (T15307). The third variable summarized the environment 10 days after the initiation of anthesis. This variable was a combination of temperature and RH variables, and is defined as the duration of time that temperature was between 15 and 30EC and corresponding RH was >90% (TRH9010). The stepwise logistic procedure was repeated using these three variables and their interaction terms. Variables and interaction terms are defined in Table 2. In addition, logistic models with only single independent variables were evaluated to assess accuracy of the models with individual variables. All models were evaluated by cross-validation prediction accuracy (percent correctly classified observations). Errors of the models with the highest prediction accuracy were analyzed to aid in the evaluation of model performance. Sensitivity (% correctly classified epidemics) and specificity (% correctly classified non-epidemics) also were determined for all the models.

RESULTS AND DISCUSSION

Cross-validation prediction accuracy of the logistic models ranged from 62% to 84% (Table 3). Four different models all correctly classified 84% of the 50 location-years from Ohio, North Dakota, Missouri and Kansas. These models utilized differed independent variables and had different levels of sensitivity and specificity. One of these four models used only the temperature and humidity combination variable TRH9010. The other three models with 84% prediction accuracy utilized at least one interaction term (product of two or three independent variables). Each of the models incorrectly classified eight cases. The number of false positives (falsely predicting a major epidemic) and false negatives (falsely predicting minor or no epidemic) is specific to each model, but four cases were incorrectly classified by all four of the identified models. Two of these four errors were false negatives. These errors appear to be the result of favorable environmental conditions or limiting factors beyond the critical time periods used by the models. Errors were not limited to a single state or location.

Logistic models utilizing only single independent variables confirmed the importance of environment during crop anthesis as critical to the development of Fusarium head blight epidemics. Models that used only independent variables that summarized temperature (T15307) and moisture (DPPT7) prior to flowering were less accurate (Table 3). Moreover, models that utilized interaction terms between pre and post-flowering environment had prediction accuracies of less than or equal to that of the model that used TRH9010 variable alone. Analysis of model errors indicated that variables summarizing environment prior to anthesis may provide models with information about potentially limiting factors, specifically, conditions that were unfavorable for inoculum production.

In some years, the environment during time periods other than those addressed by the models may influence inoculum level and head colonization, thus further enhancing or diminishing disease development and yield losses. However, we have shown here that fairly narrow time periods around crop anthesis were very useful for predicting epidemics. The usefulness of these models for making real-time disease forecasts will depend on the availability and accuracy of weather forecasts.

The models with the highest level of accuracy as determined by this analysis will be further validated with data collected during the 2000 growing season. It is possible that one of these models or some other similar model will be used to provide wheat producers with reasonably accurate regional Fusarium head blight forecasts. During these finial stages of model validation it will be essential to develop the necessary infrastructure to operate the risk assessment model(s) and deliver reliable disease forecasts at a regional level.

Table 1. Summary of information used to develop prediction models for wheat Fusarium head blight.

State	Location	Years
Ohio	Wooster	16
Ohio	Hoytville	2
Ohio	S. Charelston	1
North Dakota	Fargo	7
North Dakota	Cando	4
North Dakota	Langdon	4
North Dakota	Carrington	2
North Dakota	Dazey	1
Missouri	Novlty	5
Missouri	Columbia	3
Missouri	Lamar	3
Kansas	Powhattan	2

Table 2. Definition of independent variables and interaction terms utilized by logistic models for forecasting wheat Fusarium head blight.

Variable	Definition
TRH9010	Duration $15 \leq T^a \leq 30$ & $RH^b \geq 90$ 10days after flowering (hours)
T15307	Duration $15 \leq T \leq 30$ & $RH \geq 90$ 7 days prior to flowering (hours)
DPPT7	Duration of rain 7 days prior to flowering (hours)
FHB1	(T15307*DPPT7)
FHB2	(DPPT7*TRH9010)
FHB3	(T15307*TRH9010)
FHB6	(T15307*DPPT7*TRH9010)

[a]Temperature (°C)

[b]Relative humidity (%)

Table 3. Cross-validation prediction accuracy for logistic models of wheat Fusarium head blight developed with information from Ohio, North Dakota, Missouri and Kansas.

No. of var. [a]	Variable(s)	Percent Correct [b]	Sensitivity [c]	Specificity [d]
1	TRH9010	84	83	84
1	FHB3	84	83	84
2	FHB1, FHB6	84	72	91
2	FHB6, DPPT7	84	67	94
7	TRH9010, T15307, DPPT7, FHB1, FHB2, FHB3, FHB6	82	78	84
2	FHB2, FHB3	82	72	88
2	FHB2, T15307	82	67	91
1	FHB6	80	72	84
3	TRH9010, T15307, DPPT7	78	72	81
1	FHB2	76	61	84
2	T15307, DPPT7	70	56	78
1	FHB1	64	44	75
1	T15307	64	33	81
1	DPPT7	62	11	91

[a]Number of variables used by the model

[b]Percentage of correctly classified epidemics and non-epidemic

[c]Percentage of correctly predicted epidemics

[d]Percentage of correctly predicted non-epidemics

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CROP RESIDUE MOISTURE AND *GIBBERELLA ZEAE* PERITHECIA DEVELOPMENT

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ABSTRACT

Gibberella zeae, which causes wheat Fusarium head blight, as well as corn stalk and ear rot, has been a devastating pathogen in many crop production regions of the U.S. where both wheat and corn are grown. The effects of temperature and moisture interactions on *G. zeae* perithecia development have not been evaluated nor has perithecia development been investigated on crop residues directly. Sensors used to continuously monitor crop residue moisture were adapted for use with corn residues. These sensors are being used to monitor moisture under controlled conditions. The quantity and rate of perithecia development are currently being evaluated at 15, 25 and 30EC and three moisture levels. Sensors are also being used to monitor residue moisture in a natural environment. Monitoring factors that influence the reproduction of *G. zeae* on crop residues will provide information critical to the development of reliable disease prediction systems and facilitate management recommendations.

FACTORS AFFECTING THE DEVELOPMENT OF WHEAT FUSARIUM HEAD BLIGHT

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OBJECTIVES

Develop a disease forecasting system for wheat Fusarium head blight based on the environment and inoculum level.

INTORDUCTON

Fusarium head blight (FHB) has been a severe problem in many of the wheat production regions of the United States (McMullen et al. 1997). The development of a reliable disease forecasting system would greatly increase the ability of wheat producers to make disease management and grain marketing decisions. Recent attempts to predict Fusarium head blight have emphasized the importance of both inoculum and environment to disease epidemics (Francl et al. 1999, DeWolf et al. 2000). However, the precise nature in which environment and inoculum interact during epidemic development remains unclear. Cooperators in OH, IN, SD, ND, and MB have agreed to follow a common protocol to create a forecasting model (De Wolf et al. 1999). We report here on how an infection bioassay may provide important insights into the interactions of inoculum, environment, and the resulting disease level.

MATERIALS AND METHODS

Adapted, FHB-susceptible cultivars were grown with standard agronomic practices in replicated plots at Wooster, OH (Hopewell SRWW), Fargo, ND (Norm HRSW), and Brookings, SD (Norm HRSW). The environment at each location was monitored by an automated weather station equipped with temperature, relative humidity, precipitation, and surface wetness instrumentation. Each day, 30 wheat spikes were collected from each plot by cutting the stem just above the first node. Twelve spikes per plot were placed into a dry growth chamber environment. A second group of 12 spikes was maintained in a saturated environment (100% RH) for 24 h. Following the 24 h wet treatment, these spikes were placed in the dry environment with the heads that had remained dry. Crop growth stage each day was evaluated using the remaining heads. FHB incidence and severity of both wet and dry spikes were recorded on the day of collection and after 12 days. For this report, viable inoculum during the flowering and milk stages of crop development was estimated by FHB incidence of the heads that received the additional moisture treatment. For comparison among locations and to judge epidemic intensity in each location, plot FHB incidence and severity were evaluated during the early dough stage.

RESULTS AND DISCUSSION

FHB intensity in the replicated plots at Fargo was the highest of the research locations during the 2000 growing season (Table 1). Plots near Wooster had the least amount of disease and plots in Brookings had a disease level intermediate to the Fargo and Wooster locations.

The spike bioassay indicated that Wooster had lower estimated inoculum levels than Fargo and Brookings, which were roughly equivalent (Fig. 1). Incidence at Fargo sharply increased in association with precipitation on days 186 and 188; whereas, incidence at Brookings was not strongly associated with a rainfall event.

Wetness parameters summarized from the beginning of anthesis and early milk stages of growth indicate that precipitation was similar between Wooster and Fargo and Wooster had the longest daily average wetness. The Brookings location had 43 mm less precipitation and 9 h per day of wetness duration. Relative humidity was similar at all three locations. Average temperature at Fargo and Brookings locations were equal; however, average temperature near Wooster was 5 degrees lower than the Dakota locations.

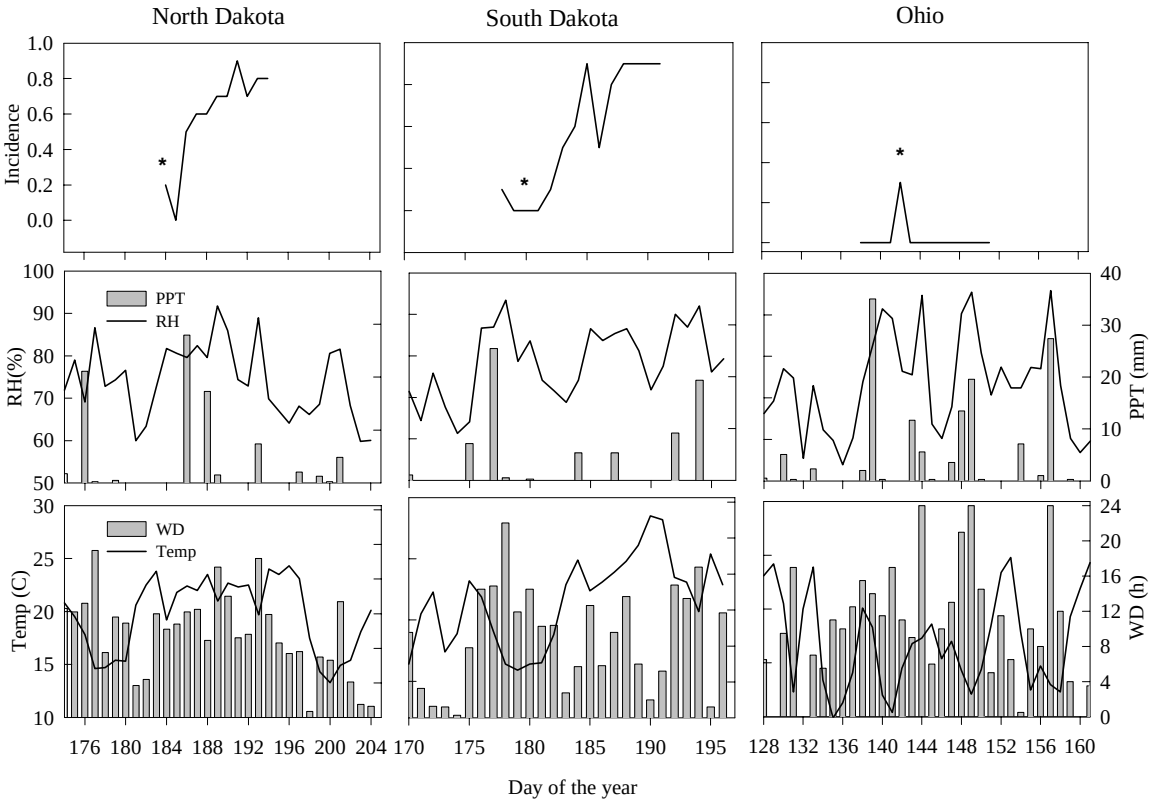
Differences in disease level between the three locations can be attributed, in part, to differences in temperature and moisture parameters, which likely influenced inoculum levels and infection periods. The low average temperature at Wooster may best explain observed low levels of estimated inoculum and disease intensity since moisture parameters were at least equivalent to the other locations. The high incidence levels on bioassays at Fargo and Brookings suggest that inoculum was abundant. Differences between the Fargo and Brookings locations in moisture levels during crop anthesis, specifically duration of surface wetness, suggest that moisture may have been a limiting factor for FHB development at Brookings.

These results demonstrate the importance of monitoring both inoculum and environment in the development of FHB epidemics. As additional information is collected, modeling fluctuations in inoculum level and infection periods based on environment should be possible. This database development is quickened by our experimental approach that utilizes multiple locations.

Table 1. Summary of environmental conditions at three research locations from beginning of crop anthesis until early milk stages of growth and Fusarium head blight (FHB) intensity in research plots at soft dough.

Variable	Location		
	North Dakota	South Dakota	Ohio
Avg. wetness duration (h)	12	9	15
Avg. temperature (C)	22	22	15
Avg. relative humidity (%)	81	80	81
Total rainfall (mm)	54	11	55
Plot FHB incidence (%)	60	38	8
Plot FHB severity (%)	31	25	4

Figure 1. Summaries of environment and incidence levels at research locations in North Dakota, South Dakota, and Ohio.



Each series of plots gives the temperature (Temp), relative humidity (RH), precipitation (PPT), wetness duration (WD), and proportional incidence level (Incidence). Viable inoculum on spikes in the field can be estimated from daily incidence because incidence values were derived from a bioassay in which wheat pikes collected from field plots were subjected to conditions favorable for infection by *G. zeae*. The * designates the beginning of crop anthesis.

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A VISUAL SCALE FOR ESTIMATING DAMAGE TO SOFT RED WINTER WHEAT KERNELS BY FUSARIUM HEAD BLIGHT

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INTRODUCTION

Fusarium head blight (scab), caused by *Fusarium graminearum*, has become an increasingly important problem in the United States due to an increase in crop acreage managed using tillage practices that leave crop residues on the soil surface (3,5,6). In 1993, the losses in United States wheat production to Fusarium head blight were estimated to exceed $1 billion, and more recent epidemics have added to the relative importance of this disease (6).

Infection by *F. graminearum* causes floret sterility and poor grain fill resulting in reduced yield and test weight (1,2,4). Diseased kernels are often shriveled and may have a white or pink discoloration. Affected grain may also contain mycotoxins, including deoxynivalenol (DON, vomitoxin) and zearalone, that have detrimental affects on animal and human health (2,7). Fusarium head blight also reduces seed quality. Seed that appears unaffected may be contaminated with mycelium or conidia of *F. graminearum*, resulting in seedling blight and foot rot when contaminated seed is planted (3).

Damage caused by Fusarium head blight can be quantified by assessing head disease incidence and severity, grain mycotoxin level, and grain yields. Researchers, interested in evaluating fungicide efficacy or the genetic resistance of wheat cultivars and breeding lines, are also concerned with kernel damage. Estimations of kernel damage are also useful to grain farmers and handlers required to assess grain marketability or feed value.

A visual scale estimating the percentage of affected kernels of hard red wheat has been proposed by R. Jones and J. Jenkins, Department of Plant Pathology, University of Minnesota. This series of photographs, representing a range of damaged kernels from 0 to 50%, were 1:1 reproductions of disease and healthy kernel mixtures on a 1000 kernel count basis. This scale proved invaluable for making visual kernel assessments, but differences between soft red wheat and hard red wheat kernel characteristics were great enough to limit the usefulness of this system. Soft red wheat is generally lighter in color than hard red wheat, thus the visual differences between diseased and healthy kernels is more difficult to discern. These differences lead to the construction of a visual scale for soft red wheat.

OBJECTIVE

To develop a visual assessment scale for soft red wheat kernels affected by Fusarium head blight.

MATERIALS AND METHODS

A visual scale for soft red wheat was prepared by creating a grain sample with a known percentage of diseased kernels from the susceptible soft red wheat cv. Hopewell. Each sample contained 200 kernels. The sample was then mixed and placed in a 5 cm-diameter container. Photographs are actual size to facilitate the comparisons with other grain samples. The visual scale can be used by passing grain samples over the photographs until the percentage of affected kernels is approximated by the damage seen in the scale. Record the appropriate percentage and continue onto the next sample. It is important to take a random, uniform sample from the harvested wheat.

DISCUSSION

There is considerable variation in the types of diseased kernels in grain samples. Harvested grain, especially grain threshed using research size equipment, may retain glumes and other plant parts. Intact spikelets should be examined carefully to confirm that Fusarium head blight caused the damage. To reduce sampling error and improve mean estimates, multiple readers may be employed, although correlation among evaluators has been high.

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INFLUENCE OF MIST-IRRIGATION VOLUME ON THE SEVERITY OF FUSARIUM HEAD BLIGHT AND SEED CHARACTERISTICS IN SELECTED CHECK CULTIVARS AND LINES OF WHEAT AND BARLEY

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ABSTRACT

A field experiment was conducted to investigate refining mist-irrigation treatments that might improve our ability to screen for resistance to *Fusarium* head blight (FHB) in wheat and barley. Mist-irrigation was applied to barley cultivars and lines Stander (susceptible, S), Robust (S), MNBrite (resistant, R), MNS93 (R); wheat cultivars were Norm (S), McVey (moderately resistant, MR), P2375 (MR), and BacUp (R). The experimental design consisted of four separate randomized complete split-blocks with four replications. One randomized complete split-block was non-misted as a control. The mist-irrigation treatments were: non-misted, 2.0, 4.0, and 8.0 mm of water per day. Split-block treatments were inoculated versus non-inoculated. Plots were inoculated with macroconidia using a CO_2 backpack sprayer to control both concentration and timing of inoculum application. Variables measured in barley plots over the different mist-irrigation treatments included FHB severity, incidence of infection, discolored kernels, and concentration of deoxynivalenol (DON) in harvested grain. Differentiation among the barley cultivars over the four variables was consistent under no mist and at the 8 mm per day volume. The variables measured in wheat plots over the same mist-irrigation treatments included FHB severity, incidence of infection, visually scabby kernels (VSK), and concentration of DON in harvested grain. Differentiation among the wheat cultivars over the four variables was more consistent than among the barley cultivars and was most consistent under no mist or at the 2 mm per day volume. We feel the lower disease levels reflect conditions in years with low FHB severity. These preliminary data also suggest that breeders could obtain useful information regarding promising breeding lines by screening for resistance to FHB utilizing inoculated plots that would be non mist irrigated.

GIBBERELLA ZEAE POPULATION DYNAMICS: A PROGRESS REPORT

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OBJECTIVE

Increase our understanding of fungal population dynamics to enhance the reliability of disease management with fungicides.

INTRODUCTION

North Dakota had been ground zero in the majority of recent FHB epidemics; consequently, agricultural industries have been displaced and small grain producers have endured economic hardships, often so severe as to preclude another season of farming. Another moderately severe, and in some North Dakota counties extremely severe, FHB epidemic occurred during the 2000 growing season. This epidemic series can be dated back to the 1993 catastrophic FHB outbreak on wheat and barley in South Dakota, North Dakota, Minnesota and Manitoba (McMullen et al., 1997).

Research on forecasting systems has suggested that a reliable model needs to take into consideration inoculation events (De Wolf et al., elsewhere in these proceedings). Inoculation has been shown to occur on multiple occasions during an FHB epidemic (Francl et al., 1999). As part of a regional forecasting system, a heuristic forecasting scheme for FHB is based on airborne spore samples of *Gibberella zeae* (Francl et al., these proceedings).

Prediction of inoculation events is being pursued as an adjunct research project to a multi-institution forecasting model development effort. This project may eventually obviate the need for airborne spore counts. Also, populations of *G. zeae* conidia may be produced on wheat leaves and these spores may then serve as a source of inoculum. We provide here the status of research on inoculation events and report on some preliminary results from wheat leaf assays for *G. zeae*.

MATERIALS AND METHODS

Inoculation timing. Fungal spore data came from two sources. First, a Burkard 7-day volumetric spore sampler was placed in the center of a wheat stubble field to sample airborne *G. zeae* ascospores continuously. The entire tape area was observed microscopically for ascospores of *G. zeae* and approximately 50 days per year for two years are available for analysis. Second, daily inoculum levels were assayed on potted Norm wheat spikes exposed to the field for 24 h. Four spikes from each pot were clipped and put in a solution of 40-ml sterile distilled water and one drop of Tween20 and shaken vigorously for 2 min. The solution was decanted and frozen until later assessment. Spores counts were assessed by colonization on Komada's selective medium and then carrot agar for colony identification of

G. zeae by observation of perithecia. To date, 54 days have been assayed over two growing seasons.

Colony forming units per spike, efficiency of fungal recovery from spikes, and spore type (ascospore or conidium) on spikes are being investigated. Relationships among environmental variables, time of day, and inoculum will be analyzed statistically. Linear regression and correlation will be used together with a critical pathway analysis to determine the variables important to inoculation timing.

Colonization of leaves. Twenty leaves formed the basic sampling unit and there were five sampling dates in 2000. Some leaves were asymptomatic (youngest on plant) while other leaf samples showed necrotic lesions. Fifty uniform pieces were cut from these leaves and plated on Komada's medium. Some pieces were surface sterilized (0.5% NaOCl for 2 min) while others were left unsterilized. After 10-12 days, colonies showing characteristics of Fusarium were transferred to half-strength PDA for identification based on colony type and conidia morphology. Colonies were plated on carnation leaf agar as needed to resolve questionable identifications.

RESULTS AND DISCUSSION

Preliminary results on inoculation were presented in the Forum last year and at the North Central Phytopathology meeting last summer (Markell and Francl, 1999, 2001). Briefly, there was a good correspondence between the Burkard and spike bioassay sources of data. Major and moderate inoculation events seemed closely linked with rainfall in excess of 5 mm. Airborne ascospore incidence exhibited diurnal periodicity and was greatest between midnight and 10:00 AM.

A final report on inoculation timing should be completed next year. Critical factors leading to inoculation events are being researched in an ongoing attempt to predict Fusarium head blight based on environment. Either aerobiota data or model estimates appear needed for successful prediction of Fusarium head blight. A successful result here would eliminate the need for spore samplers and save much of the operating cost of the NDSU scab forecasting system.

Preliminary data on leaf colonization from the 2000 growing season in North Dakota show that various species of Fusarium can be found between the three-leaf and early milk growth stages (Table 1). Furthermore, both epiphytic and apparently pathogenic relationships can be noted, often in temporal progression. This work will be expanded in 2001 and will include the following contrasting parameters: samples from fields with and without corn, wheat, or barley residue; symptomatic (i.e., necrotrophic lesions) and asymptomatic leaves; and surface sterilized vs. unsterilized pieces.

If indeed fungal colonies routinely occur on leaves, then this avenue of pathogen movement to the spike should be investigated further. Conidiation and splash dispersal are likely to occur during extended warm, wet weather. Also, one might hypothesize that inhibition of this potential source of inoculum by a fungicide may decrease the severity of an FHB epidemic.

Table 1. Incidence of *Fusarium* species on symptomatic (necrotrophic lesions observed) and asymptomatic (youngest) wheat leaves collected from a wheat-on-wheat field on the NDSU Experimental Research Station, Fargo. Samples were collected on 1 June (#1), 9 June (#2), 19 June (#3), 26 June (#4), and 10 July 2000 (#5), sterilized in 0.5 % NaOCl for 2 min or left unsterilized, isolated on Komada's medium, and identified on half-strength PDA.

Fusarium species	Date Pathogen Detected			
	Asymptomatic leaves		Symptomatic leaves	
	Sterilized	Unsterilized	Sterilized	Unsterilized
F. acuminatum	#5	#1,#2,#5	#2,#3,#5	#1,#2,#3,#5
F. avenaceum		#5	#5	#5
F. equiseti	#2,#5	#1,#2,#3,#4,#5	#2,#3,#4,#5	#1,#2,#3,#4,#5
F. graminearum	#4,#5	#3,#4,#5	#3,#4,#5	#3,#4,#5
F. poae			#2	#2
F. sambucinum	#2,#5	#2,#5	#2,#5	#1,#2,#5
F. sporotrichioides	#5	#1,#4,#5	#2,#4,#5	#1,#2,#4,#5

LITERATURE

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DESCRIPTION AND EVALUATION OF THE NDSU REGIONAL WHEAT DISEASE FORECASTING SYSTEM

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ABSTRACT

A wheat disease forecasting system has been implemented in the North Central region. Daily infection periods of three leaf diseases were predicted in 23 localities by computer models using environmental data from automated weather stations. Airborne spores of *Gibberella zeae* were sampled at 17 locations and heuristic forecasts for FHB were derived from these counts as well as temperature and wetness data. Information was delivered via the Internet and a toll-free telephone number. The system correctly predicted epidemics in 1999 and 2000. Operational problems were encountered in sampling efficiency and weather-related incidents. The system continues to be evaluated for biological realism, economic thresholds, and facilitation of user interaction. A quantitative FHB forecasting model may be ready to replace the present rule-of-thumb guidance as early as the 2002 season.

INTRODUCTION

Predictions of impending plant disease epidemics can increase appropriate pesticide use, agricultural productivity, and returns to management. Key elements of a successful forecasting system include accurate prediction for multiple and economically important plant diseases, timely and understandable communication, and cost effective remedies. Most existing forecasters are devoted to high-value crops because of the large reward for sound disease management in those cropping systems. Many of these systems consist of self-contained computer programs and environmental loggers positioned on-site. In contrast, few forecasters for major field crops exist despite the large aggregate amounts of pesticides applied. System development is hindered by the minimal marginal returns to added disease management expense and the relative economic inefficiency of an on-site system.

Diseases have considerably reduced wheat production in North America in the 1990's. Scab or Fusarium head blight (caused by *Gibberella zeae*) has caused billions of dollars of crop loss in a series of epidemics (McMullen et al., 1997). Leaf rust (*Puccinia triticina*), Stagonospora blotch (*Phaeosphaeria nodorum* and *Ph. avenaria*), and tan spot (*Pyrenophora tritici-repentis*) have been perennial foliar diseases amounting to perhaps 5-10% average crop loss per year (Long et al., 1998; McMullen and Nelson, 1992).

A regional forecasting system for three wheat diseases was initiated in North Dakota and Minnesota in 1999 and deployed again in 2000. The system was composed of environmental and aerobiota input data, computer and rule-based models to predict disease, compila-

tion of results, and two conduits of information delivery. This report describes the system and its evaluation and discusses the challenges faced in its operation.

METHODS

Data for infection period models came from bioassays in a field environment (Francl, 1995) and literature reports. Tan spot and Stagonospora leaf blotch infections within a 24 h period were predicted with accuracies of more than 80% by back propagation neural network models (De Wolf and Francl, 2000). Model inputs were cumulative growing degree days, daily average temperature and relative humidity, total daily precipitation, and hours of wetness. Leaf rust infection was based on hours of wetness and a minimum temperature threshold during the wet period.

The forecasting system in 1999 employed environmental data from 17 automated weather stations of the North Dakota Agricultural Weather Network (NDAWN) to provide regionally specific information. The deployed model relied on a wetness duration estimate, which a generalized regression neural network predicted from logger data input (Chtioui et al., 1999). For the year 2000, six additional NDAWN sites were selected to provide input for leaf disease models so that more wheat producers can take advantage of the system.

A foliar disease control advisory involved scouting for a 50% disease incidence threshold on either the penultimate or antepenultimate leaf. Scouting began at stem elongation and ended at the early milk growth stage. Once leaf disease reached the threshold, six to eight predicted infection periods were allowed to accumulate and a second confirmatory scouting was recommended. If disease progress was evident, a labeled fungicide spray was recommended.

A heuristic advisory for head blight infection was based on airborne spore concentration, wetness duration, and proximity of the managed crop to an inoculum source (infested residue). To estimate inoculum concentration, Burkard cyclonic flow volumetric samplers were placed near each of 17 NDAWN weather stations in fields with wheat stubble on the soil surface. Air samples were collected three times a week and examined under a microscope for sexually produced ascospores of *Gibberella zeae* and conidia of its anamorph, *Fusarium graminearum*

During the critical part of the wheat growing season, foliar infection period predictions were updated daily and head blight information was updated within 24 h of sample collection. Forecasts were provided via the Internet (www.ag.ndsu.nodak.edu/cropdisease) and a toll-free telephone message. A computer program automated the interface between the NDAWN environmental database, disease prediction models, and web pages. For each location, a summary of spore counts, system forecasts, and environment was placed on the web site after the 2000 season ended.

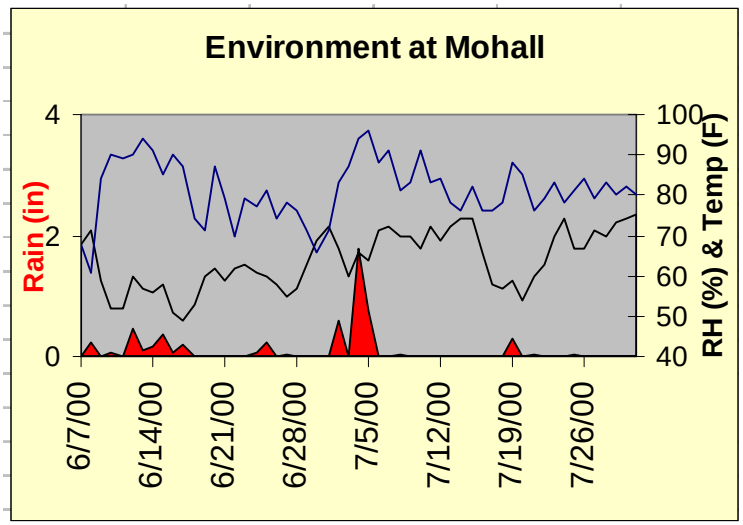
System evaluation in 1999 included a replicated field trial (n=4) on the North Dakota Agricultural Experiment Station in Fargo. The systemic fungicide azoxystrobin was applied at 120 g/ha active ingredient to the spring wheat cv. Grandin based on growth stage or according to forecast system advice. An untreated check and twice treated check were included.

Yield parameters and disease suppression were analyzed with ANOVA to judge treatment effectiveness. Similar trials were conducted in Fargo and Mohall, ND in 2000 but results are not included in this report.

After the 1999 growing season, the Burkard samplers were evaluated for sampling efficiency of *Sordaria fimicola* and *G. zeae* ascospores. Sporulating fungal cultures were placed in a container that had an opening for the machine orifice. Replicated tests (n=12) were conducted on three samplers that were powered by a regulated DC transformer.

RESULTS AND DISCUSSION

In 1999, the forecasting system correctly predicted a widespread tan spot epidemic and minor impacts due to Stagonospora blotch and Fusarium head blight. The web site received >7,000 hits from 1,408 distinct hosts. In 2000, the system web page received 15% more visitors, correctly forecasted foliar disease epidemics, and alerted growers to the presence of airborne *G. zeae* spores (Fig. 1).



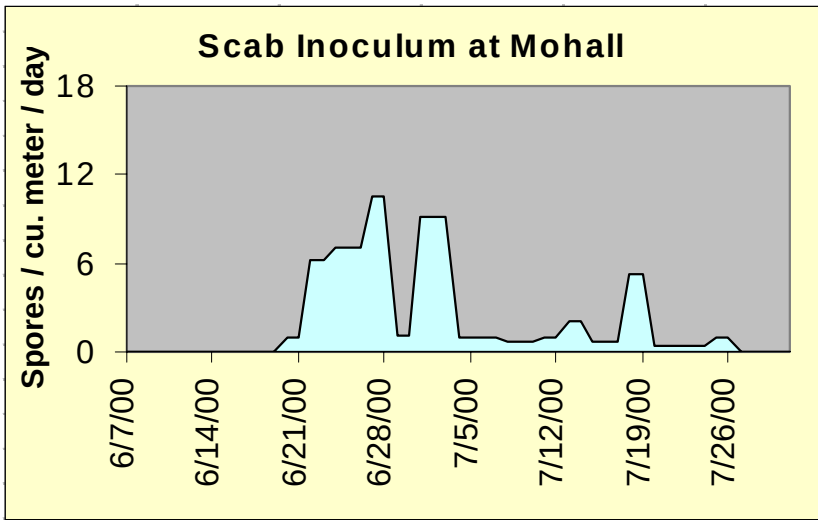


Fig. 1. Airborne spore counts and environmental summary for the Mohall, ND location. Much of the wheat crop in the area flowered around 16 July.

Fungicide trial results indicated that leaf disease was severe enough to warrant control because the forecasting system resulted in statistically significant disease suppression and yield enhancement over the control (Table 1). A single fungicide application at flag leaf emergence or heading increased protein but not yield.

Table 1. Effect of azoxystrobin fungicide application timing on disease and spring wheat yield in a replicated trial at Fargo, 1999.

Fungicide treatment	Flag leaf disease (%)	Yield (g/m2)	Protein (%)
Untreated	18 c	236 c	15.4 b
Flag leaf emergence	7 ab	244 c	15.6 ab
Heading	2:00 AM	245 c	15.7 a
Forecasting system (flowering)	9 ab	274 a	15.4 b
Heading and flowering	1:00 AM	293 a	15.8 a

During the 1999 season, two power outages prevented web page updates for 24-48 h; however, the recorded telephone message continued to provide information prior to re-initialization. On 19 June 2000, the NDSU campus was flooded. The web site was off-line for about 72 h and the phone line was down for two weeks. Also, frequent rains in both years caused problems in physically accessing the sampling sites. Timely spore counts were possible only through extended laboratory hours processing samples.

Sampling efficiency was questioned and addressed after the 1999 growing season. The Burkard cyclonic sampler was designed to spin particles within a metal cylinder until deposition into an eppendorf collection vial. However, debris collected on the walls during the season and tests showed that freshly liberated ascospores stuck to the walls of the sampling chamber. Wall adherence averaged 22% of the total sample for *S. fimicola* and 75% for *G. zeae*.

Spore sample collection in 2000 included a rinsing step to ensure better performance. The Burkard cyclonic sampler, a newly designed model, was chosen because of the potential for sample assay methods other than microscopy. For example, a PCR assay presently is being compared with results from microscopy and culturing on selective media (L. Francl, unpublished).

A user survey to assess system functionality is planned for the fall of 2000. In addition, data continue to be collected on prediction accuracy based on bioassay data and economic thresholds from fungicide trials. Fusarium head blight epidemic severity is related to multiple inoculation events and wet weather (Francl et al., 1999). A quantitative FHB forecasting model presently is under development (E. De Wolf et al., these proceedings) and incorporation into the NDSU forecasting system could happen as early as the 2002 season.

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PATHOGENICITY AND VIRULENCE OF EIGHT *FUSARIUM GRAMINEARUM* ISOLATES ORIGINATING IN FOUR REGIONS OF MEXICO

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INTRODUCTION

The expression of wheat host plant resistance to head blight caused by *Fusarium graminearum* Schw. varies widely, depending on environmental conditions (rainfall, temperature) and the inoculum used (age, concentration, incremental substrate, and isolates). It is important to have good control of these factors to avoid variation in the expression of resistance.

At present, a mixture of highly virulent pathogen isolates is commonly used as inoculum in screening wheat for Fusarium head blight (FHB) resistance in the belief that there are no vertical races in *F. graminearum,* as noted in the literature. There are, nonetheless, significant differences in pathogenicity among isolates that can greatly influence the measurement of resistance levels (Mesterhazy, 1997).

In our program differences in pathogenicity observed during FHB resistance evaluation made us suspect there was significant cultivar x isolate interaction. This led us to initiate the study reported here, whose main objective was to evaluate and confirm the presence of cultivar x isolate interaction.

MATERIALS AND METHODS

During the 2000 crop cycle in Atizapan, Toluca, Mexico, a trial was carried out in which four resistant (Sumai # 3, Frontana, Catbird, and Sha4/Chilero) and one susceptible (Flycatcher) wheat cultivars were inoculated with eight different *F. graminearum* isolates. The test isolates originated in Tepatitlan (isolates 3, 4, 5, 6), Jesus Maria (2), and El Tigre (1) in the state of Jalisco, and in Patzcuaro (7, 8), state of Michoacan.

The trial was planted with three replications; the cultivar was the main plot and the isolate, the sub-plot. The inoculum was increased in mungo bean medium, and its concentration adjusted to 50,000 spores/ml after growing five days. Twenty wheat spikes per plot were inoculated at flowering using the cotton method (Gilchrist et al., 1997).

Supplementary moisture in the form of mist irrigation was provided on the four rainless days. The different treatments were evaluated 35 days after inoculation by counting the number of affected spikelets per spike. Results were analyzed using categorical data analysis.

RESULTS AND DISCUSSION

Results of the analysis of variance (Table 1) showed highly significant differences at 0.001% between isolates, cultivar, and cultivar x isolate interactions. Isolate 1 from El Tigre was the most virulent, and 7 and 8 from Patzcuaro the least virulent (Table 2). The cultivar Frontana showed the best resistance to the eight isolates used (Table 3). Table 4 shows the absolute ratio of infected:healthy grains for each wheat cultivar with every isolate.

Table 1. Analysis of variance of blighted spikelets of five wheat cultivars inoculated with eight individual *Fusarium graminearum* isolates, Atizapan, Toluca, Mexico, 2000.

Source	DF	Chi Square	Prob
Cultivar	4	565.02	***
Isolate	7	178.10	***
Cultivar x isolate	28	106.38	***

Table 2. Analysis of contrast among the five test wheat cultivars.

Cultivars	2	3	4	5
1	***	***	***	NS
2		*	***	***
3			***	***
4				***

Table 3. Analysis of contrast among the eight *Fusarium graminearum* isolates.

Isolates	2	3	4	5	6	7	8
1	***	***	***	***	***	***	***
2		**	NS	NS	NS	NS	NS
3			*	**	**	**	**
4				NS	NS	*	*
5					NS	NS	NS
6						NS	NS
7							NS

Table 4. Absolute ratios of infected:healthy grains on four resistant and one susceptible wheat cultivars inoculated with eight different *Fusarium graminearum* isolates from four regions of Mexico. Atizapan, Toluca, Mexico, 2000.

Cultivars	Isolates							
	1	2	3	4	5	6	7	8
Sha4/Chil	0.194	0.098	0.096	0.103	0.093	0.135	0.085	0.090
Catbird	0.111	0.060	0.880	0.880	0.054	0.046	0.053	0.062
Sumai#3	0.090	0.046	0.079	0.055	0.053	0.065	0.059	0.054
Frontana	0.055	0.051	0.058	0.035	0.039	0.051	0.039	0.043
Flycatcher	0.210	0.127	0.131	0.132	0.131	0.084	0.108	0.082

CONCLUSION

The results confirm the presence of cultivar x isolate interaction.

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LOCAL GENETIC DIVERSITY OF *GIBBERELLA ZEAE* POPULATIONS FROM CORN STUBBLE, WHEAT STUBBLE AND INFECTED WHEAT HEADS

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ABSTRACT

Small-scale genetic structure of *Gibberella zeae* populations is being investigated to provide information on the epidemiology of this pathogen. Four target population complexes, one each from Michigan, Minnesota, North Dakota and South Dakota, are being characterized using amplified fragment length polymorphisms (AFLP) technology. A target complex is composed of an infected wheat field and the corn and wheat stubble fields that surround it. *Gibberella zeae* overwinters on the stubble and the colonies produce inoculum for infecting wheat heads in the subsequent year. Corn and wheat stubble was collected in June 1999 at the time of wheat anthesis in the adjacent field. This ensured that the stubble *G. zeae* populations were sampled at the time of wheat head infection. Infected wheat heads were collected two to three weeks after flowering. Preliminary results indicate that individuals do vary in their AFLP banding pattern, and populations are polymorphic. We are now increasing sample size for each population to obtain good assessments of population structure. We will report on the progress of the work in relation to the study's objectives. The first objective is to determine whether *G. zeae* populations isolated from corn and wheat stubble differ genetically. Since the timing of infection differs for corn and wheat, it is possible that they will differ genetically due to differences in the genetic structure of *G. zeae* inoculum. If these stubble populations differ genetically, then we will utilize the unique genetic features to investigate the relative importance of wheat versus corn stubble as inoculum sources in causing wheat scab. The relative importance of ascospores versus conidia as inoculum sources in causing scab will be determined from the frequency of multilocus clonal lineages within the *G. zeae* population causing scab.

AFLP LINKAGE MAP OF *GIBBERELLA ZEAE*

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ABSTRACT

A genetic linkage map of *Gibberella zeae* (*Fusarium graminearum*) was constructed by crossing nitrate nonutilizing (*nit*) mutants of *G. zeae* strains R-5470 (from Japan) and Z-3639 (from Kansas). Ninety-nine *nit+* progeny were selected and analyzed for polymorphisms using AFLP markers. Thirty-one pairs of two-base selective primers revealed 1072 polymorphic markers that mapped to 441 unique loci on nine linkage groups. The total map length of the genome from this analysis was 1036 centimorgans with an average interval of 2.3 map units between loci. Three linkage groups had high levels of segregation distortion. Selection of *nit+* recombinant progeny accounts for two of the skewed regions. One linkage group appeared to have an intercalary inversion. Loci governing trichothecene toxin amount and type (deoxynivalenol versus nivalenol) were mapped. A linkage map will be useful in population genetics studies, map-based cloning, QTL analysis, ordering genomic libraries, and comparisons with related species.

SITES OF ACTION OF TYPE II RESISTANCE TO FHB IN WHEAT: NING 7840 RETARDS SPREAD OF *F. GRAMINEARUM* WITHIN RACHIS

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ABSTRACT

Sumai 3 and its derivatives are excellent sources of genes conferring Type II resistance to Fusarium Head Blight in wheat. Type II resistance is defined as reduction in the rate of disease progression within a spike from a single point of infection. Observations are generally made on the number of visibly discolored (scabby) spikelets over a period of time. Our goal was to identify a measure of resistance that provides a higher level of precision that could be used in mapping and manipulating Sumai 3 resistance genes. Theoretically, there are several ways that a plant can reduce the rate of increase in scabby spikelets including: 1) Reduced spread from initially infected spikelet to rachis, 2) Reduced spread within the rachis, and 3) Reduced spread from within the rachis to adjoining spikelets. In addition, a plant may influence the fungus's change from a biotrophic to a necrotrophic growth habit. Ning 7840 (a Sumai 3 derivative) and a susceptible genotype (Norm) were grown in the greenhouse. Just prior to anthesis forty spikes of each genotype were single floret inoculated, then misted for 3 days. Seven and fourteen days post inoculation 20 spikes of each genotype were evaluated for the number of scabby spikelets, visible symptoms of disease in the rachis, and presence of the fungus in the rachis via a bioassay of rachis sections on PDA. The entire experiment was replicated 3 times and analyzed using Sas Proc GLM. Visible symptoms in the rachis were an accurate reflection of the presence of the fungus in both Ning 7840 and Norm, and the spread of the fungus in the rachis was significantly greater than reflected by the number of scabby spikelets. The ratio of the spread of scab in the rachis (according to visual symptoms of rachis portions infected) to the number of scabby spikelets in Ning 7840 was 2.4 and 3.4 for 7 and 14 days post inoculation (respectively), and for Norm was 2.8 and 2.2 for 7 and 14 days post inoculation (respectively). Subsequent field sampling of seven genotypes from the Uniform Scab Nursery (3 - 4 infected heads per genotype) showed 2 genotypes where the visual symptoms in the rachis were significantly greater than the presence of the fungus in the rachis according to the bioassay. In addition, in contrast to the study of Ning7840 and Norm, the presence of the fungus in the rachis was not always significantly greater than the number of scabby spikelets. Overall, the data suggest that there may be separate gene systems conferring resistance to spikelet infection vs. rachis infection. Although the visual symptoms of scab in the rachis were not significantly different from the presence of the fungus in the rachis according to the bioassay for Ning 7840 and Norm, this may not be the case for all genotypes. This poster was presented at the International Symposium on Wheat Improvement for Scab Resistance, May 5-11, 2000, Suzhou and Nanjing, China.

TEMPORAL PATTERNS OF ASCOSPORE DISCHARGE BY *GIBBERELLA ZEAE* FROM COLONIZED CORN STALKS UNDER NATURAL CONDITIONS

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OBJECTIVE

To document and characterize temporal patterns of ascospore discharge by *Gibberella zeae* from mature perithecia in corn stalk tissues under natural conditions.

INTRODUCTION

Gibberella zeae (anamorph: *Fusarium graminearum*), the predominant causal fungus of Fusarium head blight of wheat and barley, overwinters in crop residues. In large regions of north central and eastern North America, corn stalks on the soil surface are believed to be the primary reservoir of overwintered *G. zeae* and the main source of windborne ascospores for infection of cereal spikes in spring. Ascospores are forcibly discharged very short distances from perithecia and may be carried by turbulent air currents to flowering cereal spikes near or distant to the perithecial substrate. Once into the planetary boundary layer of the atmosphere (two times crop canopy height up to cloud level), ascospores are subject to vertical mixing and potential long distance dispersal. Previous investigators (Fernando et al., 2000; Paulitz, 1996) have examined patterns of ascospore capture from air within and above crop canopies, but, due to limits in experimental design, were unable to discriminate newly discharged ascospores from those redistributed locally in turbulent air or settled from higher levels of the atmosphere during periods of nonturbulence.

A comprehensive project to elucidate the aerobiology of ascospores of *G. zeae* is underway at Cornell University. A critical component of ascospore aerobiology is understanding when and under what circumstances ascospores are forcibly discharged from mature perithecia in substrates such as overwintered corn stalks on the soil surface. The time of day for peak spore discharge may be important relative to the likelihood that ascospores will be exposed to turbulence and transported into the planetary boundary layer. We are interested also in the environmental cues that affect the initiation and duration of spore discharge events. We are assessing natural patterns of ascospore discharge from a corn stalk substrate in order to formulate reasonable hypotheses about ascospore discharge that are testable by subsequent experimentation and observation. This paper reports initial results of those studies.

MATERIALS AND METHODS

Production of perithecium-bearing substrate

Fully mature dent corn stalks were collected from a production field in November 1999. Segments 2-cm long and centered on a node were cut from harvested stalks. Twenty five cut stalk pieces were placed in 1-gallon plastic jugs and soaked in 500ml distilled water for 24 hours. Jugs were plugged with non-absorbent cotton wrapped in two layers of cheesecloth. After the soaking period, the water was drained and the stalk pieces were sterilized in an autoclave for one hour with slow exhaust. Stalks were autoclaved three times over three consecutive days. Fifteen mycelial plugs from a pure culture of *G. zeae* (Gz014NY98) grown on PDA were added to each jug. Stalks were incubated at room temperature for two weeks under a 12 h photoperiod of near UV light. The stalks were shaken periodically in the jugs. Perithecia formation did not occur in the jug cultures. Fully colonized stalks then were placed over two wetted filter papers in a glass deep Petri dish and incubated under near UV light at room temperature for nine days or until perithecia were visible. Ascospore maturity was confirmed by microscopy and discharge ability was confirmed by placing agar media above the stalks.

Spore trapping experiments

A Burkard seven day recording volumetric spore trap (adjusted to sample air at 10 L/min) was used to collect discharged ascospores and record spore counts on an hourly basis.

A specially designed platform to support four corn stalk pieces bearing mature perithecia was placed in front of the orifice of the spore trap. The same stalk pieces were left in place for the duration of the experiment. Ascospores were collected on Melinex tape previously treated with a thin film of adhesive (Silicone grease and hexane in a 10:1W/V ratio). Sections of the tape were mounted in lactophenol cotton blue and scanned at right angles to the direction of rotation to determine the total number of ascospores discharged on an hourly basis. Tapes were scanned at a magnification of 400 at 2-mm intervals. Weather data (relative humidity, rainfall, wind velocity and direction, and temperature) were collected on an hourly basis using a weather station (Davis Weather Monitor II, Davis, California) located within a meter of the spore sampler. Data were collected between day of year 156 (4 June) and day of year 183 (2 July).

RESULTS AND DISCUSSION

The number of ascospores released from the corn stalks bearing perithecia and captured on Mellinex tape ranged from 0 to 3862 ascospores per hour. Typically there was a background level of 5 or more ascospores per hour during the collection period in June 1999 which was characterized by frequent rain events and cool to moderate temperatures.

There were six major events of ascospore release defined by peak counts of more than 1000 ascospores per hour. These events are summarized in Table1. These events ranged in duration from 4 to 14 hours and each was initiated and terminated during daylight hours. Peak spore release (>1000 per hour) occurred over intervals of 1 to 7 hours and the maxi-

mum discharges generally coincided with daily periods of highest temperature and lowest relative humidity. No singular weather variable was found that preceeded the five dates of major spore release in contrast to the other days of observation. The last five discharge events occurred on consecutive days during the warmest portion of the collection period. In fact, events five and six overlapped on June 26. Event five diminshed from 2372 spores in one hour down to 483 spores in the following hour immediately following a 10% mid-day rise in relative humidity. Thereafter, the ascospore counts rose again to above 1000 per hour for the next three hours (event six) in conjunction with another decrease in relative humidity. It appears that decreases in relatively humidity following periods of high humidity are somehow associated with the discharge of ascospores.

We found that most ascospores were being discharged during daylight hours when atmospheric turbulence is highest. This pattern provides the maximum opportunity for ascospores to be moved into the planetary boundary layer where vertical mixing occurs up to the cloud level and the potential for long distance dispersal is greatest. We are presently conducting experiments under controlled climatic conditions to better understand the effects of moisture, relative humidity, temperature, and light on the discharge of ascospores from mature perithecia. Our observation of peak ascospore discharge during daylight hours is in stark contrast to published reports (Fernando et al., 2000; Paulitz, 1996) of peak ascospore capture in or near inoculated plots during nighttime hours. We suggest that nighttime peaks in these studies may also be due to capture of airborne spores that settle during periods of nonturbulence. Obviously, these seemingly contradictory results need to be reconciled by further experimentation on the aerobiology of this fungus.

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Table 1. Summary of major events of ascospore release by *Gibberella zeae* from a corn stalk substrate placed near the orifice of a volumetric spore sampler under natural conditions.

		Event		Peak discharge (>10^3 per h)	
Event No.	Day of Year	Interval	Duration	Interval	Duration
1	170	2 pm – 6 pm	4 h	2 pm – 3 pm	1 h
2	174	9 am – 7 pm	10 h	12 pm – 6 pm	6 h
3	175	6 am – 8 pm	14 h	10 am – 5 pm	7 h
4	176	9 am – 7 pm	10 h	2 pm – 5 pm	3 h
5	177	6 am – 2 pm	8 h	11 am – 1 pm	2 h
6	177	2 pm – 9 pm	7 h	2 pm – 5 pm	3 h

FUSARIUM HEAD BLIGHT: INOCULUM DETECTION, DISEASE PROGRESS, AND ENVIRONMENTAL INFLUENCES

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OBJECTIVES

South Dakota State University is part of a collaborative project studying epidemiology of Fusarium head blight (FHB) on wheat under different environments throughout the upper mid-west. The ultimate goal is to develop a disease forecasting system. Primary objectives include: 1) monitoring inoculum dynamics and disease development in relation to temperature, humidity, and precipitation at locations throughout the upper mid-west; and 2) to evaluate tools and techniques for incorporation into a useful and efficient disease forecasting system.

INTRODUCTION

It has been observed that FHB occurs at epidemic levels when hot, humid conditions and frequent precipitation have occurred at anthesis (Bai and Shaner 1994, McMullin et al. 1997, Parry et al. 1995). By investigating the relationship of FHB incidence and severity to environmental conditions, a disease forecasting system may be developed to provide producers with the capability of making better management decisions. Environmental conditions are thought to influence the FHB disease cycle, but it is not certain which factors are critical, and which are most predictive of epidemics. The objective of this study is to relate certain environmental factors to the development of inoculum, the delivery of that inoculum, and the progress of the disease after infection for a study site in South Dakota.

MATERIALS AND METHODS

Spring wheat (cv. “Norm”) susceptible to FHB was planted into strips 1.4 m by 45 m using a 7-row grain drill. Two adjacent strips were planted on each of three planting dates (3 April, 25 April, and 2 May), referred to as planting date (PD) 1, 2, and 3, respectively. Multiple dates were used to help ensure that susceptible host stage and pathogen inoculum would be present concurrently. Each planting was divided into three replicate plots. Each plot was further divided into two subplots, one sampled and one unsampled. The unsampled subplot was used to assess final disease levels for each plot.

Environmental 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, this proceeding).

Daily airborne inoculum levels were monitored during the sampling period using a Burkhard Cyclone sampler (Burkhard Manufacturing). A wash of the cyclone unit was performed daily to ensure uniform sampling. The sample and wash were plated on Komada's medium for spore enumeration. Counts were reported as CFU per day. Inoculum on wheat spikes was estimated by washing heads using protocols described by Francl et al. (1999), with some modification (sampled heads were not covered prior to sampling). On each day, five primary spikes per replicate were collected and placed in a flask with 50 ml of sterile deionized water, shaken vigorously for 60 seconds to dislodge spores, then discarded. A 0.5 ml aliquot of the wash was then spread-plated onto each of three plates of Komada's medium. Plates were then incubated 10-14 days. Colonies were described and counted after incubation. Colonies were reported as CFU per spike per day.

Daily sampling for disease progress was conducted in each planting date when plants were between late emergence (GS 59) (Zadoks et al. 1974), and mid-milk (GS 75) following protocols described by Francl (1998). In each sampled plot, 24 spikes were collected by cutting stems below the upper-most node. Each sample was divided into two subsets, one labeled "dry", the other labeled "wet". Both sets were rated for the number of infection sites, and FHB severity (% of infected spikelets). One subset of daily samples ("dry") were placed directly into large incubator (RH < 50%), with their stems submerged in floral preservative to maintain freshness of tissue. The remaining subset ("wet") was treated similarly, but was subjected to 24 hours incubation in a moist chamber (100% RH) prior to incubation in the dry chamber. After an incubation period of six days, each spike was again rated for FHB infection sites and severity. After 12 days, spikes were rated for disease severity. Final FHB incidence and severity in the plots were surveyed by sampling 100 primary spikes per rep when plants reached mid-milk.

RESULTS AND DISCUSSION

In the period of seven days prior to sampling, cool wet conditions were experienced. Temperature in the canopy averaged 17°C and a total of 15.2 mm precipitation was received from seven rain events. There was little recovery of spores by either method in the few days following this cool wet period. Though moisture was high, the cool temperatures could have slowed fungal development at the soil surface, delaying the onset of ascospore production.

The Burkhard Cyclone Sampler did not perform as well as expected, however the data were considered to be a rough estimate of airborne spore concentration. Few spores were recovered on the first six days of sampling. Spores were recovered from the samples over the next 18 days at levels between 15 and 223 colony forming units (cfu) per day (Fig. 1). Head washing also resulted in very little spore recovery during the first six days of sampling. Thereafter, recovery of spores ranged from 4 to 207 cfu per head (Fig. 2). There was strong positive correlation between the overlapping sampling periods among planting dates. There was also positive correlation between Burkhard spore recovery and head washing spore recovery for PD 1 and PD 2 (r = 0.54 and 0.58, respectively).

Precipitation, canopy temperature, leaf wetness, and soil wetness for the anthesis periods of each planting date are summarized in Table 1. During susceptible periods, PD 1 and PD 2 were of similar temperature and received similar precipitation. Planting date 3 was warmer,

received much less precipitation during anthesis, and also had lower soil and leaf wetness duration. Soil wetness duration appeared to relate to airborne ascospore levels (Fig 1). Peaks in soil wetness duration were often followed by increased airborne ascospore levels though calculated correlation was negligible. Table 2 details the final disease ratings for all plantings. Planting dates 1 and 2 had moderate levels of disease. Inoculum and moisture levels were greater during anthesis periods of PD 1 and 2 than for PD 3, which would account for the higher incidence and severity. Moderately low canopy temperature may have slightly inhibited infection and FHB development during anthesis of PD 1 and 2. Dry conditions and reduced inoculum levels during anthesis of PD 3 probably resulted in reduced disease incidence. Severity was also lower in PD 3, again due to the drier conditions present.

Table 1. Environmental conditions over susceptible periods in each planting date.

Plant. Date	Time period (susceptible)	Avg. canopy temp (oC)	Precip. (mm) / events	Leaf wetness duration (hrs)	Soil wetness duration (hrs)
1	DOY 173-179	19	33 / 2	10.5	14.3
2	DOY 178-183	18	26 / 2	14.6	20.3
3	DOY 184-188	24	1-May	7.5	9.9

Table 2. Final disease ratings.

	Plant Date 1		Plant Date 2		Plant Date 3	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Rep 1	39	26	50	26	13	15
Rep 2	34	25	28	22	13	20
Rep 3	28	26	36	27	13	19
PD Mean	34	26	38	25	13	18
Overall: Disease Incidence = 28% Disease Severity = 23%						

Total incoming solar radiation varied from 4 to 17 MJ $m^{-2}d^{-1}$ (Fig. 1). It was observed that peaks in solar radiation corresponded to peaks in airborne inoculum estimates, though calculated correlation was not large (r = 0.49). Daily solar radiation may have affected airborne inoculum due to light requirements of the fungus, rapid changes in relative humidity, or increased spore escape from the boundary layer by way of increased convection of air away from the soil surface.

Disease progress was measured in terms of disease incidence (infected heads / total heads sampled) and disease severity (infected spikelets / total spikelets). There was almost always greater disease incidence in sample sets subjected to 24 hours of high moisture incubation than in those with no additional moisture, as expected. Little correlation was observed between airborne inoculum (Burkhard data) and disease incidence on those samples given

no additional moist incubation as read at six days post-sampling. Shifting the Burkhard data to compensate for any lag between airborne inoculum and initial infection (i.e. shifting the Burkhard data ahead 1, 2, 3, or 4 days) did result in increased correlation coefficients relative to the 6 day-post readings of samples, in some cases. For PD 1, with no shift, r = (-)0.39; with 2 day shift, r = 0.36; 3 day shift, r = 0.66; and 4 day shift, r = 0.74. For PD 2, with no shift, r = 0; with 2 day shift, r = 0.71; with 3 day shift, r = 0.59. Such pattern, however, was not found for PD 3.

The increase in correlation following shifting suggests that estimates of airborne inoculum may precede disease development by up to several days. This lead time would be very valuable for application of disease forecasting models to management decisions. There was weak to moderate correlation between inoculum levels found on heads and disease incidence at 6 days post-sampling for PD 1, 2, and 3 (r = 0.61, 0.22, and 0.39, respectively). Shifting the data generally did not improve correlation in these relationships.

Shifting of some of the data (i.e. airborne inoculum estimates) to compensate for lag periods proved useful. This indicates that this type of data may be more useful in forecasting systems in the future. Estimates of airborne inoculum did not relate well to infection sites on detached heads. This may suggest that the presence of inoculum is simply one requirement for infection. If inoculum fails to contact the susceptible head, or conditions are unfavorable for infection, disease may be reduced, or fail to develop. Head washing was shown to be a more reliable estimate of inoculum levels than spore trapping. High correlation among head washing data (overlapping portions of planting dates) showed consistency in the method, and the correlation of spores per spike to daily disease incidence showed relevance of the head washing method.

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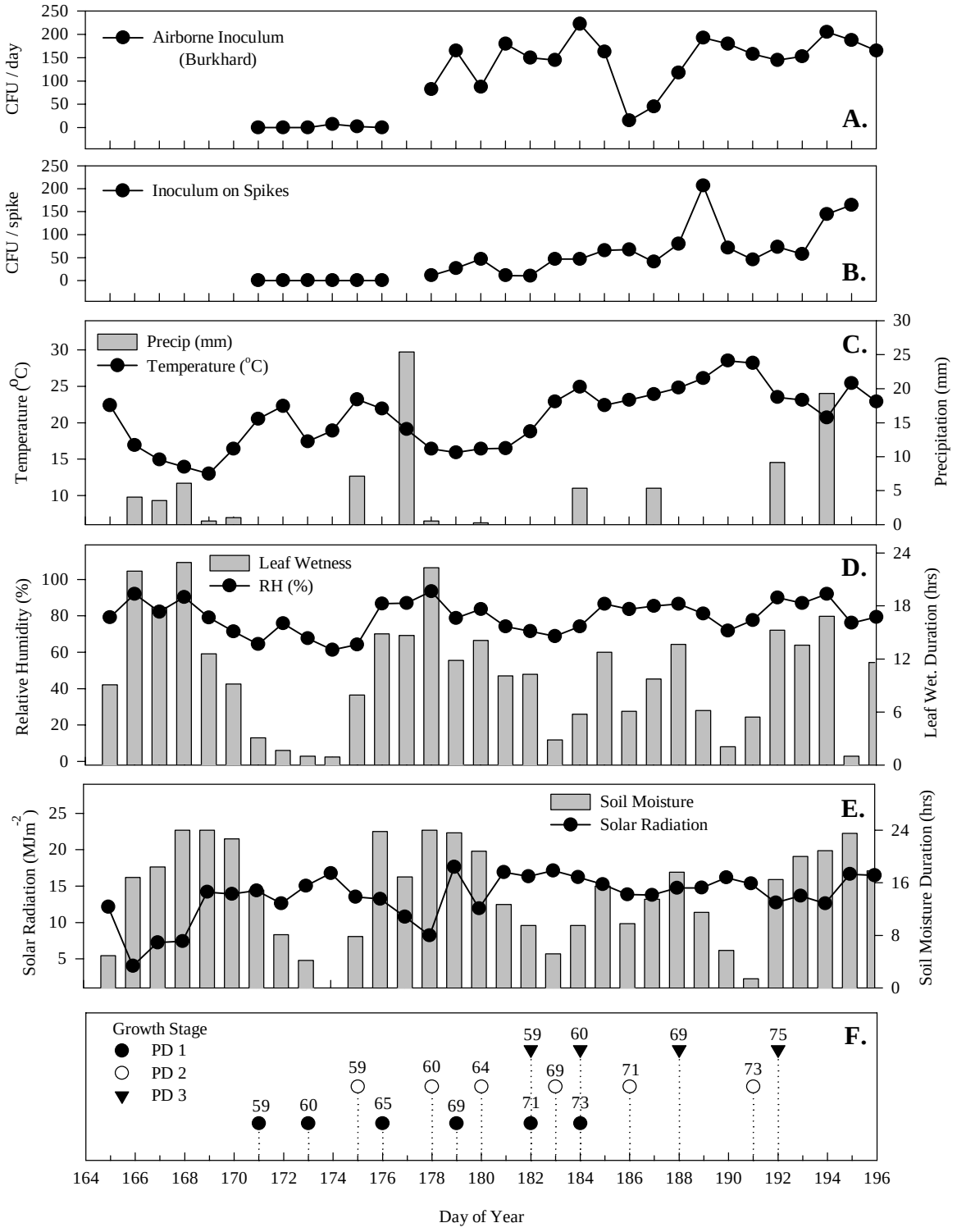

Fig. 1. Inoculum and environmental data over susceptible periods. (A): Airborne inoculum; (B): Inoculum on sampled spikes; (C): Precipitation and canopy air temperature; (D): Leaf wetness duration and relative humidity in the canopy; (E): Soil moisture duration and solar radiation; and (F): Growth stage (Zadoks) for each planting date.

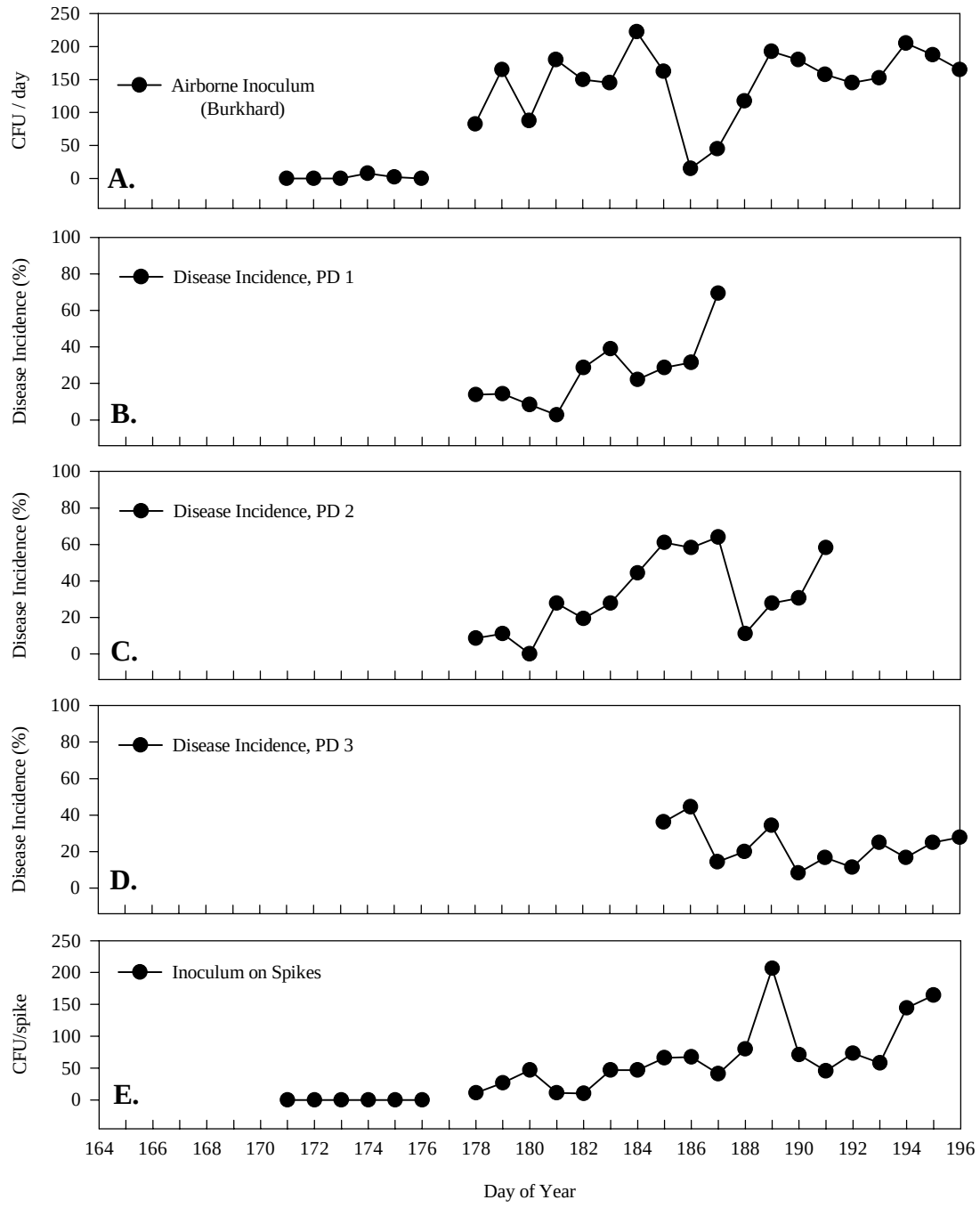

Fig. 2. Disease incidence on sampled spikes (no additional "wet" incubation) at six days post-sampling for all planting dates (B, C, and D) with Burkard Sampler estimates of airborne inoculum levels (A) given as 'cfu/ml' of sample volume (3 ml total). Inoculum estimates from detached heads (wash procedure) are given as 'cfu/spike' (E).

A SENSOR FOR MONITORING WETNESS AT THE SOIL-AIR INTERFACE

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INTRODUCTION AND OBJECTIVES

Development of epidemiological models useful to plant disease forecasting involves gathering information about environmental factors including temperature, vapor pressure, and precipitation. One aspect of the environment that has been difficult to study is moisture at the soil-air interface. The microclimate at the soil-air interface has many influences including air and soil temperature, vapor pressure of the air, precipitation, soil characteristics, and vegetation. Soil-surface moisture, often associated with dew formation in the canopy and precipitation events, is presumed to be one of the critical environmental factors affecting the development of *Gibberella zeae*. The objective of this project was to develop and test an instrument that would directly measure moisture at the soil-air interface. The instrument must be able to provide continuous measurements without altering moisture levels in the measurement area. It must also be compatible with data logging equipment. The information will be critical in understanding the effects of soil wetness on inoculum production under field conditions.

MATERIALS AND METHODS

Sensor Development. Sensors were constructed to assess soil surface wetness by measuring resistance across a plane between two electrodes. Electronically, the sensor consisted of an AC half-bridge circuit, encased in epoxy resin, with exposed wires for contacting the soil surface. Internal resistors, along with the variable resistance of the exposed sensing elements, compose the half-bridge circuit, and allow for the determination of resistance across the two exposed wires. The circuitry is similar to that found in commercial wetness sensing grids, integrating a reference resistor (R_r = 1 kW), with a fixed resistor (R_f = 100 kW) in series with the sensing elements (R_s = variable resistor). The fixed and reference resistors were encased in the instrument frame (11.5 cm by 11.5 cm by 2.3 cm) (Fig. 1). The exposed electrodes consisted of 18-gauge galvanized steel wires. An excitation voltage (V_x) was supplied to the instrument by an external source; in this case, a CR10X datalogger (Campbell Scientific, Inc.). This excitation voltage is compared ratiometrically to a voltage (V_s) measured at a point between the reference resistor (R_r) and the sensing elements (R_s + R_f). The ratio of these voltages (V_s/V_x) is equal to the ratio of the reference resistor (R_r) to the sum of all resistors ($R_r + R_s + R_f$) (see Eq. 1). To find the resistance across the sensor, the equation is solved for R_s (see Eq. 2). When there is no free moisture between the sensing elements, the circuit is open and resistance is infinite. When moisture is present, the circuit closes and resistance across the sensing elements can be measured. Resistance is higher when moisture levels are low, and approaches zero when free water is present. Excitation voltage must be supplied to the sensor in AC form, as ion polarization will occur otherwise, and sensors will decay.

Eq. 1: $V_s/V_r = R_r / (R_r + R_s + R_f)$

Eq. 2: $R_s = R_r / (V_s/V_x) – R_r – R_f$

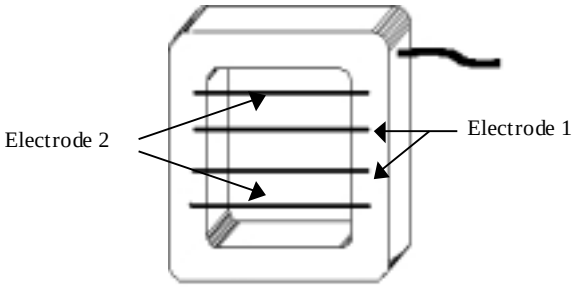

Fig. 1. Soil-surface wetness sensor. Polyurethane frame with 18-gauge galvanized steel wires (electrodes 1 and 2).

Laboratory testing and calibration. Initial testing was conducted by placing the sensors on synthetic sponges. The sponge and sensor were then placed under a desk lamp to speed drying times. Sensor output was recorded using the CR10X datalogger. Several readings were taken from the dry sponge at ten minute intervals, then water was added to the capacity of the sponge. Readings were taken every ten minutes until the sponge was very dry. Several repetitions of this procedure were conducted for each sensor. The data were plotted for each repetition and the results of each run were compared among reps and sensors.

Further testing was done using a thin layer (5 mm) of sieved soil mix (silty loam soil, vermiculite, and peat) in a small plastic container. A known weight (50 g, oven-dried) of soil was used to facilitate calculation of moisture content after addition of water. The sensor was placed atop the soil layer, and the entire assembly was placed on a digital balance. A specific weight of water was added to the soil using a misting sprayer to evenly wet the soil. Every ten minutes, the resistance across the sensor was recorded as well as the weight of the remaining water. This procedure was repeated several times with each sensor.

Measuring wetness duration and inoculum production. The soil surface moisture sensors were integrated into an automated weather station placed in field plots. Five sensors were placed at various locations throughout the plot area. Sensors were located either under the canopy, or between plots on bare soil. Measurements were taken every 2 minutes, and averaged over 30-minute intervals using the CR10X datalogger. Soil surface moisture duration in hours per day was estimated based on the period of time that sensor output was below a threshold value. When sensor output was above the threshold level, the soil was considered dry at the surface.

Airborne inoculum levels were monitored using a Burkhard Cyclone Sampler (Burkhard Manufacturing). Inoculum on wheat spikes was estimated by washing heads using protocols described by Francl et al. (1999), with some modification (sampled heads were not covered prior to sampling). Both methods are described in detail elsewhere in this proceeding (Osborne and Jin, this proceeding). Data were reported as colony forming units (CFU) per day, or per spike.

RESULTS AND DISCUSSION

Laboratory calibration. The sensors performed well in laboratory tests. In sponge trials, there was significant variability among sensors (P = 0.03), likely due to variation in components or construction (each sensor was hand-made). For individual sensors, there was no significant difference among reps, indicating consistency over time. In thin soil layer trials, individual sensors consistently indicated the point at which the surface was completely dry by a marked increase in sensor resistance, usually on the order of 100 kW or more over a ten minute period. Different sensors did not always have similar output values for specific water content, but each was consistent over reps. Visual or tactile estimates of surface-moisture compared favorably to sensor output on the sponge and on thin soil layer. As the substrate dried, sensor measurements resulted in a three-part response curve (Fig. 2).

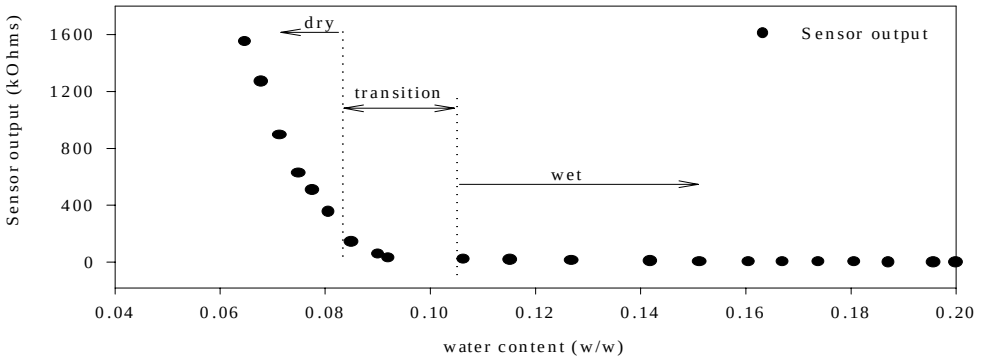

Fig. 2. Thin-soil layer calibration. Sensor output plotted against water content in the microcosm. Visual comparisons are indicated by arrows and dashed dividers.

Sensor output was very low (0 to 100 kW) when the soil was visibly wet. Output was very high (10^3 to 10^4 kW) when the soil was visibly dry on the surface. The transition period was short in laboratory tests where the drying process was increased using a heat lamp, but should be different over time for substrates with different physical characteristics. Sandy soil, for example, dries more quickly than clay soils. Therefore, the sensor response in sandy soil would be more rapid, and the transition period would be shortened. Though the sensor output varied in response to substrate differences, one could determine the wet/dry threshold for each particular application. A sensor which indicates wet or dry surface conditions and afford calculation of wetness duration would be adequate for many epidemiological modeling applications.

Effects on inoculum under field conditions. Soil surface wetness measurements were highly variable among sensors at certain times. This was expected due to the placement of the sensors in varying levels of canopy cover and at different locations with respect to drainage. Sensors in the lower portion of the field indicated wet conditions for longer periods of time than sensors on higher ground. This matched visual observations of the field. Sensors under

canopy coverage tended to indicate moisture longer than those on the bare soil. During long periods without precipitation (periods greater than 4 days), wet/dry indications from all sensors agreed 86% of the time for the 48 hours preceding precipitation. Four sensors agreed an additional 9% of the time. For the period of 48 hours following major precipitation events (5 mm or more), all sensor indications agreed 57% of the time, and four of five agreed an additional 12% of the time. This indicates that the sensors exhibit a degree of uniformity which is satisfactory under field conditions, with the intended application.

For purposes of comparing inoculum detection and soil surface moisture, data from all sensors were averaged to approximate wetness level for the plot area (Fig. 3a). Airborne inoculum levels (as estimated by the Burkhard sampler) appeared to increase as soil moisture duration increased (Fig. 3b). Decreased inoculum appeared to correspond to lower soil moisture duration. When compared to head washing data (Fig. 3c), increased spore recovery appeared to lag peaks in soil moisture duration by one day. Reduction in numbers of spores recovered also lagged reductions in soil moisture duration by one day. These preliminary results indicated that the sensors could serve as a useful tool for estimating soil surface moisture for applications such as disease forecasting, where duration of wetness may be a critical factor in the development of inoculum. To further test this idea, controlled studies are being conducted to monitor perithecial development, and spore discharge under various wetness regimes.

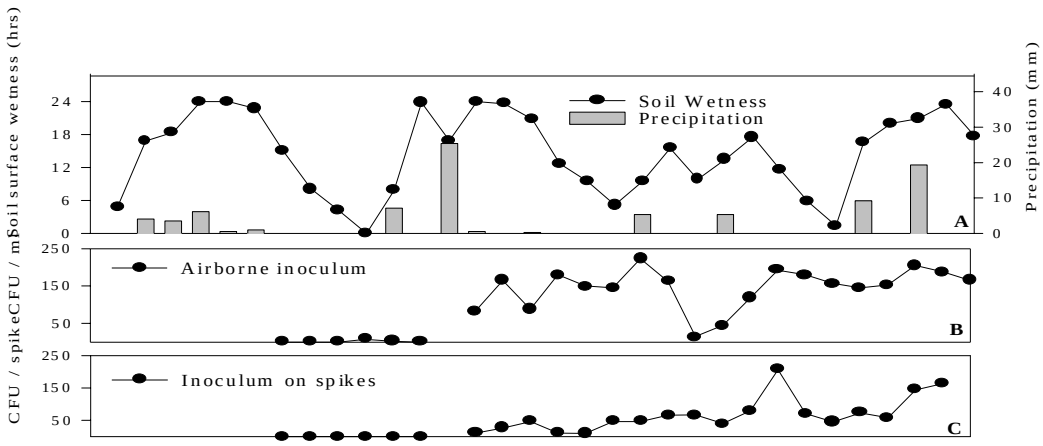

Fig. 3. Environmental parameters and inoculum measurements. (A) soil wetness duration and precipitation; (B) airborne inoculum collected by Burkhard spore collector; and (C) inoculum washed from detached spikes.

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MEASURING DIFFERENCES IN THE ABILITY OF STRAINS OF *FUSARIUM GRAMINEARUM* TO SPREAD WITHIN WHEAT HEADS

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ABSTRACT

We have been characterizing isolates of *Fusarium graminearum* that differ in their ability to spread, cause disease and accumulate mycotoxins in inoculated wheat heads. Identification of isolates that differ genetically for virulence and toxin production will allow the subsequent isolation of genes that are important for controlling for these traits. Knowledge of the determinants of disease spread may suggest novel strategies for disease control. Isolates of *Fusarium graminearum,* representing 8 genetic lineages of this species complex and other *Fusarium* species that cause FHB have been tested. Pathogenicity tests have been conducted on two cultivars of wheat (Norm and Pioneer 2375) very susceptible to the disease. Plants were inoculated at early to mid-anthesis by placing a 10 µl inoculum droplet containing approximately 10^4 conidia into the fifth spikelet from the base of the head. Two weeks after inoculation, disease symptoms (necrosis and/or bleaching of the spikelet) were recorded for 10 spikelets per head including the inoculated spikelet as well as 5 spikelets above and 4 below the point of inoculation. Glumes from the rated spikelets were then plated on mung bean agar medium to detect the presence of the fungus and the remainder of the spikelet, in some instances, was tested for the presence and level of mycotoxin. Symptom expression, the presence of the fungus and mycotoxin concentration were recorded for each spikelet from ~20 inoculated heads. Distribution of symptoms, fungal colonization and mycotoxin accumulation differed significantly among isolates.

SPATIAL PATTERNS OF FUSARIUM HEAD BLIGHT IN NEW YORK WHEAT FIELDS DURING THE EPIDEMIC OF 2000

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INTRODUCTION

Spatial pattern analysis can be a useful tool in understanding the epidemiology of plant diseases including the nature of inoculum sources (Madden, 1989; Madden and Hughes, 1999). There was a widespread epidemic of Fusarium head blight (FHB) in New York winter wheat in June 2000. We assessed spatial patterns of disease incidence in four distant winter wheat fields in order to gain preliminary insights about probable sources of inoculum for spike infection.

MATERIALS AND METHODS

In each of four fields, the incidence of spikes with symptoms of FHB was assessed in 60 quadrats of size 0.093 m^2 (1 ft^2), 20 spikes per quadrat, during the late dough stage. All 60 quadrats were sampled along a single row transect, at 1.5 m intervals, in fields 1,2 and 3. In field 4, samples were assessed in six rows of 10 quadrats each. The six rows were within 41 m of each other. Within rows, the quadrats were 4.5 m apart. The BBD (Beta Binomial Distribution) program (Ver. 1.3) (Madden and Hughes, 1994) was used to calculate the index of dispersion and fit the binomial and beta-binomial distributions to disease incidence data for each field.

RESULTS AND DISCUSSION

Mean incidence of FHB was less than 1% in the first three fields, but was four times higher in field 4. The index of dispersion was significantly different from its expected value assuming randomness for field 4 only. The binomial distribution adequately described the incidence of FHB in fields 1, 2 and 3, but the beta-binomial was a better descriptor than the binomial in field 4 (Table 1).

The pattern of FHB incidence in fields 1,2 and 3 appeared to be completely random. This is consistent with the hypothesis of a mainly external initial inoculum source of airborne ascospores of *Gibberella zeae*, and little or no in-field source of inoculum from crop residues clustered on the soil surface. Corn residues were not observed in fields 1, 2 or 3. In field 4, spikes affected by FHB appeared to be somewhat aggregated within particular quadrats. Since the window of spike susceptibility is brief, it was assumed that only primary inoculum present at the time of anthesis contributed to observed symptoms. A very few small remnants of corn stalks from a corn crop two years earlier were still visible on the soil in field 4. Perithecia of *Gibberella zeae* are produced on corn residue left on the soil surface for up to two years after the crop has been harvested (Khonga and Sutton, 1988). The observed

aggregation of FHB in field 4, together with the observed corn residue, suggests that at least a portion of the inoculum for spike infection was derived from within-field sources.

We consider these results as preliminary circumstantial evidence that inocula from sources external to wheat fields as well as from residues within wheat fields contribute to FHB epidemics in New York.

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Table 1. Characteristics of winter wheat fields sampled for Fusarium head blight in 2000 and statistics describing the pattern of disease.

Field	Cultivar	Previous crop		Beta-binomial parameters[c]			
		1998	1999	D[a]	P_{lrs}[b]	p	θ
1	Caledonia	wheat	oat	0.8	1	0.0525	0
2	AC Ron	corn	pea	1.11	0.55	0.06	0.0046
3	Caledonia	corn	pea	0.86	1	0.0417	0
4	unknown	corn	soybean	1.77*	0.0006	0.2375	0.0408

[a] Index of dispersion. D > 1 suggests aggregation of disease incidence. * indicates $P < 0.001$ for a test of whether D differs from its expected value for a random pattern of disease incidence.
[b] Probability associated with a likelihood ratio test of whether the beta-binomial distribution is a better descriptor of the observed disease incidence than the binomial distribution.
[c] Moment estimates of the parameters of the beta-binomial distribution.

INFLUENCE OF LOCAL VERSUS REGIONAL FACTORS ON INCIDENCE OF SEED INFECTION BY *FUSARIUM*

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INTRODUCTION

A widespread epidemic of Fusarium head blight affected winter wheat grown in western New York in 1996. We investigated whether seed harvested from fields in close proximity had more similar incidences of infection by *Fusarium* than did seed from fields that were more distant.

MATERIALS AND METHODS

One hundred seeds each from 23 seedlots, harvested in 1996 and representing four adapted winter wheat cultivars (Cayuga, Geneva, Harus, NY Batavia), were assayed for infection by *Fusarium* spp. using the freezing blotter method (Limonard, 1966). Geographic coordinates for each field were converted to planar coordinates by the Corpscon software program (Ver. 5.x, U.S. Army Corps of Engineers). Fields were on average 70 km apart (median = 62.8 km; range 1 m to 179 km). Out of the 253 possible pairwise comparisons of distances between fields, 247 could be classified within the mesoscale [10 to 200 km apart, *sensu* Francl et al. (1999)] level.

Spatially referenced (by planar coordinates) counts of the number of *Fusarium*-infected seeds were analyzed by the spatial analysis by distance indices (SADIE) software program (Perry et al., 1996). SADIE is a form of spatial correlation analysis that is conditioned on the existing heterogeneity of counts within a specified area. The SADIE index I_a is a measure of the spatial association. Values of $I_a > 1$ would indicate that fields with similar levels of seed infection are closer together than expected by chance alone. Significance testing is done by permuting the observed data set under the hypothesis that there is no association among fields in seed infection level. For this study, 5,967 randomizations were done.

RESULTS AND DISCUSSION

Percent seed infection by *Fusarium* spp. ranged from 0 to 24 (mean 8.4). The evidence for associations among fields in terms of seed infection by *Fusarium* was not strong ($I_a = 1.569$, $P = 0.0508$). Results therefore support the hypothesis that winter wheat fields are spatially random at the mesoscale with respect to *Fusarium* seed infection. Therefore, site-specific conditions at the local scale (100 m to 50 km) are of greater importance in determining the actual levels of seed infection than overall regional weather patterns. For example, two of the fields in the analyzed data set were only 1 m apart, yet percent seed infection in one (5%, cultivar Cayuga) was three times that in the other (18%, cultivar NY Batavia). Differences in flowering date, cultivar resistance to *Fusarium* or local inoculum variability may significantly impact seed infection levels.

Large-scale weather patterns affect the overall mean seed infection incidence in a given region, but the range of seed infection incidence in any year is more strongly influenced by local variables.

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THE BETA-BINOMIAL DISTRIBUTION DESCRIBES THE INCIDENCE OF SEED INFECTION BY *FUSARIUM GRAMINEARUM* AMONG SEEDLOTS IN A RREGION

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INTRODUCTION

In cereal seed health assessments, an individual seed is either infected by *Fusarium graminearum* or not. If repeated samples are drawn from a well mixed seedlot, the incidence of seed infection within such samples is expected to follow a binomial distribution. The mean incidence of infection is described by the binomial parameter, *p*. If a number of different seedlots are assayed for the same pathogen, the probability of seed infection can be expected to vary from lot to lot for any of a number of reasons, including local environment, inoculum availability, and the relative susceptibilities of cultivars to seed infection. That is, *p* may vary across seedlots. A common way of accounting for nonconstant *p* is to assume that *p* itself is a variable with a beta distribution. If *p* has a beta distribution, the resultant mixture distribution is a beta-binomial distribution (Madden and Hughes, 1995). Here we demonstrate that the beta-binomial distribution is a superior (to the binomial distribution) and adequate descriptor of cereal seed infection incidence by *Fusarium graminearum* across several seedlots from a production region.

MATERIALS AND METHODS

The binomial and beta-binomial distributions were fit to seven separate data sets on the incidence of seed infection by *Fusarium graminearum* in three cereals (Table 1). Data sets 1 to 3 are on the infection of wheat seed in Kansas by *F. graminearum* (Love and Seitz, 1987). Summaries of data sets 4 to 6, on the infection of barley and oat seed in Manitoba by *F. graminearum*, were previously published (Clear et al., 1996).

The binary form of the index of dispersion (D) (Madden and Hughes, 1995) was calculated. Values of D > 1 suggest that the variance in seed infection incidence among lots is greater than that expected assuming seed infection incidence across seedlots is distributed binomially (a phenomenon called overdispersion). The quantity has a distribution (N-1 degrees of freedom) under the null hypothesis that seed infection incidence is randomly distributed, where N is the number of seedlots (Madden and Hughes, 1995).

The BBD software program (Ver. 1.3) (Madden and Hughes, 1994) was used to calculate D and to fit the binomial and beta-binomial distributions to data sets. A likelihood ratio test assessed whether the beta-binomial provided a better fit to the data than the binomial distribution.

RESULTS AND DISCUSSION

All data sets were overdispersed (Table 2). Therefore, seed infection incidence by *F. graminearum* varies across seedlots in a manner that cannot be explained by binomial variation only. Extra-binomial variation may be due to cultivar differences in seed infection, differences in inoculum availability, flowering date or to site-specific environment. The beta-binomial distribution was a significantly better descriptor than the binomial distribution of seed infection incidence for all seven data sets examined (Table 2, Fig. 1).

The parameters of the beta-binomial distribution, estimated from a relatively small random sampling of seedlots, are a concise summary of the regional incidence of cereal seed infection by *F. graminearum*. Moreover, a distributional approach to describing seed infection incidence allows one to estimate probabilities associated with seed infection incidences not in the empirical data set.

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Randy Clear of the Canadian Grain Commission supplied the data on seed infection incidence in Manitoba and Quebec cereals.

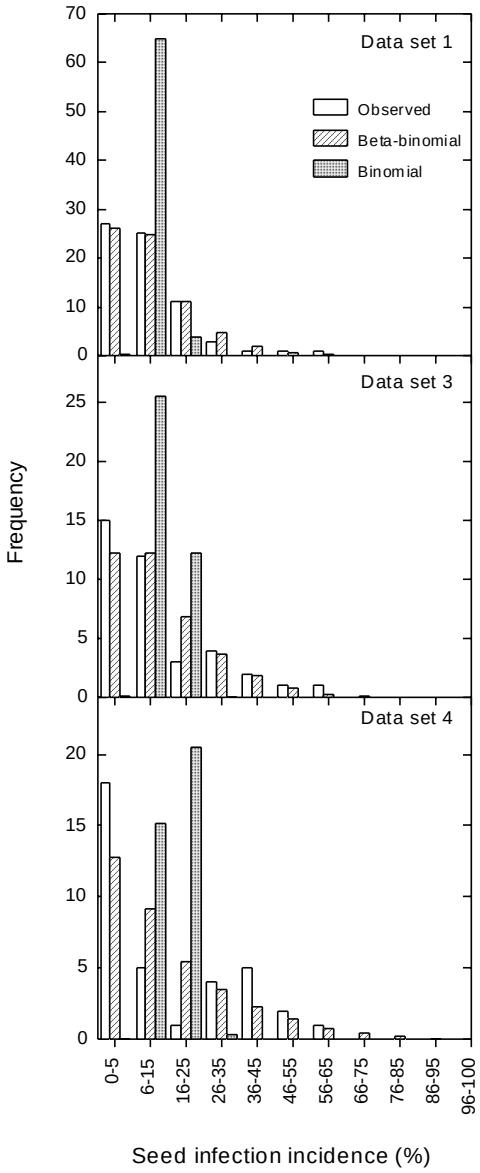

Figure 1. Fit of the binomial and beta-binomial distributions to seed infection incidence by *Fusarium graminearum* across cereal seedlots.

Table 1. Data sets used to assess the fit of the binomial and beta-binomial distributions to *Fusarium graminearum* seed infection incidence.

Data set	Source	Crop	Year	No. of seedlots	No. of cultivars	No. of locations
1	Kansas	wheat	1982	23	2	12
2	Kansas	wheat	1983	24	2	12
3	Kansas	wheat	1982	36	18	2
4	Manitoba	barley	1993	78	?	78
5	Manitoba	barley	1994	45	?	45
6	Manitoba	oat	1993	30	?	30
7	Quebec	wheat	1989	31	9	31

Table 2. Results of fitting the binomial and beta-binomial distributions to seed infection incidence by *Fusarium graminearum*.

Data set	D[a]	P_{LRS}[b]	$\hat{p}$[c]	$\hat{\theta}$[c]	P_{χ^2}[d]
1	39.89	< 0.0001	0.1537	0.4955	0.161
2	4.51	< 0.0001	0.0276	0.0525	n.d.
3	8.13	< 0.0001	0.3847	0.0718	0.605
4	41.49	< 0.0001	0.4215	0.6365	0.007
5	13.81	< 0.0001	0.4379	0.2558	n.d.
6	41.03	< 0.0001	0.1443	0.5769	0.090
7	2.42	< 0.0001	0.0425	0.156	0.002

[a] Index of dispersion. Values of D>1 indicate that the variance in seed infection incidence is greater than that expected if seed infection incidence is binomially distributed. A test was used to determine whether D differs from its expected value assuming seed infection incidence follows a binomial distribution (Madden and Hughes, 1995). In all seven instances $P < 0.001$.

[b] Significance level for a likelihood ratio test of whether the beta-binomial distribution fits the data better than the binomial distribution (Turechek and Madden, 1999).

[c] Maximum likelihood estimates of the parameters of the beta-binomial distribution.

[d] Probability associated with a goodness-of-fit test of the beta-binomial distribution. $P > 0.05$ indicates a significant fit of the beta-binomial to the data at a=0.05, for example. n.d. means that it was not possible to do a goodness-of-fit test.

SAMPLING SPORES OF FUSARIUM GRAMINEARUM

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INTRODUCTION AND OBJECTIVES

In order to develop effective control measures for Fusarium head blight, it is necessary to understand the epidemiology of the disease. One important epidemiological question is how weather influences production and release of inoculum, infection, and symptom development. This study is part of a 4-state collaborative study (North Dakota, South Dakota, Indiana, Ohio) aimed at generating a body of comparable data over diverse environments to answer these questions. Here we report results of studies conducted in Indiana during the 2000 growing season.

MATERIALS AND METHODS

Inoculum levels were monitored at two sites at the Purdue University Agronomy Research Center (PARC) during the 2000 wheat season. At one site (Field 53), wheat cultivar Clark was sown into disked corn residue. At the other site (IPM), wheat cultivar INW9513 was sown following soybeans in a plot that was adjacent to a plot that had corn residue from the previous crop year on the soil surface. The distance from the corn residue to the edge of the wheat plot (where inoculum was monitored) was about 3 m.

Burkard cyclone spore samplers were operated at both sites. Sampling began in field 53 on 1 May, when wheat was in the early boot stage (GS 44) and on 8 May in the IPM field when heads were 50% emerged (GS 55). The samplers drew in 16.5 L of air per minute. Each day at 1100 hours, the Eppendorf tube into which airborne particles were collected was replaced in each sampler.

Spores were recovered from the Eppendorf tube by adding 1 ml sterile water, shaking the tube on a Vortex mixer for 1 minute, and spreading 0.3 ml of the suspension over Komada's medium in each of two petri plates. The plates were placed in an incubator programmed to maintain 25 °C during a 12-hr photoperiod and 20 °C during a 12-hour dark period each day. After 1 week, colonies of Fusarium graminearum were counted on each plate. A sample of the remaining spore suspension was examined under a hemacytometer for conidia or ascospores of Gibberella zeae.

Beginning 12 May, when wheat in field 53 was in mid anthesis (GS 65) and heads in the IPM field were three-fourths emerged (GS 57), 5 heads were collected each day at about 1030 hours. In the laboratory, the 5 heads were placed in 50 ml of sterile water that contained 2% Tween 20. They were shaken vigorously for 1 minute, then 1-ml samples were plated onto each of 5 petri dishes containing Komada's medium. Plates were placed in the incubator described above. After 1 week, colonies of Fusarium graminearum were counted on each plate.

On 30 May (GS 77) scab incidence was estimated by counting the number of blighted heads in each of ten 60-cm lengths of row. Incidence was estimated again on 6 June (GS 79), but the samples were 30 cm long.

A Campbell weather station was operated in field 53, at the same location as the Burkard sampler. This unit recorded various weather parameters at 30-min intervals.

RESULTS

Spores of G. zeae were recovered by both samplers from the beginning of the sampling period until early June, when sampling ceased (Figs. 1 and 2). At both sites, there were days when no propagules were recovered. From days 131 (9 May) through day 138 (17 May), there was general agreement in the number of spores sampled at each site. From day 139 (18 May) through the end of the sampling period (28 May), however, there was little agreement between data from the two sites. Peaks in spore numbers in field 53 occurred when few spores were recovered at the IPM field. There was an irrigated head blight nursery about 100 m to the southeast from the spore sampler in field 53. The peak in spore numbers seen on days 142 and 143 in field 53 but not in the IPM field may have been an effect of moisture provided to corn residue by the irrigation during a period when no rain fell. Despite this discrepancy during the latter part of the sampling period, there was a significant correlation between spores collected at both sites ($R=0.39$, $P=0.04$).

During the first half of the spore sampling experiment, rain fell at least every 2 or 3 days. During this time, the number of spores collected in field 53 and the amount of rainfall were not significantly correlated ($R=0.51$, $P=0.06$). Spores were collected on every day during which rain fell. However, spores were also collected on days with no rain. The same pattern was seen for spores collected in the IPM field for the full duration of the experiment, and for this data set the correlation was significant ($R=0.71$, $P=0.002$). We also examined the relation between number of spores collected and the number of hours that relative humidity was less than 75% each day. There was no correlation at either site. A more thorough analysis of the effect of weather on number of spores in the air will be conducted when data from several cooperating states have been pooled.

The numbers of spores recovered each day from heads collected at the two sites were correlated ($R=0.48$, $P=0.02$). At both sites, there was no correlation between the number of spores recovered by the Burkard sampler and the number of spores recovered from heads during a given 24-hour period. However, if the daily pattern of spores collected by the Burkard sampler is compared with the number of spores collected from heads, there is a rough concordance for the data from field 53 (Fig. 1). When we compared the number of spores from heads on a given day against number of spores recovered by the Burkard unit on the previous day, the correlation was significant for the field 53 data ($R=0.61$, $P=0.001$), but not for the IPM field data.

Scab incidence in field 53 was 2.9% on 30 May (GS 77) and 6.4% 1 week later. Scab incidence in the IPM field was 4.4%. Incidence of scabby kernels in the grain harvested from field 53 was 2.2% and the level of DON was 2.6 ppm.

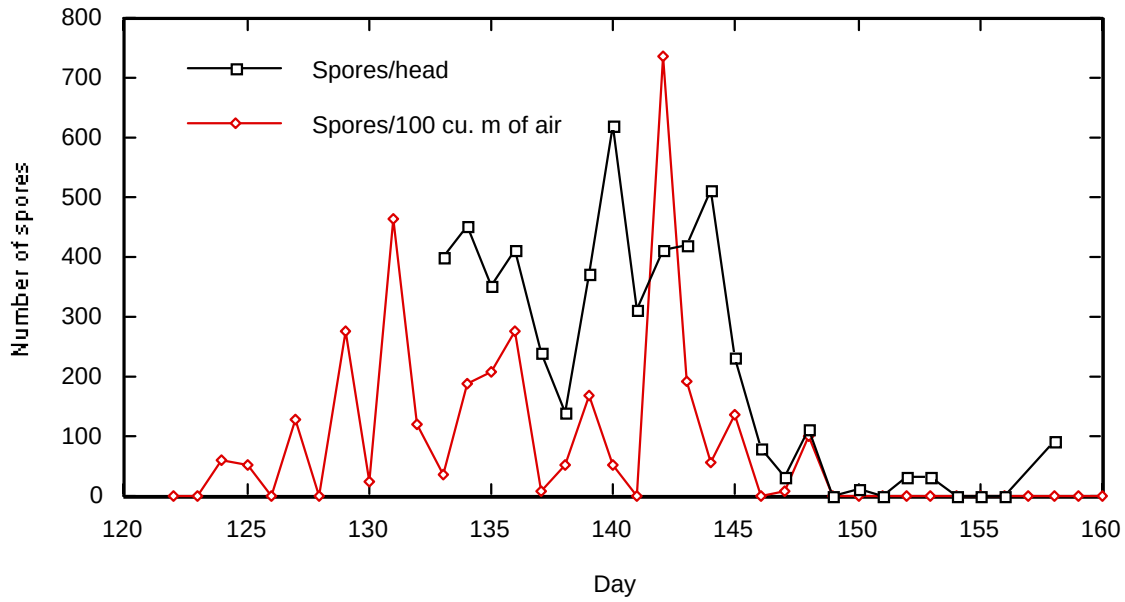

Figure 1. Number of spores of Gibberella zeae collected each day in field 53 of the Purdue Agronomy Research Center, beginning at 1100 hours, with a Burkard volumetric spore sampler or recovered from heads.

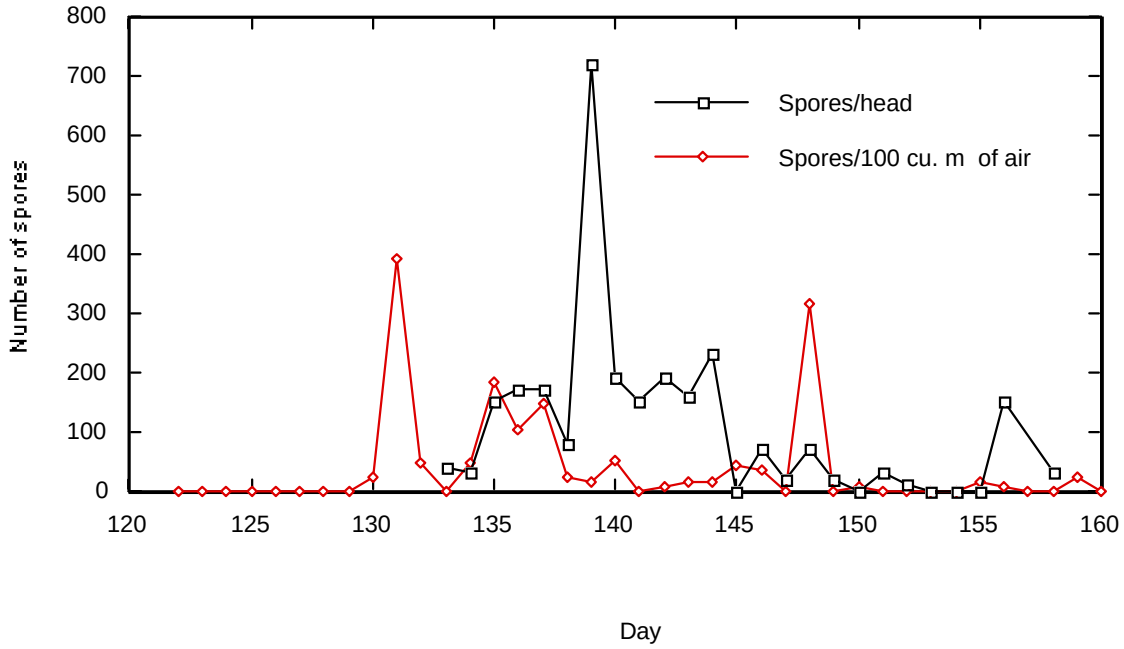

Figure 2. Number of spores of Gibberella zeae collected each day in the IPM field of the Purdue Agronomy Research Center, beginning at 1100 hours, with a Burkard volumetric spore sampler or recovered from heads.

DISCUSSION

There were no periods of sustained rainfall from the time of wheat anthesis through grain filling, in contrast to what occurred during the same wheat developmental period in 1996, when a major head blight epidemic occurred. Nonetheless, the Burkard volumetric spore samplers collected spores of F. graminearum on many days during anthesis and early grain filling. Spores were also recovered directly from head washings during this period. The incidence of head blight was light to moderate, as was the incidence of scabby kernels. This suggests that weather conditions may have been limiting for infection rather than for production of inoculum.

There was a weak correlation between spores collected by the Burkard samplers at two locations separated about 1 km apart. This suggests that local conditions as well as general weather conditions influence production and release of spores.

There appears to be a large discrepency between the number of spores recovered from heads and the number of spores collected from a volume of air during a 24-hour period. We will look at this relation further by using wind speed data to estimate the volume of air intercepted by a wheat head during the course of a day.

Limited inferences about the influence of weather on spore release and dispersal can be made from one year's data. A 4-state collaborative study, now in its second year, will provide a greater number of data points from which to draw inferences about the effect of weather on production and dispersal of inoculum, infection, and disease development

FUSARIUM HEAD BLIGHT IN BARLEY IN ONTARIO IN 2000

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OBJECTIVES

The objectives of this study was to identify the percent of kernels infected with *Fusarium* species in spring barley cultivars included in the Ontario Spring Barley Performance trials, and from a nearby commercial field of variety Chapais, with a high level of disease, to check the pathogenicity of three isolates of *F. graminearum* collected from barley on winter wheat seedlings, and to evaluate fungicide efficacy against FHB in spring barley, using the fungicides with known response against FHB in wheat.

INTRODUCTION

Fusarium head blight (FHB) epidemics are common in Ontario wheat, and corn, but have been rarely reported in barley (*Hordeum vulgare*). FHB is observed frequently in barley grown in the Red River Valley of Western Canada and North Central USA, while in Eastern Canada FHB was observed for the first time on barley in 1986 (R. Clear, personal communications), but there is no published information. This year, visible FHB symptoms on spring barley were noticed as tan or brown florets in the spike. In harvested grain, Fusarium damaged kernels (FDK) were also observed. Tekauz et al. (2000) noted that FHB in barley may result from a combination of factors such as a fundamental shift in the pathogen population, new varieties being grown in the region, and environmental conditions promoting disease. According to Campbell et al. (1999) DON contamination in Eastern Canada was particularly severe in recent years (1996, 1997, 1998), when some barley samples contained up to 8-9 mg/kg of toxin. After artificial inoculation with *Fusarium graminearum* in Ridgetown, ON, in 1997 (Schaafsma, and Tamburic-Ilincic, 1997) mean DON level across entries were higher in barley then in wheat (3.98 ppm, vs 2.23, respectively), as well as percent of seeds infected with *F. graminearum* (49.46, vs 15.81, respectively).

MATERIALS AND METHODS

Forty six spring barley cultivars from the Ontario Spring Barley Performance trials were evaluated for percent of kernels infected with *Fusarium* spp. Eighteen of them were two-row feed barley, while twenty eight were six-row feed barley. After harvest, the seed was surface sterilized in a 3 % sodium hypochlorite solution for three minutes, air dried and placed on acidified potato dextrose agar (PDA). These were incubated for seven days under light on a 12:12 hr light/dark cycle, at room temperature. *Fusarium* spp. colonies were then transferred to carnation-leaf agar (CLA), and incubated as above. The identification was done according to Nelson et al. (1983), and Burgess et al. (1988).

Two varieties of spring barley (Chapais- six-row barley , and Morrison- two-row barley) were grown in a randomized complete block design with four replications. The fungicides applications were made at 50 % anthesis for each variety. Deoxynivalenol (DON) content was estimated using competitive ELISA test (Sinha and Savard, 1996). Individual spikes were collected from a commercial field of Chapais barley on the Elora Research Station then dried and threshed in bulk. Seeds were treated as previously described to obtain Fusarium colonies.

Pathogenicity tests were conducted on Knop medium (1 g KNO3; 0.12 g KCl; 0.25 g KH_2PO_4; 0.25 g $MgSO_4$ x H_2O; in trace FeCl x 6 H_2O; 15 g agar, and 1 L distilled water) in glass tubes under laboratory conditions, according to Levic and Tamburic (1996). Subcultures of the three isolates of *F. graminearum* from spring barley varieties (Chapais- isolate from the commercial field, AC Stephen, and C231-041), were grown on PDA medium at room temperature for 1 week. Plugs of inoculated PDA (4x4 mm) were placed on the medium, then the surface sterilized kernels from three wheat varieties (AC RON- highly susceptible, Harus-susceptible, and Freedom-moderate resistant) were placed 2 cm above. Controls were fungus-free. Fourteen-day-old seedlings were evaluated using scale from zero (no symptoms) to five (the mycelium covered seed, roots discoloured, and seedling growth stopped).

RESULTS AND DISCUSSIONS

Fusarium spp. colonies were isolated from sixteen cultivars of spring barley from Ontario Performance trial (34.8%). Differences in susceptibility to *Fusarium* were found among cultivars, based on percent of seed infected with *Fusarium* spp. For the group of barley varieties tested, two-rowed types had less FHB than six-rowed types. These results are consistent with those of Tekauz et al. (2000). AC Sirius, AC Stephen, and C231-041 were the most susceptible genotypes to FHB. *F. graminearum* was the predominant species (68 %), followed by *F. sporotrichioides* (10 %), *F. poae* (8 %), *F. equiseti* (6 %), *F. verticillioides* (4 %), *F. subglutinans* (2 %), *and F. proliferatum* (2%). The same *Fusarium* species have been identified from wheat, and corn crops grown across Ontario (1996-2000). There was correlation between percent of *Fusarium* spp. and *F. graminearum* isolated from infected seed (r= 0.967). Thirty eight percent of the seed from the commercial field of Chapais barley was infected with *F. graminearum*.

Morrison (two-row barley) responded better to protection by fungicides than did Chapais (six-row barley). Mean DON content was (1.8 versus 6.5 ppm). The highest rate of FOLICUR, reduced DON level significantly on the variety Morrison.

The *F. graminearum* isolates from barley crop, were pathogenic on the three wheat varieties tested (Table 3). The differences in the wheat genotype's reactions to barley isolates which were similar to their reactions to wheat isolates (DAOM178148).

Table 1. (previous page) Fusarium head blight reaction of 46 spring barley cultivars after naturally infection at Elora Research Station, Guelph, Ontario, 2000.

Treatment #	Cultivar	Percent seed infected	Percent seed infected by F. graminearum
1	AC KINGS	0	0
2	AC PARKHILL	1	1
3	AC SIRIUS	9	7
4	AC STERLING	0	0
5	ALMONTE	0	0
6	BELMORE	0	0
7	FORMOSA	0	0
8	MORRISON	0	0
9	SUNDERLAND	0	0
10	VIKING	0	0
11	CM96503	0	0
12	T123-172	0	0
13	T125-053	0	0
14	T169-055	0	0
15	T193-198	1	0
16	96/1110	0	0
17	96/1114	0	0
18	AB168-11	0	0
19	AC ALMA	2	1
20	AC HAMILTON	0	0
21	AC LEGEND	1	1
22	AC STEPHEN	9	5
23	AC WESTECH	0	0
24	ACCA	1	0
25	BRUCEFIELD	0	0
26	CHAPAIS	5	5
27	FOSTER	2	1
28	GRANT	1	0
29	LEGER	0	0
30	MYRIAM	0	0
31	NELLYGAN	0	0
32	OAC BAXTER	3	2
33	OAC KIPPEN	0	0
34	SANDRINE	0	0
35	VIVIANE	0	0
36	AB186-3	0	0
37	ABI89	0	0
38	CM862.6	1	0
39	CI62-120	0	0
40	C166-050	0	0
41	C229-004	0	0
42	C231-041	7	5
43	OBS4065-125	3	2
44	OBS4065-157	1	1
45	OBS4181-43	3	3
46	OS94-544	0	0
Total		50	34

Table 2. Fusarium head blight control in spring barley (Chapais and Morrison) with foliar application of fungicides. Ontario, 2000.

Treatments	Rate product/ha	Chapais DON (ppm)	Morrison DON (ppm)
Control		6.5	2.3
FOLICUR 432 F	289 mL	6.8	1.8
FOLICUR 432 F	364.6 mL	5.9	1.3
TILT 250 EC	500 mL	6.7	1.6
Mean		6.5	1.8
LSD (P=.05)		1.5	0.8

Table 3. The pathogenicity tests of *F. graminearum* isolated from barley on three winter wheat cultivars AC RON, Harus, and Freedom, using scale from zero (no symptoms) to five (the mycelium covered seed, roots discoloured, and seedling growth stopped).

Isolate	AC RON	Harus	Freedom
Control	0	0	0
DAOM178148	4.8	4.61	3.72
# 22	3.99	2.75	3.64
# 42	5	4.5	2.42
Chapais	4.55	4.82	4.3
Mean	4.59	4.17	3.52
LSD (P=.05)	0.5	1.52	1.01

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THE MECHANISM OF FORCIBLE DISCHARGE OF ASCOSPORES IN *GIBBERELLA ZEAE*

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ABSTRACT

In *Gibberella zeae*, ascospores are forcibly discharged from perithecia forming on debris and are believed to form the primary inoculum of head blight disease. The mechanism of forcible discharge of ascospores has not been well studied in any ascomycetous fungus. We are using physiological and genetic studies to elucidate this mechanism. It is clear that osmotic pressure builds up within the ascus and serves as the force behind discharge. We have identified several factors that are likely involved in this increase in pressure. In addition, the use of insertional mutagenesis to isolate discharge minus mutants is in progress. Analysis of discharge minus mutants will provide insight into this mechanism. This work should identify novel targets for control of *Gibberella zeae*.

AFLP MARKERS INDICATE LITTLE DIVERGENCE BETWEEN U.S. CORN BELT POPULATIONS OF *FUSARIUM GRAMINEARUM* (*GIBBERELLA ZEAE*)

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ABSTRACT

In 1999, we surveyed wheat-scab populations from five states within the U.S. Corn Belt. We isolated *Fusarium* samples either directly from scabby wheat heads, or from individual seeds within contaminated seed-lots. We have examined 42 polymorphic AFLP loci for 71-75 randomly selected *Fusarium* isolates from each tested population. The percentage of thesse isolates that we identified as *Fusarium graminearum* (*Gibberella zeae*) ranged from a low of 79% (56/71) from an Illinois seed-lot, to 100% (71/71) from a Kansas wheat head sampling. Divergence among *F. graminearum* populations from these five states was low. Pairwise G_{ST} values ranged from only 0.013 to 0.040, and indicate high rates of effective migration (*Nm*) between populations. We observed a positive, but statistically insignificant, correlation ($r = 0.46$, $p = 0.18$) between inter-population geographic separation and G_{ST} values. These data provide support for the hypothesis that regional populations of *F. graminearum* within the U.S. Corn Belt are part of a single, largely panmictic, metapopulation.