PROGRESSION OF *FUSARIUM* SPECIES ON WHEAT LEAVES FROM SEEDLING TO ADULT STAGES IN NORTH DAKOTA

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ABSTRACT

A more complete inventory of inoculum sources would help our understanding of epidemics of Fusarium head blight (FHB). We hypothesize that *Fusarium* species can colonize and survive on leaves in the US northern Great Plains. In 2001, two hard red spring wheat cultivars Alsen (resistant to FHB) and Oxen (susceptible) were planted in a wheat stubble or plowed area in three replicate subplots, each 1 X 2 m. Twenty healthy (asymptomatic) and diseased (with necrotic leaf spots) leaf samples of each cultivar were collected from the 4-leaf to the early milk stage. Leaf samples were collected on 6/7, 6/14, 6/21, 6/28, and 7/5. The leaves were cut into 2 cm pieces. Ninety-eight leaf pieces of each sample were plated on Komada's medium. Forty-nine of 98 leaf pieces were surface disinfected with 5% sodium hypochloride prior to plating. The leaf pieces were incubated at 22 C with an alternating cycle of 12 h light and 12 h dark for 10 days. Different colony types of Fusarium species were counted and transferred onto ½ strength PDA for species identification. Twelve *Fusarium* species were isolated: *acuminatum, avenaceum, equiseti, graminearum, moniliforme, proliferatum, poae, sporotrichioides, subglutinans, scirpii,* and *semitectum*. *Fusarium graminearum* and *Fusarium sporotrichioides* were the most prevalent species after *Fusarium equiseti* throughout the season. *Fusarium graminearum* was isolated from healthy disinfected leaves (0-6%, depending on date sampled), healthy nondisinfected leaves (2-12%), diseased disinfected leaves (2-18%), and diseased nondisinfected leaves (4-52%). *Fusarium sporotrichioides* was isolated from healthy disinfected leaves (0-14%), healthy nondisinfected leaves (0-16%), diseased disinfected leaves (0-18%), and diseased nondisinfected leaves (2-42%). *Fusarium avenaceum* was isolated from both healthy (0-6%) and diseased (0-10%) leaves but *F. poae* was observed only on diseased leaves (0-22%). Diseased leaves produced pathogenic Fusaria at a higher frequency than healthy leaf samples but a difference between the plowed and stubble areas was not detected. The results indicate that *Fusarium* species associated with FHB, such as *graminearum*, *sporotrichioides, avenaceum,* and *poae*, can survive parasitically and saprophytically on leaves throughout the season; furthermore, these leaves may contribute additional inoculum for FHB development in the northern Great Plains. Management of foliar pathogens may help in reducing FHB intensity by decreasing the amount of inoculum.

EFFECT OF *FUSARIUM GRAMINEARUM* INFECTION DURING WHEAT SEED DEVELOPMENT ON SEED QUALITY

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OBJECTIVES

1) Determine the time of infection of *Fusarium graminearum* during wheat seed development and its effect on 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. Head scab epidemics can not only result in significant yield losses, but also cause serious reductions in seed quality. In contrast to seeds used for other purposes, seeds planted to regenerate the crop must be alive and possess those physiological traits that allow germination and seedling establishment (TeKrony and Egli, 1991). The pathogen may affect both the physical and the physiological aspects of seed quality, including seed size, composition, germination, and vigor. In addition, losses in food-grain quality are caused by production of the fungal mycotoxin deoxynivalenol (DON), which, in high levels, renders grain unsafe for human or animal consumption, and is detrimental to planting seed quality.

Previous studies have examined seed germination, vigor and DON concentrations of mature wheat seeds based on visual assessment of kernel disease severity. However, relatively few studies have investigated the development of *F. graminaerum* infection during seed development and maturation and its influence on planting seed quality. Likewise, little information is available regarding when peak infection occurs during seed development and maturation and how these infection levels relate to seed germination and vigor and the production of DON. 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 FHB research initiative and the farmer.

MATERIALS AND METHODS

Replicated plots of four soft red winter wheat cultivars differing in Type II resistance to *F. graminearum* were established following corn in a chisel plowed and disked seedbed on Spindletop Farm in Lexington, KY in October of 2000. Cultivars included one susceptible (Pioneer 2552), two moderately resistant (Roane, Coker 9474) and one resistant (Pioneer 25R18) line. The corn seed inoculation procedure used to initiate FHB epidemic conditions was modeled after the method of Paulitz (1996) and inoculum was distributed among field plots on March 24, 2001. Mist irrigation was initiated on April 10, 2001and continued

through June, 4 to stimulate FHB epidemic conditions. Ambient air temperature and precipitation were measured at the field site. At anthesis (Feekes 10.2), approximately 1,200 spikes in each of two replications of each cultivar with anthers extruded in mid-spikelet were identified. At four days after anthesis (DAA) 75 previously marked heads were harvested, and harvesting continued at four-day intervals for a total of ten harvests in each cultivar.

Fresh weight, dry weight and seed moisture were determined at each harvest for all cultivars. Thirty heads from each cultivar were evaluated at each harvest for disease incidence and severity according to Stack and McMullen (1998). One-hundred seeds from each harvest were assigned a numerical rating as an indicator of disease severity and classified as normal, normal-discolored, brown-shriveled, chalky-shriveled, and white tombstone (levels 5 to 1 respectively) prior to plating for infection.

One hundred fresh seeds from seed of approximately 35 heads was separated from chaff, surface sterilized, plated on modified PDA medium, and evaluated for F*usarium graminearum* infection approximately seven days post-harvest. Standard germination (AOSA, 1999) accelerated aging germination, a stress vigor test, and the conductivity test for membrane integrity (Hampton and TeKrony, 1995) were conducted for seed of all harvests.

RESULTS AND DISCUSSION

Anthesis occurred in all cultivars between May 10-14, 2001. Physiological maturity (PM, maximum seed dry weight) occurred between 30 and 32 days after anthesis (DAA). F. graminearum seed infection (freshly harvested seed) increased in all cultivars from = 20% at 10 DAA, to maximum levels (>95%) at 37-40 DAA, which were maintained until the final harvest (~50 DAA, Fig. 1A). Seed infection for Coker 9474 was slightly lower than that of P-2552, P-25R18, and Roane until 30 DAA, when all cultivars began to exhibit similar infection levels. Average seed infection over all 10 harvests was significantly higher for P-2552 and Roane than P-25R18 and 9474 (63.2 and 62.7% vs. 56 and 51% respectively). Two large precipitation events preceded the most abrupt increases in seed infection with one of these occurring after irrigation was stopped (23 DAA). Roane and P-2552 provided consistently higher visual estimates of spikelet infection than P-25R18 and 9474 at each harvest date. A significant linear relationship was shown between visual estimate of spikelet infection (severity) and *F. graminearum* seed infection for the first 5 harvests in all cultivars (r^2 = 0.858, Fig. 1A, inset).

Standard germination (SG) of untreated seed for the four cultivars was highly variable in early harvests (Fig. 1B) ranging from <40% (Coker 9474) at 10 DAA, to above 80% at 19 DAA. Germination declined to unacceptable commercial quality (<80%) in all cultivars by 25 DAA and continued to decline to approximately 30% at the last harvest. Although the germination of all cultivars was low, P-25R18 showed the highest germination at PM (57%), which was significantly higher than the other three cultivars for the remaining harvest dates. Standard germination was significantly lower for P-2552 (43%) across all harvests compared to P-25R18, Roane, and 9474 (mean = 51% for all 3 cultivars respectively). Standard germination of treated seeds was significantly higher than untreated (Fig. 1B, inset) for the last five harvest dates, with germination maintained above 50%. Germination of treated seed

of P-25R18 was greater than other cultivars, with SG ranging from 75% at PM, to ~65% at nearly 50 DAA. Overall means for treated seed across all cultivars was 61% compared to 48% in untreated seed. As expected, there was a significant correlation between SG (untreated) and *F. graminearum* seed infection (r2 = 0.567).

Small grain crops are most susceptible to infection during the flowering period and infection continues up to the soft dough stage of kernel development (McMullen and Stack, 1997). Type I resistance to initial infection appeared to be slightly more prevalent in Coker 9474, based on isolation of *Fusarium graminearum* from seed, and the visual rating of spikelet infection. Interestingly, this advantage was not readily obvious when measuring seed quality. Seed infection levels were very high in all cultivars, including the Type II resistant Pioneer 25R18. Concurrently, germination was very poor in all cultivars, with P-25R18 showing marginal improvement in the last five harvests compared to the others. High levels of field inoculum resulted in very high seed infection levels, which may have masked the advantages of Type II resistance ascribed to P-25R18, but provided a selective environment for evaluation of Type I resistance. Measures of seed quality (SG, seed vigor, etc.) could potentially be useful in assessing varieties earlier in development for Type I resistance and also resistance to kernel infection, as described by Mesterhazy (1995). This could serve as an additional protocol for breeder selection of promising germplasm. Retention of seed germination after PM may be of great interest to seed producers, since a crop could be harvested earlier (swathing at PM) to avoid late season disease pressure and declines in seed quality.

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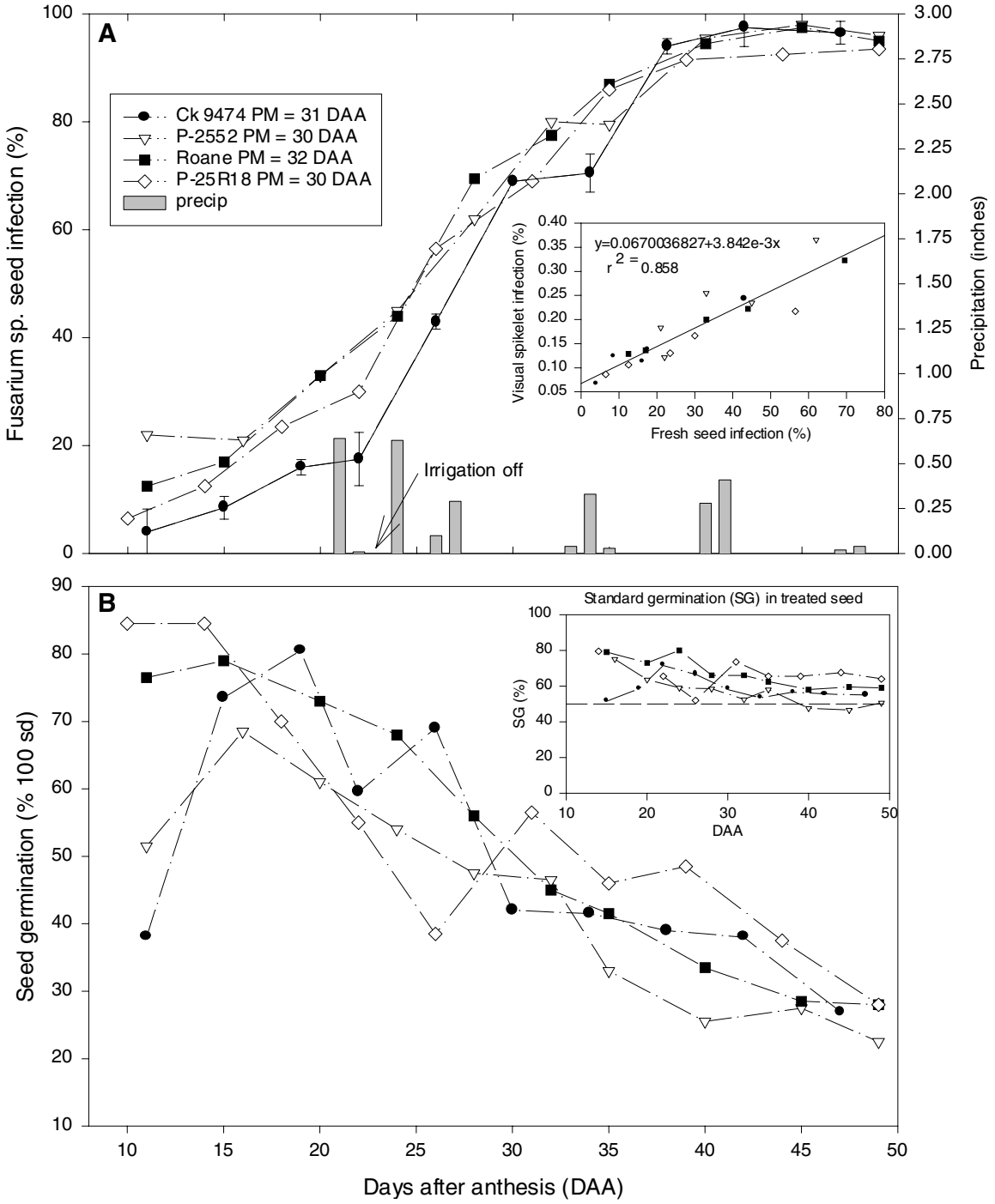

Figure 1. *Fusarium graminearum* seed infection during development in four varieties of wheat and effects on germination of treated and untreated seed.

ARE *GIBBERELLA ZEAE* SEXUAL SPORES THE CRITICAL INOCULUM LEADING TO FHB EPIDEMICS?

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) causes Fusarium head blight (FHB) epidemics in wheat and barley and ear rot in corn. Fungal infections decrease yield and often contaminate grains with trichothecene mycotoxins that are harmful to human and animal health. To understand and control fungal disease, the factors and conditions that lead to epidemics must be identified. The fungal life cycle in the field can include growth on plant debris in the soil during which two types of spore may be produced: sexual spores known as ascospores and asexual spores called macroconidia. Observations in the field suggest that the sexual spores may be a primary source of inoculum for FHB epidemics. In order to test the role of sexual spores, we deleted the entire mating type locus (MAT) that controls sexual reproduction. *G. zeae* MAT-deletion strains appear similar to wild-type (GZ3639) in morphology and in their ability to make macroconidia but no longer make sexual spores. In greenhouse tests, macroconidia from MAT-deletion strains caused disease and produced trichothecenes following inoculation into wheat heads. To test the importance of sexual spores in disease development, we conducted a field test in Spring 2001 in which we compared the ability of GZ3639 and a MAT-deletion strain to cause FHB on wheat. In order to focus our experiment on ascospores, we chose autoclaved corn stalk pieces as an inoculum source because GZ3639 can produce a large amount of ascospore-containing perithecia on it. We inoculated three plots (3m x 3m) of Wheaton wheat, with either sterile corn stalks, corn stalks inoculated with GZ3639 or corn stalks inoculated with a MAT-deletion strain. We scattered 100 stalks per plot between wheat rows at three weeks and at one week prior to flowering. Seed yield data and trichothecene analysis indicated that the MAT-deletion strain caused less disease and resulted in less trichothecenes than GZ3639. These results suggest an important role for sexual spores in FHB disease severity and toxin level and suggest that new control strategies that target *Fusarium* ascospore production might lead to significant reduction in the negative impact this fungus has on agricultural products.

WHAT IS KNOWN ABOUT INFECTION PATHWAYS IN FUSARIUM HEAD BLIGHT?

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ABSTRACT

The epidermis of the exposed outer surfaces of the florets of wheat and barley and the glumes subtending wheat spikelets consists of very thick-walled cells. These armored cells apparently are not penetrated directly from outer surfaces by *Fusarium graminearum* or other head blight Fusaria. However the glume, as well as the palea and lemma that enclose the floret, each have several rows of stomates which can be entered by hyphae of *F. graminearum.* Whether such stomatal entry leads to significant fungal invasion of florets or glumes is uncertain, although thin-walled chlorenchyma cells located beneath stomates are postulated to be sites of colonization following stomatal penetration. Another possible avenue of entry is the mouth at the apex of the floret. Exposed adaxial surfaces of the awn, palea and lemma near the floret mouth can be colonized by *F. graminearum* mycelia which then extend along adaxial surfaces basally into the mouth opening where they can colonize caught and retained anthers or the apical brush of the caryopsis (abstract of Lewandowski and Bushnell). The mycelia can also colonize interior surfaces of the palea and lemma . Another potential pathway of entry is the crevice between the palea and lemma, especially near the floret base (Kang and Buchenauer, Mycol. Res. 104:1083-1093, 2000; abstract of Lewandowski and Bushnell). Whether entering from mouth or crevice, the fungus can develop abundantly on and within interior tissues of the lemma and palea. Surface tissues of the ovary and lodicules are especially susceptible (Tu, D.S., Ph. D. Thesis, Ohio State Univ., 1950). However, the mode of penetration into these interior tissues has not been determined. Within tissues, *F. graminearum* can grow between cells instead of entering them, establishing a biotrophic relationship with host tissues. How and where the intercellular fungus penetrates cells for subsequent growth within cells is not known. Eventually, virtually all interior floret tissues can become heavily colonized. Once established within the floret, the fungus colonizes and follows vascular tissues in the floret stalk through the rachilla or rachis into other florets. Wall appositions containing callose and lignin have been implicated as factors reducing fungus spread in heads of resistant wheat (Kang and Buchenauer, Physiol. Mol. Plant Pathol. 57:255-268, 2000). Furthermore, defense response genes are known to be activated in wheat heads inoculated with F*. graminearum.* However, the molecular and physiological responses of heads to invasion by head blight Fusaria are largely uninvestigated, whether in resistant or susceptible plants. On the fungal side, *F. culmorum* produces cell wall degrading enzymes in infected floret tissues (Kang and Buchenauer, J. Phytopathology 148:263-275, 2000). Fungus-produced trichothecene toxins such as deoxynivalenol (DON) contribute to virulence of *F. graminearum*. DON, which is a potent inhibiter of protein synthesis and is postulated to inhibit activation of defense response genes, can induce complete loss of chloroplast pigments at sublethal concentrations (abstract by Seeland and Bushnell). Nevertheless, there is much to be learned about pathogenesis in Fusarium head blight at both the molecular and cellular levels.

INFLUENCE OF ENVIRONMENT ON INOCULUM LEVEL AND FUSARIUM HEAD BLIGHT SEVERITY

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OBJECTIVES

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

INTORDUCTON

Epidemics of Fusarium head blight (FHB) have had a devastating impact on wheat production throughout North America (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 FHB have emphasized the importance of both inoculum and environment to disease epidemics (Francl et al. 1999, DeWolf et al. 2001). However, the interactions between environment, inoculum and disease level have not been sufficiently quantified to allow the development of a disease forecasting system. This report will summarize the information obtained during 2001 to further illustrate the findings of a cooperative effort among researchers in OH, IN, SD, ND, PA and MB to create a FHB forecasting model (De Wolf et al 1999, De Wolf et al. 2000).

MATERIALS AND METHODS

Adapted, FHB-susceptible cultivars were grown with standard agronomic practices in replicated plots near Wooster, OH (Hopewell SRWW), Fargo, ND (Norm HRSW), and State College, PA (Hopewell). The environment at each location was monitored by an automated weather station equipped with temperature, relative humidity, precipitation, and surface wetness instrumentation. Each day, 5 wheat heads were collected from each of three replicated plots (n=15) by cutting the stem just above the first node. Heads where then transported to a laboratory for further processing. In the laboratory, the 5 heads from each rep were placed in a 250 ml flask containing 50 ml of sterile distilled water plus Tween 20 (1 drop/100ml). Flasks were shaken vigorously for 2 min to dislodge spores from the spikes, and 1 ml of the spore suspension transferred to replicate plates of Komata's media. The plates were incubated for 10 to12 days and daily inoculum level estimated from the number of *Gibberella zeae* cfu's observed. Other *Fusarium* species were also recovered from the wheat heads, but only *G. zeae* will be considered for this report. Growth stage of the wheat plots was assessed daily, and FHB incidence and severity evaluated during the early dough stage.

RESULTS AND DISCUSSION

Disease incidence and severity were higher at the Ohio location than at the North Dakota and Pennsylvania locations during the 2001 growing season (Table 1). Inoculum level as estimated by the number of *G. zeae* colony forming units per wheat spike per day was greater at the North Dakota location relative to the other locations considered in this analysis (Figure 1). The North Dakota inoculum levels were punctuated by a single prominent peak that reached a maximum of 138 cfu's per spike per day. This peak in *G. zeae* inoculum was associated with 4 consecutive days of precipitation, and coincided with crop anthesis at this location. In comparison, the number of *G. zeae* cfu's observed at the Ohio and Pennsylvania locations ranged from 1 to 30. The highest levels at these locations did not occur until near the end of anthesis, and peaks in inoculum level were not strongly associated with precipitation events.

The average wetness duration summarized from the beginning of anthesis until the early milk stages of growth was 4 h less at the North Dakota location than at the Ohio and Pennsylvania locations (Table 1). The North Dakota location also received equal amount of precipitation as did the Pennsylvania location, and 23 mm less precipitation than the Ohio location. Average daily temperature at the Ohio location was within 2 degrees of the Pennsylvania location; however, average temperature at the North Dakota location was >9 degrees higher than the other locations.

Differences in disease level among the locations can be attributed, in part, to differences in temperature and moisture parameters, which likely influenced inoculum levels and infection periods. For example, the disease intensity observed at the North Dakota location may be explained in part by high inoculum levels, and consecutive days of favorable moisture and temperatures during crop anthesis. In contrast, the combination of low temperatures and low inoculum levels during anthesis may explain the low disease intensity observed at the Pennsylvania location. The high disease intensity observed at the Ohio location relative to the Pennsylvania location despite similarities in average wetness duration, temperature, and relative humidity were likely influenced by the frequency and magnitude of precipitation events during crop anthesis.

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.

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Table 1. Summary of weather conditions during crop anthesis and Fusarium head blight (FHB) intensity observations in research plots at three North American locations.

Variable	Location		
	Pennsylvania	North Dakota	Ohio
Avg. wetness duration (h)	13	9	13
Avg. temperature (C)	15	25	13
Avg. relative humidity (%)	75	79	80
Total rainfall (mm)	26	26	49
Plot FHB incidence (%)	0	67	71
Plot FHB severity (%)	0	16	42

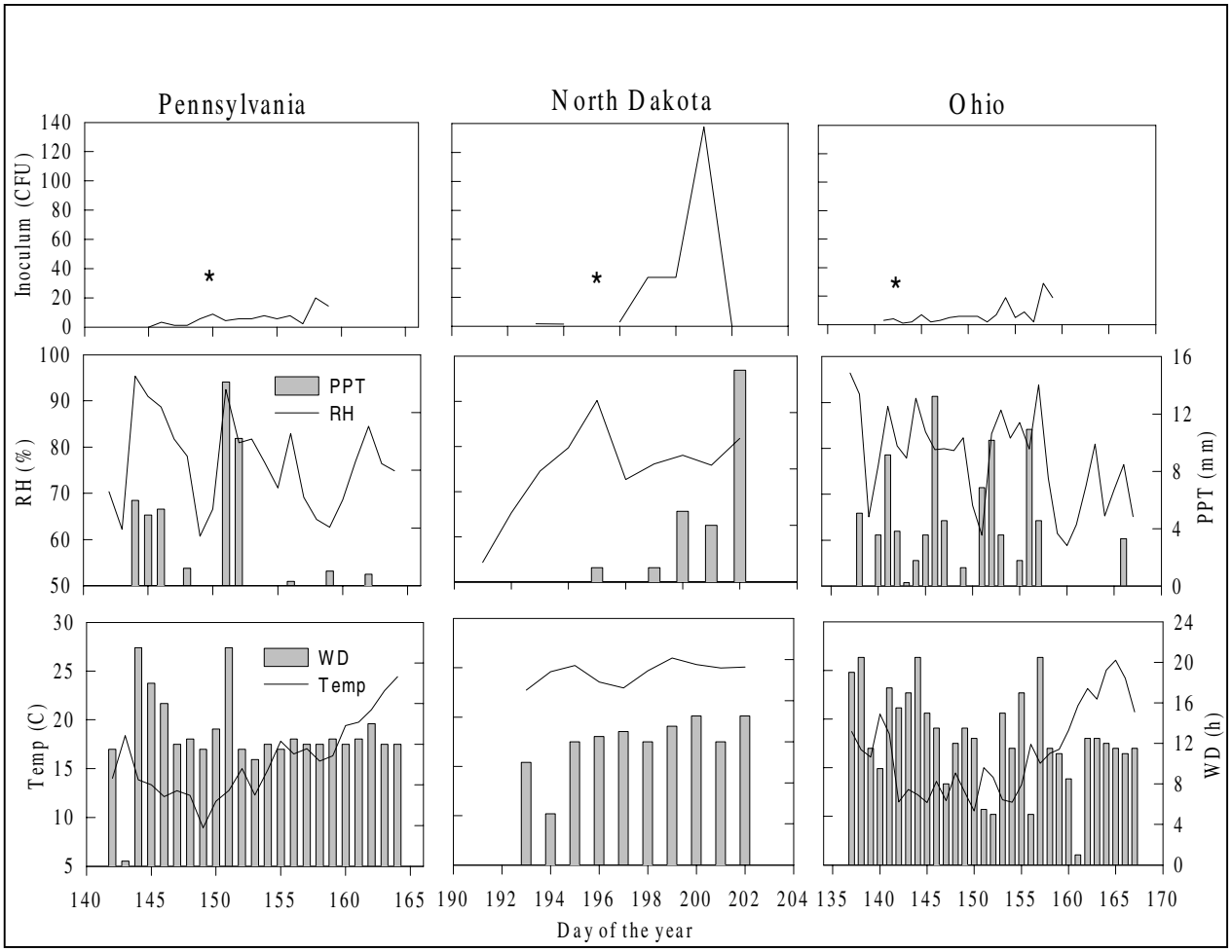

Figure 1. Summaries of environment and inoculum levels at research locations in Pennsylvania, North Dakota, and Ohio. Each series of plots gives the temperature (Temp), relative humidity (RH), precipitation (PPT), wetness duration (WD), and *G. zeae* inoculum level. Inoculum level was estimated from the number of colony forming units rinsed from wheat spikes collected from replicated plots of susceptible wheat varieties. The * designates the beginning of crop anthesis.

THE NEPAL *GIBBERELLA ZEAE* PROJECT

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ABSTRACT

Sampling of a small area (< 12 km2) of Lamjung district in the Nepal Himalaya has identified *Gibberella zeae* populations with an unusually high level of biodiversity. From smallholder farms in this area, we collected *G. zeae* strains from five kinds of representative samples: seed samples of wheat, rice, and maize, and samples of soil debris and weeds. We classified the genetic diversity of 575 strains using a species-specific, sequence characterized (SCAR), polymorphic marker, that placed all of the strains into one of five previously identified SCAR types (1). Each of the four major SCAR types, designated 1, 2, 3, and 5, was represented in each of the five kinds of samples, but the relative proportions of the SCAR types varied. On wheat, for example, SCAR type 3 comprised 62% of strains, type 5 comprised 22% of strains, and types 1, 2 and 4 were rare. In contrast, type 2 comprised half of the strains isolated from maize.

To investigate associations between genotype, virulence, and toxin production, we characterized 250 representative strains for virulence on wheat heads in the greenhouse by measuring average % spikelets blighted for ten replicate heads at 17-19 days after inoculation. We also measured production of the trichothecene toxins nivalenol (NIV) and deoxynivalenol (DON) by GC-MS analysis of seeds pooled from ten replicate heads. One-third of the strains tested produced only DON and two-thirds produced NIV. In SCAR types 1, 2, and 5, >95% of the strains in each type produced the same toxin, either DON or NIV, but in type 3 equal numbers of DON-producers and NIV-producers were present. Virulence on wheat was influenced significantly both by SCAR type and toxin type, with higher virulence on wheat associated with DON production. As previously suggested (1), types 2 and 3 may show some host specialization, since type 2 is rare on wheat and has the lowest virulence on wheat, while type 3 is relatively common on wheat and has high virulence on wheat. The Nepal population of *G. zeae* thus appears to differ from the North American populations studied to date, which have not demonstrated host specialization (2). Characterization of virulence of Nepal *G. zeae* strains on maize is being conducted by Gyanu Manandhar at the Nepal Agriculture Research Council.

Because the SCAR analysis measures genetic variability at only one locus, additional studies are in progress using amplified fragment-length polymorphisms to obtain information on fine genetic structure of the population of *G. zeae* from Nepal. These genetic studies should elucidate the level of genetic differentiation between the SCAR types and their potential for interbreeding and genetic recombination. This work should allow us to determine whether Nepal is a center of diversity for *G. zeae*, and to evaluate the potential of the Nepal *G. zeae* population for generation of novel genotypes of potential concern for wheat head blight management worldwide.

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COMPARISON OF TWO METHODS FOR ESTIMATING SCABBY KERNELS IN FUSARIUM-INFECTED SPRING WHEAT

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ABSTRACT

Grain from eight spring wheat lines was used to compare two methods for estimating scabby kernels. The wheat was grown in replicated field plots inoculated with six rates of Fusarium-colonized grain inoculum including a non-inoculated fungicide-protected control. Percentage of tombstones (TMB) was determined by separating grain into sound and scabby kernels and counting kernels in each category. Percentage of visually scabby kernels (VSK) was estimated by matching the sample to standards generated by mixing healthy and scabby kernels of a hard red spring wheat. Mean, minimum, and maximum values for 191 samples examined were 17.5%, 1%, and 40% for TMB and 14.3%, 0%, and 50%, for VSK, respectively. The Pearson correlation coefficient of TMB with VSK was 0.78 for the individual samples. Correlation of TMB with VSK for the means of the 48 cultivar-inoculum treatments was highly significant (r = 0.92). The results indicate that the two methods tested are comparable as visual estimates of damage to wheat grain from *Fusarium* infection.

EFFECT OF BURNING WHEAT AND BARLEY RESIDUES ON SURVIVAL OF *FUSARIUM GRAMINEARUM* AND *COCHLIOBOLUS SATIVUS*

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ABSTRACT

Cereal residues left on the fields due to minimum tillage increase the inoculum potential of pathogenic fungi. The effect of residue burning on the viability of Fusarium head blight (*Fusarium graminearum)* and spot blotch/common root rot (*Cochliobolus sativus*) pathogens was studied in wheat and barley residues burned one month after harvest using a flame thrower. Remaining residues were collected and stored at -10 C until isolations were made on PDA and Komada's media in late fall. Wheat node counts in straw pieces ranged from 58-137 and from 161-487 in burned plots and in control plots, respectively. Recovery of *F. graminearum* (FG) and *C. sativus* (CS) were significantly (P=0.001) reduced in burned residues (FG, 6%; CS, 10%) in comparison with the non-burned residues (FG, 26%; CS, 40%). Recovery of both pathogens was almost nil from totally charred residues. Recovery of *F. culmorum*, *F. avenaceum,* and *F. sporotrichioides*, and other fungi followed the same patterns. Our data shows that residue burning can reduce the inoculum potential of pathogens present in residues, and may assist in the management of destructive diseases such as Fusarium head blight.

MANIPULATING ARTIFICIAL EPIDEMICS OF FUSARIUM HEAD BLIGHT IN WHEAT WITH INOCULUM CONCENTRATION

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ABSTRACT

The poor repeatability of artificial Fusarium head blight (FHB) epidemics generated in field nurseries is likely due to methodological problems of inoculation, the variability of the fungus, and the confounding effects of environmental parameters on disease development. The ability to manipulate disease development through the application of macroconidia inoculum was examined in field experiments in St. Paul MN. Six concentrations of macroconidial inoculum (0, 6250, 12500, 25000, 50000, and 100000 macroconidia per ml) were applied to 2.4 m long two-rowed plots of the spring wheat cultivars Norm (susceptible), McVey (moderately resistant - MR), Pioneer 2375 (MR), and BacUp (resistant). The experimental design was a split-plot with four replications, with cultivar as the main plot and inoculum concentration as the sub-plot. Inoculum was applied to each plot at the appropriate concentration at anthesis and 3 and 7 days after anthesis. Plots were mist-irrigated to promote disease development for 30 minutes immediately following inoculation events and daily (8 mm per day) between inoculation and the final disease assessment. Visual assessment of twenty primary heads in each plot was used to determine disease incidence (DI) and disease severity (DS) at 14, 19, and 24 days after anthesis. Grain was harvested at maturity and used to determine 200-grain weight, visually scabby kernels (VSK) and deoxynivalenol concentration (DON). Disease levels increased with increasing inoculum concentrations irrespective of the wheat cultivar examined. The mean DI and DS (all wheat cultivars) for the six inoculum concentrations was 28%, 42%, 43%, 64%, 78%, and 93%; and 7%, 14%, 18%, 27%, 41%, and 55%, respectively. Differences among the inoculum treatments were evident in DI and DS at all three assessment and cultivar rankings were consistant with previous evaluations (DS - BacUp, 9%; McVey, 20%; Pioneer 2375, 32%; and Norm, 49%). Visual differences in disease symptoms were evident in the VSK and DON levels of harvested grain. By increasing inoculum concentrations we were able to generate increasing levels of disease, even in 2001 when a lack of rainfall between anthesis and the last inoculation date made environmental conditions highly adverse to FHB development. Information gleaned in this study will improve our ability to manipulate the level of disease in field nurseries screening germplasm for resistance to Fusarium head blight.

MODIFICATION OF A CROP RESIDUE MOISTURE SENSOR FOR APPLICATIONS IN THE EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Corn residue remaining on the soil surface is a major source of *Gibberella zeae* inoculum in regions where small grains and corn are incorporated into crop rotations. However, the role of temperature and moisture in *G. zeae* perithecia development on corn residues have not been investigated. Sensors that can be used to continuously monitor the moisture status of crop residues were adapted for use with corn stalks. The modified sensors incorporate improvements that should improve sensor durability in a corrosive environment, and increase compatibility with other environmental monitoring instrumentation. Preliminary results indicate that the modified sensors have similar range in moisture sensitivity when compared to previously used moisture sensors. The modified sensors will be used to help evaluate the effect of temperature and moisture on perithecia development in growth chamber and wheat field environments. In the future it maybe possible to predict inoculum release events based on environmental variables including the temperature and moisture status of crop residues.

FUSARIUM GRAMINEARUM INFECTION AND MOVEMENT IN FLORAL COMPONENTS OF WHEAT SPIKES

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ABSTRACT

Many plant breeders screen wheat cultivars for Type II resistance to Fusarium head blight resulting from *Fusarium graminearum* (Schwabe) infection by visual spikelet evaluation following the single floret inoculation system. The objective of this study was to determine the relationship between the level and rate of *F. graminearum* infection in floral components of wheat spikelets and the visual ratings of spikelet infection in the greenhouse. Several spikes of VA96W-326 were inoculated in a middle spikelet at anthesis, misted at high RH for 3 days, and harvested at 5, 10, 15, and 20 day intervals post inoculation. The spikelets were labeled in relation to location above and below the point of inoculation (PI). Fresh seed from the left floret of each spikelet and dry components [glume, lemma, seed, palea] from the corresponding right floret of each spikelet and the rachis (below spikelet) were plated on a modified PCNB agar to determine *F. graminearum* infection. *F. graminearum* infection in both florets increased with maturity with the most significant change occurring between 5 and 10 days. After 5 days, *F. graminearum* infection occurred primarily at the PI for all components, except the glumes, which showed no infection. After 10 and 15 days, a large increase in infection was observed in all components in all spikelets below the PI, while infection declined sharply to zero above the PI. Rachis infection was greater than infection in all other components after 10 and 15 days. There was no significant difference in seed infection between fresh seed from the left floret and the corresponding dry seed of right floret from the same spikelet. Greenhouse ratings of individual spikelet infection showed a strong relationship with the bioassay of the corresponding seed infection at 10 and 15 day harvests, but not at 20. This study of one cultivar, VA96W-326, shows maximum infection of floral components 15 days after inoculation, which should be related to the timing of visual ratings of spikelet infection in the greenhouse.

INFLUENCE OF CULTIVAR AND PLANTING DATE ON FUSARIUM HEAD BLIGHT DEVELOPMENT ON WINTER WHEAT IN OHIO

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ABSTRACT

Scab or Fusarium head blight, caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a devastating disease of wheat and barely in the United States and around the world. Scab outbreaks of varying intensity have been common and widespread across much of the eastern half of the United States, affecting yield and grain quality. This disease has caused losses up to $1 billion per year. In addition, reductions in grain yield, kernel size, test weight, and associated DON accumulation in the grain often result from severe disease epidemics. Host resistance and cultural practices have long been considered effective means of disease control. No single disease management strategy is likely to control this disease because of lack of availability, excessive cost, or negative impacts on soil conservation. To control Fusarium head blight, multiple disease management strategies will be required. Therefore, the objectives on this research were: i) to evaluate disease development on three wheat cultivars planted in three different dates, and ii) to determine the relationships between cultivar and/or planting date with yield and DON content.

Seeds of three wheat varieties (Hopewell, Elkhart, and Paterson) treated with Raxil-Thiram, were planted using 24 seeds/ft of row on three planting dates (18 Sep., 2 Oct., and 16 Oct, 2000) in Ravenna silt loam at the Ohio Agricultural Research and Development Center near Wooster. Prior to planting, the field was moldboard plowed, then 200 lb/A of granular fertilizer (6-24-24) was broadcast over the field and incorporated with a disc. In addition, 200 lb of ammonium nitrate was applied on 28 March. For each variety and each of the planting dates, there were three replicate plots. Each plot was 35-ft long, and consisted of 7-rows with 7 in. between rows. Corn stalks colonized by *Fusarium* sp. were placed between plots. In each plot, disease assessments were made three times a week (June 11 - June 26) for both incidence and severity in a one ft. area across the plant rows at 10 locations. Each location was marked with a tall flag that remained in the field during the period of assessment. Disease incidence was calculated as the percentage of heads with disease, and severity was calculated as the average percentage of affected florets per head. Plots were harvested on 17 of July. Yield (bu/A) was determined from harvested grain adjusted to 13.5% moisture. Harvested grain was visually assessed for the damage kernels, and grain was analyzed for DON content.

Disease development varied in the three planting dates among the three cultivars. There was more disease in the last planting date (16 Oct.). Elkhart cultivar had the maximum disease incidence and severity (80.2 and 44.3%, respectively). On the other hand, Patterson cultivar had the lowest disease incidence and severity (58.2 and 30.1%, respectively).

No significant differences were found in yield among the three cultivars through the three planting dates, but there were significant differences among cultivars in DON content through the three planting dates. In general, DON content was highest in the last planting date for the three cultivars. Elkhart had the highest level (10.4 ppm) of DON, and Patterson had the lowest level (2.2 ppm) of DON content. There were significant differences in test weight among cultivars. Elkhart and Hopewell had significantly higher test weight at the mid-planting date (59.9 and 59.7 lb/bu, respectively).

In conclusion, there were higher disease and DON level on the last planting date, but higher test weight was found at the mid-planting date. Elkhart had the highest maximum disease, the highest DON, and highest test weight. On the other hand, Patterson had the lowest maximum disease, the lowest DON level, and the lowest test weight.

SPATIAL PATTERN OF SCAB INCIDENCE DURING FUSARIUM HEAD BLIGHT EPIDEMICS ON WINTER WHEAT IN OHIO

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INTRODUCTION

Fusarium head blight of wheat (*Triticum aestivum L.*) caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a limiting factor in wheat and barely production. It reduces wheat yield in many production regions of North America (Bai and Shaner 1994; Parry et al.1995; McMullen et al. 1997). When environmental conditions are favorable, the disease can cause yield losses up to $1billion (McMullen et al. 1997). The analysis of spatial patterns of plant diseases is an important component of epidemiology. Information on disease patterns is a useful ecological characteristic that helps define a population such as diseased wheat heads (Gilligan, 1988; Campbell, and Madden, 1990; Madden, et. al., 1995).

Despite the economic importance of Fusarium head blight, there is little information showing the spatial patterns (dispersion) of infected heads and the changes in patterns over time as disease incidence increases. This information would be useful for better understanding the spatio-temporal dynamics of Fusarium head blight. Additionally, data may determine more efficient sampling procedures that may result in more precise estimates of mean disease intensity and properly analyzing data from different treatments. Thus, the objective of this study was to quantify the spatial pattern of Fusarium head blight incidence in wheat fields.

MATERIALS AND METHODS

Disease Assessments.

Epidemics of Fusarium head blight of wheat were monitored over time in two fields at the Ohio Agricultural Research Development Center (OARDC) in Wooster, and at a single time in two fields at the OARDC Northwest Branch near Hoytville, which is located in a major wheat production region of Ohio approximately 120 miles northwest of Wooster. In the Wooster fields, disease incidence was assessed twice a week in June 2001. In each field, three transects with 15 sample points per transect, spaced at 0.75-cm intervals, for a total of 45 sample points per field were established. Each sample point was marked with a flag that remained in the field throughout the assessment period. In the Northwest Branch fields, there were ten rows (transects) with ten sample points per row, spaced at 1-m intervals for a total of 100 sample points per field. At each sample point, the incidence of scab was recorded for a 1-ft sub-transect across the plant rows.

Data Analyses
Heterogeneity Analyses: Distribution and indices.

The beta-binomial and the binomial distributions were fitted to data on the incidence of diseased heads per transect for each individual field assessment using the computer program BBD, Version 1.2 (Madden and Hughes, 1994). The beta-binomial has two parameters, *p*, which is the expected probability of disease (a measure of disease incidence), and θ , a measure of the variation (heterogenity or aggregation) in disease incidence per sample unit. Values of θ greater than 0 indicate aggregation. The binomial has a single parameter representing the probability of disease. A good fit to the binomial distribution is suggestive of a random spatial pattern of disease incidence, while a good fit to the beta-binomial is suggestive of an aggregated (overdispersed) spatial pattern of disease incidence. Standard X^2 goodness-of-fit tests were calculated for each distribution to determine the most appropriate distribution.

For each field and assessment date, the index of dispersion, *D*, was also calculated. *D* is the ratio of the observed variance of incidence among the sampling units to the expected binomial (i.e. random) variance (Madden and Hughes, 1995).

The effect of disease aggregation is to inflate or increase the observed variance above the expected binomial variance. Therefore, values of $D > 1$ suggest spatial aggregation. *D* has a X^2 distribution under the null hypothesis of randomness. A large test statistic and small significance level (<0.05) indicate that one should reject the null hypothesis of randomness (=binomial) in favor of aggregation (overdispersion). Moreover, the so-called C(α) test, which is more specific than the test of *D,* was used to test for overdispersion. Here, the alternative hypothesis is not just overdispersion but overdispersion described by the beta-binomial.

RESULTS AND CONCLUSIONS

Mean disease incidence per field, an estimate of the expected probability of a head being diseased (*p*), ranged from 0.018 to 0.693, with a median among fields of 0.024 (Table 1). As anticipated, *p* increased over time within all fields.

The program BBD successfully calculated maximum likelihood estimates of *p* and θ for all the data sets. Where there was a sufficient number of disease classes for the test to be preformed, the frequency distribution of diseased heads could be described by the beta-binomial distribution in over 75% of the data sets and by the binomial distribution in 58% of the data sets.

The values of θ ranged from 0.00 to 0.073, with a median of 0.011. Estimated θ in over 90% of these data sets were greater than 0 (Table 1) indicating overdispersion.

The index of dispersion *D*, ranged from 0.88 to 4.50, with a median of 2.22. *D* and θ were both positively correlated with the estimated parameter *p*, with correlations of 0.52 and 0.65, respectively (Figs. 1 and 2).

The X^2 test for D (Madden and Hughes, 1995), and the C(α) test both had indicated significant heterogeneity in about 90% of the data sets (Table 1).

In conclusion, it was found that heads of wheat infected with scab were aggregated to highly aggregated within the wheat fields. Moreover, the degree of aggregation was high and increased over time as incidence increased.

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Table 1. Statistics for describing the spatial pattern of the incidence of Fusarium head blight in four wheat fields in Ohio in 2001.

Field	Disease assessment date	Estimated beta-binomial parameters [a]				C(α) test [b]	
		p	se(*p*)	θ	se(θ)	*z*	P(z)
F 1 (Wooster)	06/11	0.071	0.0092	0.053	0.0179	8.54	<0.001
	06/14	0.081	0.0088	0.039	0.0105	8.32	<0.001
	06/18	0.204	0.0110	0.018	0.0077	6.21	<0.001
	06/21	0.304	0.0097	0.021	0.0089	2.86	0.002
	06/25	0.587	0.0171	0.047	0.0113	14.91	<0.001
F 2 (Wooster)	06/11	0.018	0.0033	0.011	0.009	3.01	<0.001
	06/14	0.030	0.0047	0.011	0.0084	2.12	0.017
	06/18	0.276	0.0147	0.031	0.0108	6.64	<0.001
	06/21	0.635	0.0162	0.047	0.0108	12.01	<0.001
	06/25	0.693	0.0113	0.013	0.0058	4.75	<0.001
F 3 (Hoytville)	06/26	0.047	0.0145	0.000	- [c]	-1.30	1.000
F 4 (Hoytiville)	06/26	0.623	0.0143	0.073	0.0134	23.20	<0.001

[a] *p*, expected probability of a leaf being diseased, estimated as the mean incidence; θ, aggregation parameter; se(θ), standard error of designated estimated parameter.
[b] *z*, standard normal statistic of the C(α) test; P(*z*): significance level of *z*.
[c] Analysis not preformed.

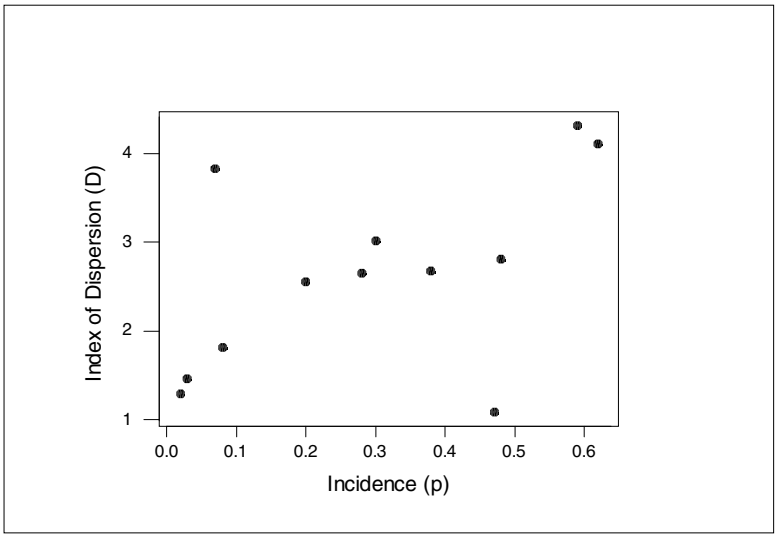

Figure 1. Index of dispersion (*D*) in relation to the mean incidence (P) of Fusarium head blight from four wheat fields in Ohio in 2001. Each point represents one field at one time.

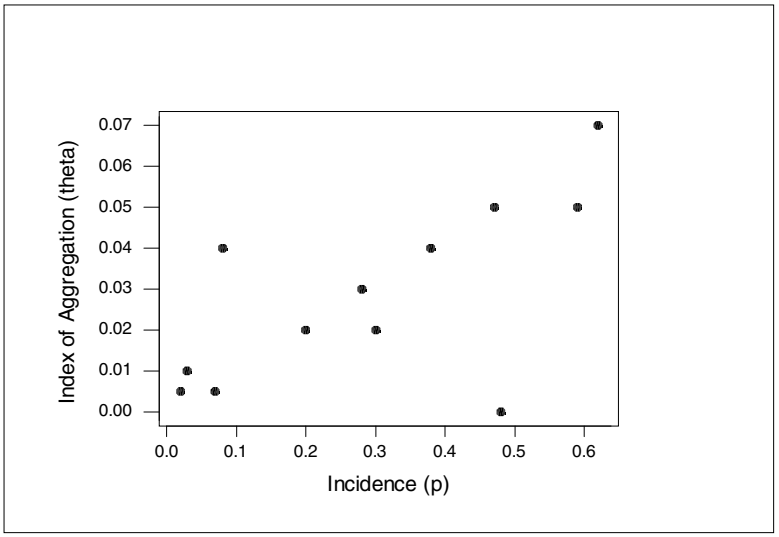

Figure 2. Index of aggregation (θ) in relation to the mean incidence (P) of Fusarium head blight for four wheat fields in Ohio in 2001. Each point represents one field at one time.

PAST, PRESENT AND FUTURE OF FORECASTING SMALL GRAIN DISEASES

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Economic losses from Fusarium head blight have stimulated recent development of forecasting systems in Canada and the U.S. so it is perhaps appropriate to review what has been accomplished to date and where research may take us in the future. Diseases of high-value horticultural crops have received the bulk of forecasting system research and development effort and relatively few systems have been deployed for forecasting diseases of low-value field crops. Despite this economic disparity, small grain producers in Europe and North America have had access to several disease forecasting systems.

The first wheat disease forecaster was implemented in 1978 in The Netherlands. Called EPIPRE, for Epidemiology and Predication and Prevention, the system emphasized scouting by trained observers who forwarded information by mail to a central computer. Epidemic progress of leaf rust, stripe rust, powdery mildew, sharp eyespot, and the Septoria leaf blotch complex were forecasted together with outbreaks of aphid pests. EPIPRE used simple algorithms of population development, such as the exponential growth model, but the management recommendation incorporated many details such as cultivar, soil type, fertilizer rate, yield goal, and cost of pesticide application. The system spread to other European countries but popular usage eventually declined.

Another European model, the IPS Modell Weizen, was introduced in 1988 and is still in use. It was primarily as a method to guide control of the Septoria leaf blotch complex on wheat; leaf and stripe rusts, tan spot, powdery mildew, and eyespot were added later. Like EPIPRE, the system relies on trained scouts to diagnose diseases in the field but management decisions are based on disease incidence thresholds without reliance on computer models.

The next example is primarily an advisory system for disease management but encompasses some forecasting capability. MoreCrop (Managerial Options for Reasonable Economical Control of Rusts and Other Pathogens) was introduced to wheat growers in the U.S. Pacific Northwest in 1991. Some 30 diseases are covered for wheat and a version for barley disease management was introduced in 1999. MoreCrop bases its advice on an expert system that uses information about climatic regions, crop managerial practices, cultivar characteristics, field disease history, and departure from normal climate. The likelihood of a particular disease is suggested by the combination of factors.

North Dakota State University deployed its Small Grain Disease Forecasting System in 1999 for wheat growers and their advisors in North Dakota and Minnesota. Leaf rust, tan spot, and Stagonospora nodorum leaf blotch infection periods are predicted daily by computer models based on local weather provided by an automated weather station network. Scouting of leaf disease incidence is required for optimal disease management. Also,

airborne spores are sampled three times a week to provide information on the activity of the Fusarium head blight pathogen. Crop growth stage, extent of recent wetness from fog, rain and dew, and near-term weather forecast are suggested as guides to determine whether infection is likely. Forecasts are provided via the Internet and a toll-free number. Producers and buyers of malting barley also monitor the spore counts as a decision aid.

The Canadian provinces of Ontario and Manitoba/Saskatchewan introduced Fusarium head blight forecasts in 2000. Both systems provide contour maps of disease likelihood based on environmental data from a weather station network. Geographical information system software interpolates these point data. However, the predictive models that generate the probability of disease differ somewhat between Ontario and the Prairie Provinces. Ontario uses weather forecasts to predict the level of DON in wheat and updates this with weather station readings as flowering proceeds. The Manitoba/Saskatchewan model uses recent environmental data in a regional forecast with less sensitivity to actual flowering date.

The Ohio State University introduced a Fusarium head blight forecast in 2001. The forecast is based on a risk assessment model developed with support of the U.S. Wheat and Barley Scab Initiative. Environmental variables concerned with temperature and rainfall are summarized for the seven days prior to the onset of flowering to predict the subsequent risk of head blight. Forecasts are provided via the Internet as a county map of Ohio with risk areas highlighted.

It is difficult to foresee precisely the future of these forecasting systems but some common outcomes may be expected to recur since similar forces are at work.

Production economics and adversity to economic risk will play key roles in whether a system will be adopted. Ten years ago in North Dakota and Minnesota, approximately 100,000 hectares of wheat were sprayed with a fungicide. Today, one million hectares of spring wheat and durum are treated and barley area treated stands near where wheat was 10 years ago.

Funding for and management of system operations are often distinct from research and development. In Canada, fungicide registration is predicated on growers having available a disease advisory system so fungicide manufacturers have a compelling interest in continued operation. Grower groups and local grants fund system operations at North Dakota State University.

System reliability is an important concern because users can be rather unforgiving of failure. Reliability encompasses not only the prediction but also all parts of information delivery. To bring expectations into balance, users should be constantly educated about the accuracy rate of models, sources of variability, and other factors that influence their personal risk profile.

Conversely, the host institution benefits immensely when the system clearly makes money for the producer. Success stories told in the agricultural media enhance adoption of the system and garner political support.

Perceived value of the system will tend to increase usage. Forecasts for multiple diseases and insect pests give the user an integrated management system that will continue to hold value even if, for example, a scab-resistant cultivar is being grown.

Adoption is also influenced by factors such as cost effectiveness, ease of use, and accessibility. In-depth instructions and details of the pathogen's biology largely go unread during the decision making process. So too, scouting for disease intensity prior to treatment may be done hurriedly or left undone entirely (looking for symptoms is not an issue for scab forecasts). However, quite a few users are likely to listen to a recorded forecast from their cell phone while they are driving between chores.

Feedback is important for system viability because it allows one to catch problems before they become unwieldy. The system manager should know who is using the system, how it's being used, and if there are any obstacles to its use. Market penetration is an important benchmark of user participation. Periodic user surveys should be incorporated into the operations of a forecaster.

Research interests of scientists working in epidemiology suggest some future avenues of small grains disease forecasting. Expansion into new states is a recurrent theme: e.g., North and South Dakota plan to deploy risk assessment models in 2002. Concurrently, models of infection period are nearing completion and also should be deployed soon. New knowledge of pathogen population biology (e.g., perithecial development, spore survival, and leaf colonization) will undoubtedly contribute to future gains in forecaster accuracy. Specificity of forecasts is another broad avenue under development; e.g., environmental wetness estimates were generated at a spatial resolution scale of 4 km^2 with great success in a pilot study. Whereas present systems provide a uniform forecast for hundreds of square kilometers, future forecasts may aid management of diseases within a single field. It is obvious that novel methods will continue to enhance forecasting systems, given the talented scientists working on the problem and advances in information technology.

POPULATION GENETICS OF *FUSARIUM GRAMINEARUM* FROM CHINA

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ABSTRACT

In order to address questions of genetic diversity, gene flow and recombination among populations of the Fusarium head blight pathogen in China, a collection of strains was made from the important grain-producing region of the lower Yangtze River Valley. Diseased wheat heads were collected at 5 meter intervals along a transect in four production wheat fields in Zhejiang Province. The four fields ranged in distance from each other from 50 km to 200 km. DNA was extracted from 204 strains, transferred to solid support and probed with 6 DNA clones that hybridize to DNA fragments characteristic for particular lineages within the *F. graminearum* complex. All 204 strains from China gave a pattern of hybridization identical to that of DNA from known strains of *F. graminearum* lineage 6. The DNAs were further hybridized to 9 clones capable of detecting polymorphic loci within lineage 6 and a telomere-containing probe that further resolved genotypes. High levels of gene flow were detected among the four China populations (mean Nm = 11. 1; range 6.6 – 65) and the populations were extremely similar (Nei's unbiased genetic identity, I = 0.96 – 0.99). As a result, the strains from the four fields were considered part of a single large population. Gene diversity for the 9 single copy loci within the population was high (h = 0.35; range = 0.17 – 0.58) and the average number of alleles per locus was 3.3. Multilocus haplotypes were constructed from the allelic information of the nine polymorphic loci. Clones, having the same multilocus haplotype, originated primarily from isolates obtained from the same wheat head. Whether high genotypic diversity is caused by frequent sexual recombination is currently being explored.

MGV1 REGULATES FEMALE FERTILITY AND PLANT INFECTION IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

The wheat scab disease caused by *Fusarium graminearum* is prevalent worldwide and can cause severe losses during epidemics. The pathogen over-winters in debris as mycelia or perithecia and ascospores are believed to be the primary inoculum. To understand molecular mechanisms regulating the infection process of this important pathogen, we isolated a MAP kinase gene *MGV1* from *F. graminearum* strain PH-1. *MGV1* is highly homologous to the *MPS1* gene in *Magnaporthe grisea* that is involved in conidiation, plant penetration, and female fertility. The *MGV1* gene appears to be dispensable for conidiation in *F. graminearum* even though it is required for female fertility during sexual reproduction. Vegetative growth of the *mgv1* deletion mutants is normal in liquid media but is reduced when cultured on solid nutrient agar plates. Mycelia of *mgv1* deletion mutants are defective in cell wall structures and hypersensitive to cell wall degrading enzymes. In infection assays with flowering wheat heads and corn silks, *mgv1* mutants are dramatically reduced in virulence and appear to be defective in spreading *in planta*. Our data suggest that *MGV1* is involved in multiple processes in *F. graminearum* related with sexual reproduction, plant infection, nutrient sensing and cell wall integrity.

DEVELOPMENT OF *FUSARIUM GRAMINEARUM* ON FLORET SURFACES OF FIELD-GROWN BARLEY

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ABSTRACT

To investigate the role of mycelial colonies on floret surfaces in head blight infection, we mapped the location and number of colonies for 1-12 days after field plots were inoculated with *Fusarium graminearum*. The experiment was conducted on the St. Paul Campus of the University of Minnesota. Four plots of Robust barley (provided by K. Evans and R. Dill-Macky) were inoculated June 25, 2001 by spraying heads with aqueous suspensions of macroconidia at 50,000 or 100,000 spores/ml. At inoculation, 90% of the heads had emerged from the boot. After inoculation, plots were mist-irrigated daily from 5-9 p.m. and 4-6 a.m., totaling 8 mm of water per day. In addition, 13 mm of rain fell on the 5th day after inoculation (DAI). For the 12 day period of floret sampling, the average maximum daily temperature was 29^{O} C; the average minimum was 18^{O}C. As viewed in the field at 13 DAI, approximately 1/3 of heads showed one or more chlorotic lesions. At 1, 2, 3, 4, 5, 6, 8, 10 and 12 DAI, florets were harvested, with bias for samples from heads showing one or more chlorotic lesions. Each day, 60 fresh (unfixed) florets were dissected and examined for presence of chlorotic or necrotic lesions; 80 additional florets were fixed and stained in lactophenol-cotton blue and then dissected and examined microscopically for presence of mycelium on floret surfaces. Mycelium and lesions were rarely seen at 1-3 DAI. At 4-6 DAI, mycelial colonies were noted on 13% of awns, 13% of lemma exteriors, 9% of lemma interiors, 57% of palea exteriors, 3% of palea interiors, and 6% of caryopses. At 4-6 DAI, chlorotic lesions were present on the lemma and palea in 8-13% of florets, and on the awn and/or caryopsis in 3-4% of florets. Thus the presence of lesions did not correlate with the presence of mycelium, especially on the frequently colonized palea exterior. There, mycelial colonies were usually located on the basal half of the palea, near the palea keel, which faces the rachis of the head. By 6-8 DAI, colonies on the palea surface often spread laterally into the crevice between the palea and lemma, sometimes extending to the interior surfaces of the palea and lemma as well as to the caryopsis. By 8-12 DAI, discrete lesions were discernible in 14% of florets on interior or exterior surfaces near the base of the lemma and palea. A second pathway to the interior of the floret was through the apical floret mouth. At 4 DAI, small discrete colonies were often present on the adaxial surface of awns, within 1-2 mm of its junction with the lemma and on adaxial tissues of the lemma and palea near the floret mouth. By 5 DAI and thereafter, colonies extended basally on the interior surfaces of the lemma and palea, sometimes colonizing any retained anthers or the brush apex of the caryopsis. By 8-12 DAI, discrete chlorotic or necrotic lesions were present on the apex of 14% of lemmas and 13% of paleas. The results indicate that under warm, mist-irrigated field conditions, colonies on the abaxial (exterior) surface of the palea (near the keel) and on adaxial (interior) surfaces of the palea and lemma facing the floret mouth serve as starting points for floret invasion.

USE OF FUSARIUM HEAD SCAB RISK ASSESSMENT MODELS IN OHIO, 2001

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ABSTRACT

During the 2001 wheat growing season, head scab risk assessment models were used to predict the risk of Fusarium head scab in Ohio. Logistic models were previously developed from hourly weather, crop growth and disease observations from 50 location-years representing three wheat production regions in the US. Model 1 utilized the duration of precipitation in hours and the number of hours when the air temperature was between 15°C and 30°C for 7 days prior to flowering. Cross validation prediction accuracy for this model was 78% for determining when disease was not severe (severity ≤ 10%), and its accuracy was 56% for predicting when an epidemic occurred (severity ≤ 10%). Model II utilized the: 1) number of hours when the air temperature was between 15°C and 30°C for 7 days prior to flowering; and 2) the number of hours when the relative humidity was 90% or above and the air temperature was between 15°C and 30°C for 10 days after flowering. Cross validation prediction accuracy for this model was 83% for determining when disease was severe (severity ≥10%). Hourly weather data from six weather stations in Ohio (Dayton, Columbus, South Charleston, Wooster, Hoytville and Toledo) were used to determine duration of weather events for the pre- and post-anthesis time periods. Disease risk probabilities were calculated using logistic equations determined by each model. Weather conditions in April and the first week of May were relatively dry and cool. Precipitation events became more frequent during mid to late May in many regions of the state with most locations reporting from 15 to 21 hours of measurable precipitation during the 7 days prior to anthesis. However, average daily temperatures for most locations in the state were generally below 15°C between 22 May and 4 June when most of the wheat was in anthesis. Calculated risk probabilities for locations ranged from 0.32 to 0.84 for Model 1 and from 0.04 to 0.78 for Model II. In no case did both models predict a high risk for head scab at any location. Based on these results the head scab risk prediction was reported to be low to moderate depending on the location in the state. Head scab risk predictions were posted on the Ohio State University Ohio Field Crop Disease web page (www.oardc.ohio_state.edu/ohiofieldcropdisease/) during the critical time of disease development through harvest. Field reports and disease surveys made about 3 weeks after anthesis indicated head scab was low to very low throughout most of Ohio. Most counties reported the average percentage of wheat heads with scab in fields to be below 2%. Some counties in central and northwest Ohio reported head scab levels ranging from 0 to 20% of the heads affected.

EFFECTS OF DEW, SPRAY VOLUME AND ADJUVANT ON FUNGICIDE CONTROL OF FUSARIUM HEAD BLIGHT IN DURUM WHEAT, HRSW AND BARLEY

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OBJECTIVES

1) Evaluate the effect time-of-day for fungicide application has on Fusarium head blight (FHB) control in respect to the presence, absence or imminent formation of dew. Applications would have morning, mid-day and evening timings.

2) Evaluate management of spray volume and/or adjuvant as a tool to compensate or enhance time-of-day effects on FHB control.

INTRODUCTION

The volume of water present on a grain head covered by dew appears, by visually estimate, to be many times greater than the water applied during a fungicide application. The potential to manage the presence of dew to increase spike coverage or uptake of fungicide on the head offers small grain producers a technique to increase the efficacy of fungicides. Spray solution volume and adjuvant are important parts of fungicide application techniques which producers can easily adjust during the day to accommodate the environmental conditions at time of application.

Five unpublished experiments in ND have included dew as a variable and were available as personal communication. Two experiments on barley to evaluate dew effect on FHB control were conducted by J. Pederson, 1998. Head coverage by fungicide and dye solution increased from 8 to 21% when spraying dew covered heads compared to dry heads later in the day. Not enough FHB occurred for evaluation. The second experiment had head coverage by fungicide and dye of 5.8 and 1.4% for dry and rain wet heads, respectively. FHB levels were low with no difference detected. Field severity of FHB on durum was reduced from 3.4% on dew covered heads in the morning to 1.3% when spraying dry heads in the afternoon, T. Gregoire 1998. Dew effect in another trial was non-significant for FHB head severity on durum, T. Gregoire 2000. A greenhouse study found no significant differences in FHB field severity between fungicide applied to dripping wet heads and dry heads, M. McMullen 2001. FHB levels, field severity, when using 0.06% v/v Induce non-ionic surfactant were 3.5 and 1.5% for wet and dry heads, respectively, compared to levels with 0.125% v/v Induce of 1.1 and 1.3% for wet and dry heads, respectively.

MATERIALS AND METHODS

A 3x2x3 factorial design with three replicates was used for a 'Plaza' durum experiment, which also included an untreated check when randomized. Treatments were, 1) Morning,

mid-day and evening times-of-day for fungicide application. 2) Two spray volumes, 10 and 20 gal/a. 3) Three levels of the non-ionic surfactant Induce, none, 0.06 and 0.125% v/v. Additional experiments on 'Robust' barley and 'Grandin' HRSW were conducted with only the three time of day treatments and an untreated check in a randomized complete block design with four replications. A spray volume of 20 gal/a and Induce surfactant at 0.125% v/v were used for all fungicide treatments on the Robust and Grandin. Fungicide was applied to the barley on July 10 at 6:45am, 3:30pm and 9:30pm, the HRSW on July 14 at 7:15am, 3:15pm and 7:00pm and on the durum July 18 at 8:00am, 2:30pm and 9:00pm.

Folicur (tebuconazole) at 4 fl oz/a applied at Feekes 10.51 was the fungicide treatment in all experiments. A hand held CO_2 pressurized boom with XR8001 flat fan nozzles oriented forward and backwards towards the grain heads at 30^{O} from horizontal was used to spray (6.7' x 20') plots. Dew quantity was measured by cutting about 50 heads in each of four places in each experiment. The peduncle was held just below the wet head and cut about one inch long. The heads were placed in a pan lined with tinfoil which was sealed to prevent moisture escape before weighing. The heads in a three square foot area were counted for each area sampled. The cut heads were air dried indoors until no free water was apparent and weight decrease with time dropped to a steady loss of a few hundredths of a gram over five minutes. Free water still in the head, but not readily visible, was found by slapping a head on a blotter paper and seeing the dark spots made by water droplets from between spiklets.

Sprinkler irrigation was used only for the Plaza durum study before flowering and 0.20 inch was applied on July 19 at 6:pm with rainfall occurring most days before and after fungicide application. FHB grain spawn was spread two days before fungicide application. The HRSW and barley trials were grown under natural conditions but were adjacent to irrigated/inoculated trials which increased FHB spore levels.

Foliar and FHB disease field notes were taken 21 to 25 days after fungicide application at soft dough stage. Foliar diseases were quantified as percent necrosis of individual leaves and FHB incidence and head severity were measured by counting infections on 25 individual heads from each plot. All plots were harvested by straight cutting with a Hege plot combine and drying before processing. ANOVA was used for statistical analysis.

RESULTS AND DISCUSSION

The morning spray for Robust barley had 56.8 grams water per 100 heads and 41.7 heads per square foot, equivalent to 272 gallons water per acre on heads. There were water droplets visible in the spiklets but few water droplets on the beards. Water droplets were visible on the flag and other leaves. No visible run-off of water during the spraying operation was apparent. The morning spray for Grandin HRSW had 238.4 grams water per 100 heads with 40.5 heads per square foot, equivalent to 1111 gallons water per acre on heads. The spiklets were full and the beards covered with water droplets due to 0.61 inch rain ending at 11:00 pm the previous night. The crop canopy was covered with water droplets and run-off of water from the beards and leaves occurred during the spraying operation. The mid-day timing, 3:15 pm, had no free water visible in the head but drops showed when heads when slapped on blotter paper. Drying showed 0.9 grams water per 100 heads or 4 gallons water

per acre in the heads. The morning spray for Plaza durum had 26.3 grams water per 100 heads and 37.5 heads per square foot, equivalent to 113 gallons water per acre on heads. The beards were heavy with water droplets but no water was visible in the spiklets. Water droplets were visible on the flag and other leaves. No run-off of water during the spraying operation was apparent.

Leaf disease, FHB, yield and test weight by time of day, solution volume and adjuvant rate on durum wheat are shown in Table 1. The 3x2x3 factorial analysis had no significant interactions between the three factors. Time of day for fungicide application showed no significant difference for leaf disease, yield and test weight. FHB incidence, head severity and field severity were reduced for mid-day application compared to morning or evening. The higher adjuvant rate, 0.125% v/v of Induce with Folicur fungicide, reduced leaf disease compared to 0.06% v/v and no adjuvant. Differences due to adjuvant were non-significant for FHB, yield and test weight. No significant differences were found between spray volumes of 10 and 20 gallons per acre.

Leaf disease, FHB, yield and test weight for morning, mid-day and evening spray timings for three crops are given in Table 2. Only the 20 gal/a solution volume and 0.125% v/v adjuvant rate was used. Leaf disease and FHB were significantly reduced by fungicide for all crops but differences between timings were non-significant. Grain yields for the durum were not different while yields for the morning and evening timings of the HRSW were significantly greater than the check. Only the mid-day barley yield was significantly higher than the check. The barley trial had serious lodging which caused variability within the trial. Test weight differences due to time of day were non-significant for barley and HRSW and all fungicide treatments raised the durum test weight compared to no fungicide.

The amount of dew and its position on the head of small grains is variable from day to day. When very high amounts of dew are present, 1111 gallons per acre measured on HRSW heads, the application of spray solution causes run-off which is assumed to move fungicide to the soil. Trends from trials with dew treatments indicate the spraying of fungicide in very wet conditions may reduce efficacy. Measured effects of fungicide application in light morning dew, mid-day and evening conditions were small and variable between experiments. Time-of-day for fungicide application appears to be a minor factor for crop producers. Use of recommended adjuvant rates and high spray solution volumes when dew is present was supported by trends in this work.

Table 1. Fusarium head blight, Foliar Disease, Yield and Test Weight of Plaza Durum by Fungicide Timing, Solution Volume and Adjuvant Rate, Langdon, 2001.

			Fusarium Head Blight				
	Leaf Necrosis			Head	Field		Test
Treatment	Flag	Flag-1	Incidence	Severity	Severity	Yield	Weight
	%	%	%	%	%	bu/a	lb/bu
Untreated Check	67.5	97.5	92.5	36.4	34.0	34.6	47.6
Fungicide Timing							
Morning	31.3	59.2	84.9	20.1	17.1	46.7	51.5
Mid-Day	30.4	65.0	77.9	17.9	14.1	45.0	51.2
Evening	30.8	61.7	87.0	20.7	18.1	44.1	51.2
LSD P = 0.05	ns	ns	4.4	2.0	2.3	ns	ns
Solution Volume							
10 gal/a	32.2	65.6	84.3	20.3	17.2	45.5	51.4
20 gal/a	29.4	58.3	82.2	18.9	15.7	45.1	51.2
LSD P = 0.05	ns	ns	ns	ns	ns	ns	ns
Adjuvant Rate v/v							
None	35.0	71.3	82.4	20.9	17.4	43.7	50.9
Induce 0.06%	30.4	60.4	84.0	19.4	16.4	46.2	51.7
Induce 0.125%	27.1	54.2	83.3	18.5	15.6	45.9	51.3
LSD P = 0.05	5.4	9.8	ns	ns	ns	ns	ns

Table 2. Fusarium head blight, Foliar Disease, Yield and Test Weight for Robust Barley, Grandin HRSW and Plaza Durum Experiments, Langdon, 2001

Fungicide Timing	Flag Leaf Necrosis			FHB Field Severity			Grain Yield			Test Weight		
	Barley	HRSW	Durum	Barley	HRSW	Durum	Barley	HRSW	Durum	Barley	HRSW	Durum
	--------- % ---------			--------- % ---------			-------- bu/a --------			------- lb/bu --------		
Morning	37.5	13.8	30.0	1.4	2.5	17.3	65.8	50.9	43.2	47.4	56.7	51.0
Mid-Day	25.0	15.0	17.5	1.9	3.6	14.3	89.9	45.6	45.5	47.7	55.8	51.0
Evening	42.5	17.5	27.5	1.5	5.1	13.9	76.6	51.3	45.5	48.1	57.0	50.9
No Fungicide	52.5	50.0	67.5	3.9	9.3	34.0	68.0	44.1	34.6	46.3	55.0	47.6
LSD=0.05	27.1	14.9	13.7	1.7	3.2	10.2	11.7	6.0	ns	ns	ns	2.1

Fungicide was Folicur 4oz/a at 20 gal/a and 0.125% v/v Induce adjuvant for all treatments
Foliar disease predominantly Septoria species.

INOCULUM DYNAMICS OF *FUSARIUM* SPECIES AND LEVELS OF *GIBBERELLA ZEAE* SPORE-TYPE RECOVERED FROM WHEAT SPIKE BIOASSAYS

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ABSTRACT

Ascospores and conidia of *Gibberella zeae* are the primary causal agents of Fusarium head blight (FHB) in wheat and barley. Although *Fusarium graminearum* is the primary causal pathogen of the disease, it may be possible that other *Fusarium* species could play a role in the successful infection by *Fusarium graminearum.* In addition, some question remains concerning the importance of ascospores versus conidia as sources of inoculum. Wheat spikes clipped and vigorously shaken in sterile distilled water and Tween20 have been used in previous studies to ascertain colony-forming units (cfu) of *F. graminearum.* This wheat-spike bioassay technique was used for three years to investigate *F. graminearum* species dynamics, and for two years to compare frequencies of *F. graminearum* spore type present on wheat at the North Dakota State experiment station in Fargo, North Dakota. *Fusarium graminearum, F. sporotrichioides, F. equiseti, F. moniliforme*, and *F. sambucinum* were present in fluctuating levels during all three years. Both spore types were observed from wheat heads, with a range of 40-80% ascospores. In addition, the wheat head bioassay technique was investigated for its efficiency to recover ascospores and conidia in equal amounts when a known aliquot of either spore type was applied directly by aerial spraying and sampled at 0.5 h, 3.0 h, 7 h, and 24 h post-inoculation. Higher levels of both spore types were recovered at earlier post-inoculation times than later times, and overall levels of recovered conidia were significantly higher than those of ascopsores. To aid in interpretation of the data, known quantities of ascospores were applied directly to Komada's agar and cfu were tabulated. More cfu were observed from conidia-inoculated plates but the amounts were not significantly different than those of ascospore-inoculated plates. Observation of both conidia and ascospore in varying ratios on wheat heads suggest that both spore types may influence disease pressure; moreover, presence of other *F.* species could potentially affect the ultimate intensity of FHB caused by *F. graminearum* if there is competition among the *Fusarium* species present.

SOYBEAN IS A HOST FOR *FUSARIUM GRAMINEARUM*

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ABSTRACT

Routine surveys of seed quality of soybean (*Glycine max*) grown South Brazil revealed unexpected infection with *Fusarium graminearum*. Seemingly symptomless seeds were surface disinfested with 1.0 % sodium hypochlorite for 2 minutes and plated on water agar or 1/4 PDA. After 8 days, spores were examined from fungal colonies growing from the seeds and determined to be members of the *F. graminearum* species complex. Seed lots varied in the percentage of infected seed, ranging from 0 - ca.20%. To determine if the fungus was pathogenic to soybean, 8 strains of the fungus derived from soybean were added to soil at a rate of 10^3 macroconidia/ ml or pods were inoculated each with 10^4 macroconidia. Seedlings grown in infested soil developed small necrotic lesions in the crown and upper tap root. Pods inoculated with the fungus developed large (>1 cm), dark brown, necrotic lesions. Younger pods inoculated with the fungus blighted and dropped from the plant. Cultures of *F. graminearum* were recovered from lesions on the crown, roots and pods of inoculated plants. The lineage of the *F. graminearum* fungus infecting soybean was determined by obtaining the DNA sequence from the EF1-alpha gene from five strains and comparing it to strains of known lineage. Strains of the fungus from soybean grown in Brazil were from lineage 2 or lineage 8. Two strains of *F. graminearum* lineage 7 from the U.S. caused similar symptoms on soybean. Mycotoxin tests on naturally and artificially infected seed are being conducted.

FUSARIUM HEAD BLIGHT: EPIDEMIC VS. NON-EPIDEMIC CONDITIONS IN SOUTH DAKOTA FOR 2001

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INTRODUCTION AND 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.

It has been observed that FHB occurs at epidemic levels when warm, humid conditions and frequent precipitation have occurred at anthesis (Bai and Shaner, 1994; McMullen et al., 1997; Parry et al., 1995). By investigating the relationship of FHB incidence and severity to environmental conditions, a better characterization of the disease can be made. 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. FHB epidemiology monitoring plots at Brookings, SD showed distinct differences in disease incidence and severity over three planting dates in 2001. The objective of this report is to compare environment and inoculum factors that may have resulted in the differential disease development.

MATERIALS AND METHODS

Spring wheat (cv. “Norm”) susceptible to FHB was planted into strips 1.4m by 45m using a 7-row grain drill. Two adjacent strips were planted on each of three planting dates (11 May, 18 May, and 29 May, 2001), referred to as planting date (PD) 1, 2, and 3, respectively. Multiple dates were initially intended to 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.

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

Daily airborne inoculum levels were monitored during the sampling period using a Burkhard Cyclone Sampler (Burkhard Manufacturing). 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 (Komada, 1975). Counts were reported as colony forming units (CFU) per day. Inoculum on wheat spikes was estimated by washing spikes using protocols described by Francl et al. (1999), with some modification (sampled spikes were not covered prior to sampling). On each day, five primary spikes per replicate were collected and placed in a flask with 50ml of sterile deionized water, shaken vigorously for 60 seconds to dislodge spores, then discarded. A 0.5ml aliquot of the wash was then spread-plated onto each of three plates of Komada's medium. Plates were then incubated 5-8 days. Colonies were described and counted after incubation. Colonies were reported as CFU per spike per day.

Disease incidence and severity was assessed in each planting date at early to late dough stage. In each replicate, 100 spikes from primary tillers were visually rated for FHB. Diseased and total spikelets were recorded for each spike. Incidence was calculated by: infected spikes divided by total spikes per replicate. Severity was calculated for infected spikes by: diseased spikelets divided by total spikelets and reported as an average over all infected spikes per replicate.

Environmental variables (Table 1) were selected (or created using existing variables) for correlation analysis against airborne spore estimates (Burkard data), spike-borne spore estimates (spike wash data), disease incidence and disease severity over planting dates and replicates. Actual vapor pressure of the air was estimated from calculations of saturation vapor pressure and on relative humidity measurements. Variables were established to represent averages over several time periods including: flowering period, 7 days prior to flowering, 4 days post-anthesis, 7 days pre-flowering+flowering period, and flowering period+4 days post-anthesis. Within each time period, for temperature and vapor pressure, variables were established to represent averages over daytime (8:00a.m. to 8:00p.m.) hours, averages over nighttime (8:00p.m. to 8:00a.m.) hours, average daily maximums, and average daily minimums. Leaf and soil-surface wetness duration, and solar radiation were totaled, and daily averages were calculated. Precipitation was totaled and number of precipitation events larger than 3mm were also indicated as variables. Wind speed was averaged over the entire time period. For Burkard and spike washing data, correlation was examined for variables representing anthesis period and earlier. For disease incidence, correlation to anthesis period variables was examined. For disease severity, correlation was examined for variables representing flowering and after.

Table 1. Environmental variables used in correlation analysis

Temperature in ^{0}C (for each time period)	Vapor Pressure (e_a) in kPa (for each time period)	Additional Parameters (for each time period)
Avg Temp	Avg e_a	Avg Daily Sol. Radiation (hrs)
Avg Daytime Temp	Avg Daytime e_a	Daily Leaf Wet. Duration (hrs)
Avg Nighttime Temp	Avg Nighttime e_a	Daily Soil Wet. Duration (hrs)
Avg Daily Max Temp	Avg Daily Max e_a	Mean Wind Speed (μ)
Avg Daily Min Temp	Avg Daily Min e_a	Precipitation (mm)
		No. Precipitation events > 3mm

RESULTS AND DISCUSSION

Major environmental parameters for each planting date are summarized in Table 2. Generally, dry conditions with moderate temperatures were experienced prior to and throughout the first and second planting date (PD1 and PD2). Leaf wetness duration (LWD) was greater during anthesis for PD2 than for PD1. Temperatures were warmer and the environment was more moist during PD3 anthesis. Inoculum levels (airborne and spike-borne) increased with time from the beginning of flowering for PD1 to end of flowering for PD3. Ascospores were present during all flowering periods, however levels were much higher for PD3. Disease levels were moderate for PD1, high for PD2 and very high for PD3 (Table 3).

Table 2. Environmental conditions over susceptible periods in each planting date.

PD	Time period (susceptible)	Avg. air temp (°C)	Avg. e_a (kPa)	Precip. (mm) / events	Mean wind spd (m/s)	LWD[a] (hrs)	SWD[b] (hrs)
1	DOY 182-188	20.4	1.730	0.3 / 0	2.19	9.8	17.7
2	DOY 188-194	23.1	1.858	0.3 / 0	1.71	12.4	0
3	DOY 200-206	23.8	2.459	27.4 / 4	2.52	14.33	24.0

a. Leaf wetness duration.
b. Soil wetness duration.

Table 3. Final disease ratings.

	Plant Date 1		Plant Date 2		Plant Date 3	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Rep 1	34	10.4	84	27.1	100	67.9
Rep 2	30	8.1	89	18.7	100	69.9
Rep 3	25	9.2	78	15.7	95	51.4
PD Mean	29.7	9.2	83.7	20.5	98.3	63.1
Overall:	Disease Incidence = 71%		Disease Severity = 31%			

For both airborne and spike-borne inoculum, highly significant correlation was observed with anthesis-period nighttime temperature and vapor pressure variables, as well as leaf wetness duration and precipitation (r = 0.96 to 0.99). Significant correlation was noted with wind speed (r = 0.61 and 0.64 for airborne and spike-borne inoculum, respectively) and soil wetness duration (r = 0.48 and 0.51). Correlation was not as strong for the pre-flowering period variables. (Table 4.) Disease incidence showed strong positive correlation to temperature, vapor pressure and leaf wetness duration. Moderate positive correlation to precipitation was also noted. Soil wetness during anthesis showed almost no correlation with disease incidence. Disease severity showed strong positive correlation to vapor pressure, nighttime temperature, leaf wetness, and precipitation. Severity showed strong negative correlation to solar radiation and moderate to strong negative correlation to daytime temperatures (depending on evaluation period).

The results of the correlation analysis may help to identify variables that are highly predictive of the various components of a forecasting or disease development model. These results reported here indicate the potential importance of nighttime conditions (especially temperature, humidity, and dew formation) on the release of inoculum into the air. The correlation of temperature and humidity with incidence values (which can be extrapolated to infection rate) may indicate their importance for spore germination and initial infection. The correlation of disease severity (an indication of spread within an infected spike) with nighttime conditions, and the negative correlation with daytime temperatures and radiation indicate that spread within infected plants probably occurs during favorable nights, and may be hindered during unfavorable days.

Table 4. Relevant correlation values for certain environmental variables against four responses.

	Airborne inoculum	Spikeborne inoculum	Airborne inoculum	Spikeborne inoculum	Disease incidence	Disease severity
	vs. anthesis period		vs. 7 days pre flowering		anthesis period	anthesis + 4 days
Night e_a	0.991	0.969	0.723	0.714	0.760	0.971
Night temp.	0.999	0.969	0.285	0.317	0.864	0.844
Day temp.	0.519	0.469	0.144	0.181	0.890	-0.330
Wind speed	0.616	0.632	-0.605	-0.655	0.097	n/a
Radiation	-0.300	-0.332	0.826	0.827	0.256	-0.878
Precipitation	0.962	0.947	0.003	0.044	0.660	0.952
LWD	0.945	0.906	0.757	0.805	0.966	0.823
SWD	0.483	0.506	0.061	0.017	-0.061	n/a

Through the continued monitoring of inoculum, disease, and environment across numerous sites within several states, local and regional FHB forecasting systems and disease development models, which would provide producers with the capability of making better management decisions, are on the horizon.

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HYPERSPECTRAL REFLECTANCE OF EIGHT SPRING WHEAT VARIETIES IN A SCAB NURSERY

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ABSTRACT

In 2001, preliminary research was conducted on the use of hyperspectral reflectance measurements for detection of disease in spring wheat. Replicated field plots were established using eight common varieties with a range of susceptibility to Fusarium head blight (FHB). Plots were situated within the FHB field screening nursery at South Dakota State University, Brookings, SD. The nursery was artificially infested by spreading colonized grain over the soil surface to serve as an inoculum source. Mist irrigation was used to increase humidity and soil wetness in the field. Beginning at heading, measurements were taken from the plots every 7 to 10 days using a hyperspectral radiometer (CI-700, CID, Inc. Camas, WA) and infrared thermometer. Digital images of all plots were also taken. The CI-700 measured reflected radiation across a waveband from 350 nanometer (nm) to 950nm, at 1nm increments. Data was compiled for each date and analyzed by performing ANOVA at each interval wavelength. The main objective of the study was to determine the potential of such data to predict disease and/or toxin levels within infected plots through elicitation of spectral signatures indicative of disease or toxin level.

SOIL-SURFACE WETNESS SENSOR: REPORT OF FURTHER TESTING

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INTRODUCTION AND OBJECTIVES

Free moisture (i.e. wetness) at the soil-air interface, or within the top few centimeters (cm) of soil is a very important factor in the development of certain plant pathogens and plant diseases. This variable has been difficult to estimate or measure with current technologies such as gypsum blocks, time-domain reflectometry, neutron probe, or tensiometers which measure moisture at greater depths. *Gibberella zeae*, causal agent of Fusarium head blight (FHB) in cereal crops resides and over-winters in corn stubble and cereal residues at or near the soil surface. From this niche, under favorable environments, ascospore inoculum develops (Paulitz, 1996; Sutton, 1982, and Parry et al., 1995) and is then available for dissemination to susceptible sites on host plants. Soil-surface moisture, often associated with precipitation events or dew formation in the canopy (Rosenberg et al., 1983) is presumed to be one of the critical environmental factors affecting the development of residue-borne pathogens (Rotem, 1978). Development of epidemiological models useful in plant disease forecasting involves gathering information about critical environmental parameters. The capacity to measure soil surface wetness and wetness duration would potentially add power to current and future models. In 2000, a sensor was developed and described (Osborne and Jin, 2000) which would be used to estimate soil wetness duration through the use of data logging equipment. The primary objective of this project was to further develop an instrument which would directly measure wetness at the soil-air interface and to conduct testing to evaluate its suitability for the intended applications.

MATERIALS AND METHODS

Initial testing of sensors.

Soil-surface wetness sensors were constructed as described by Osborne and Jin (2000). Sensors were first tested on synthetic sponges. Sensing elements were held in contact with the sponge using rubber bands. The sponge and sensor were placed under a 150W lamp to speed drying. Readings were taken from the sensor using the CR10X datalogger. Several readings were taken from the dry sponge at two-minute intervals, then water was added to the capacity of the sponge. Readings were taken every two minutes until the sponge was very dry. Several repetitions of this procedure were conducted for each sensor. The data was plotted for each repetition and the results of each run were compared statistically among replicates and sensors using analysis of variance procedures.

Further testing was performed using a thin (approx. 5mm) layer of soil (screened to pass a 2mm sieve) in a plastic container. A known weight of oven-dry soil (100g) was used to facilitate moisture content calculation after addition of specific weights of tap water. The sensor was placed atop the soil layer, secured with rubber bands and the entire assembly

was placed on a digital balance. The balance was zeroed and 20g of water was added to the soil layer using a trigger-pump misting sprayer in order to evenly wet only the soil (droplets landing elsewhere were removed with absorbent paper). The system was allowed to set undisturbed for 20 minutes to allow for water absorption into soil micropores. Thereafter at five-minute intervals, the resistance across the sensor and the weight of water remaining was recorded. This procedure was repeated several times with each sensor. Data was analyzed as described for sponge trials.

Preliminary field testing of the soil-surface moisture sensors was conducted by integration into an automated weather station placed in the 2000 FHB epidemiology study field plots at South Dakota State University, at Brookings, SD. Five sensors were placed into the field shortly after planting, and operated until just before harvest, or for approximately three months. Sensors were evaluated for response to known wetting events (precipitation) and for durability of construction.

Calibration of sensors.

Each sensor was calibrated against both volumetric moisture content as well as tactile estimates of surface wetness using three soil types in thin layer trials. A sandy loam, silt loam, and clay loam were obtained to provide a range of soil types for calibration. For each soil type, six different gravimetric moisture contents (2, 4, 6, 8, 10, and 12% water) were established in thin soil layer pans. Soil water content levels were established by adding water to oven-dry soil, then mixing to homogenize each pan. Water was replaced as needed by weighing each pan and misting the soil until the desired weight was reestablished. Each pan was classified as 'wet' or 'dry' based on appearance and tactile estimates made by touching the soil surface with bare fingers, using slight pressure. Soils were deemed to be wet if the surface appeared darker than a dry check pan of the same soil type, and felt damp to the touch. Mottled (dark/light) surface was considered evidence of wetness if accompanied by supporting tactile estimation. By this method, sandy loam soil was determined to be wet at 4% moisture and above. Silt loam and clay loam soils were wet at 6% moisture and above. Sensors were placed onto a soil pan selected at random, allowed 30 seconds to settle, then a measurement was recorded. This was repeated until each pan had been measured 12 times by each sensor. Data were transformed using natural log transformation, then analyzed by determining mean and standard deviation for each moisture level/sensor combination. Calibration values were determined for each sensor based on the mean (transformed) for the smallest soil water content which was determined to be wet (i.e. 4% for sandy loam, 6% for clay loam and silt loam). Confidence intervals (C.I.) (90%) were calculated for the selected mean (transformed) value, and the upper limit was selected. The upper limit of the CI was then transformed back to kOhms by inverse natural log transformation, and that value was then used as the wet-dry calibration value for each sensor.

Advanced Field Testing.

In 2001, six sensors were again integrated into the automated weather station at the FHB epidemiology monitoring plots in Brookings, SD by placing pairs of sensors across three sites within a 100ft radius of the weather station. Additional sensors were placed into similar studies in North Dakota, Ohio, Indiana, and Pennsylvania. Data was collected every 30 minutes. The sensors were placed into study sites onto soil that had been cleared of large

debris, or large soil peds. The area was to be smooth, to allow good sensor-soil contact. The sensors were to be depressed slightly, so as to bury the wire elements partially (but not completely) into the soil. Results of the field trials were examined in spreadsheet format. Calibration values were applied to raw data for each 30-minute period resulting in a binary response (wet or dry). The consistency across sensors for indicating wetting events was noted. The duration of wetness was calculated for 24-hour periods, and totaled over the study duration and compared among sensors.

RESULTS AND DISCUSSION

Initial testing and calibration of sensors.

The sensors performed well in all trials. Visual and tactile estimates of surface moisture compared favorably to sensor measurements on the sponge and on soil. For sponge trials, resistance values across the sensing elements were plotted against time. The resultant curves were smooth, and increased more or less exponentially. Sensors were consistent over replications, however measurements differed across sensors. The variation can likely be attributed to variations in sensor construction (sensors were individually constructed). On thin soil layers in the laboratory, sensors responded in a manner similar to the sponge trials. Initial field testing showed the sensors to be quite uniform in sensing wetness events (precipitation or heave dewfall). The sensors were durable, and appeared to be in good condition after the initial field trial.

Calibration values were determined for each sensors. Values determined ranged from 30 kOhms to 120 kOhms, with variation between sensors and across soil types.

Advanced field testing.

Sensors were generally very consistent for indication of wetting events. Variation of (+/-) 30 minutes was observed in some cases, however, most sensors responded to wetting events within the same 30-minute sampling period. Differences are likely attributable to sensor variation or variation in microclimate across sensor sites. Duration of wetness (Table 1) varied among sensors for specific wetting events. When considered over several events, however, the difference among sensors was insignificant (Table 2). The variation for singular events again could be attributed to microclimatic differences of sensor sites, sensor construction, or errors in calibration values. An error of (+/-) 30 minutes per event may be expected due to the sampling period used in the evaluation. The analysis shows that sensors within pairs were not significantly different over all events, and differences among pairs (or sites) were also not significant. For individual events, sensors within pairs tended to be similar while pairs tended to be different, suggesting site differences attributable to canopy or microclimate variability. Calibrations may also account for variation given that duration was estimated using the calibration values derived on specific soil types in the greenhouse. When larger calibration values were applied (potentially overestimating wetness duration) sensor uniformity increased greatly for individual wetness events. This technique may be more useful for model development as long as any potential overestimation remains consistent over locations and time.

Table 1. Soil wetness duration (hours) for six sensors following precipitation events.

Wetness Event	Precip (mm)	SSWS 1	SSWS 2	SSWS 3	SSWS 4	SSWS 5	SSWS 6
1	<1	10.5	15.0	0.0	1.0	17.5	15.0
2	18	32.5	54.5	36.5	85.0	83.5	59.5
3	4	15.5	15.5	19.0	16.5	24.5	15.5
4	15	112.5	108.5	109.5	123.5	115.0	108.0
5	<1	4.0	2.0	0.0	0.0	4.0	0.0
6	47	320.5	344.0	317.0	346.0	347.0	322.0
7	3	21.5	21.5	3.0	0.0	12.0	0.0
8	10	54.5	54.0	36.0	16.0	32.5	23.0

Table 2. Analysis of variance (ANOVA) for paired sensors.

Source of variation	df	MS	P > F
Pairs	2	270.2	0.9786
Sensors(Pairs)	3	209.1	0.9970
Error	42	12507	

Sensor durability was very good. Sensors remained intact and in good condition over the course of one field season. Calibrations will be checked during the winter to determine if they remain stable over time.

CONCLUSIONS

Overall the sensors performed well. In laboratory tests, sensors responded smoothly to changes in substrate moisture content, and were consistent in replicate trials. Calibration values were determined for three soil types, and allowed for wet/dry differentiation compared to tactile estimates. Field trials were considered successful in that sensors responded in a uniform manner to precipitation or heavy dew. Care must be taken to apply proper calibration values to data in order to best estimate the field conditions. Laboratory derived values may not be representative of field conditions. Sensors were durable over the course of a field season and remained in good condition.

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EFFECT OF *GIBBERELLA ZEAE* ASCOSPORES AND *FUSARIUM GRAMINEARUM* CONIDIA ON FUSARIUM HEAD BLIGHT SEVERITY AND DEOXYNIVALENOL PRODUCTION IN BARLEY

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OBJECTIVE

To determine if ascospores of *Gibberella zeae* and conidia of *Fusarium graminearum* induce quantitatively similar levels of Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration in inoculated barley plants.

INTRODUCTION

FHB, caused by *F. graminearum*, threatens the existence of the malting barley industry in the Upper Midwest region of the USA [1,2]. Researchers searching for resistance against FHB use either conidia of *F. graminearum* or ascospores of the perfect stage of *G. zeae* for inoculum. In some screening programs, ascospores are used for inoculum in field tests and conidia for growth chamber/greenhouse tests. An important factor to consider in the choice of inoculum for these tests is whether the relative infectivity of ascospores and conidia is similar. A previous study comparing the infectivity of the two spore types in wheat was conducted in the greenhouse (4). The objective of this study was to compare *G. zeae* ascospores and *F. graminearum* conidia for their ability to cause disease and produce DON in barley under both growth chamber and field conditions.

MATERIALS AND METHODS

The six-rowed barley cultivar Stander (susceptible to FHB) and the two-rowed line CIho 5415 (moderately susceptible to FHB) were inoculated in both growth chamber and field experiments. Inocula of both spore types were derived from *F. graminearum* isolate KB176. Conidial inoculum was produced on PDA (50% potato dextrose), whereas ascospore inoculum was produced on carnation leaf agar (5). Plants at the early to mid-dough stage of development were inoculated (10,000 conidia/ml) in the growth chamber and field using methods modified from Salas et al. (1) and Prom et al. (3), respectively. Greenhouse grown barley plants were inoculated, given a 24-hr moist period (22ºC with 100% RH), and then placed in growth chambers at 25/20ºC (12 hr 5000 lux light /12 hr dark) for two weeks. Plants were misted twice a day to increase humidity and infection. In the growth chamber experiments, each treatment (ascospores and conidia) consisted of two to four replicates, and each replicate of three to five spikes. DON assays were made for each replicate.

For the field experiments, plants at the early to mid-dough stage were spray-inoculated at dusk and immediately covered with plastic bags to maintain high humidity. Bags were removed the following morning to avoid excessive heat development. Each treatment (as-

cospores, conidia, and control) consisted of four replicates, and each replicate was composed of five to ten spikes. DON assays were performed on each replicate.

RESULTS AND DISCUSSION

Growth chamber and field data for FHB and DON were analyzed separately due to low FHB infection of field-inoculated plants. Ascospore inoculum induced slightly higher FHB levels than conidial inoculum on Stander and CIho 5415, but the difference was only statistically significant for CIho 5415 in the field test (Figs. 2a and 3a). In a similar study on wheat, Stack (4) found that ascospores and conidia induced quantitatively similar levels of FHB in single floret inoculations. No statistically significant differences were detected between the spore types for DON production, except again in the case of CIho 5415 in the field where ascospore inoculum produced a higher DON level than conidial inoculum (Figs. 2b and 3b). The general trends observed for FHB severity were not always reflected in the corresponding DON concentrations, particularly for CIho 5415 in the growth chamber test and Stander in the field test (Figs. 2 and 3). When FHB and DON data of the two barley accessions were pooled, ascospore inoculum always resulted in slightly higher levels than conidial inoculum; however, the only significant difference detected was for FHB under field conditions (Table 1). When FHB and DON data of the two spore types were pooled, cultivar Stander exhibited significantly higher DON and FHB levels than the two-rowed accession CIho 5415 in both experiments, except for FHB under field conditions (Table 2). This result was not unexpected because six-rowed accessions are generally more susceptible than two-rowed accessions. The results from the field should be interpreted with caution because natural inoculum was present in the plots as indicated by the disease levels on controls (Fig. 3).

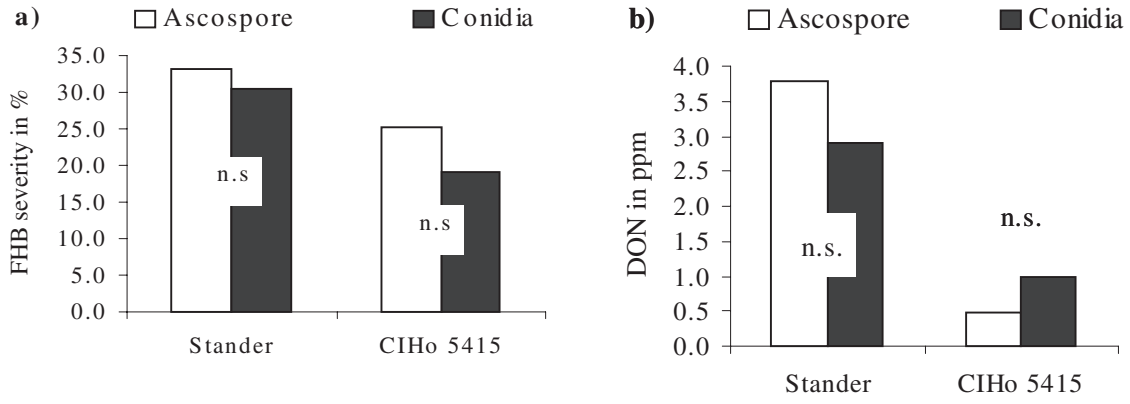

Fig. 2. Effect of conidia (*Fusarium graminearum*) and ascospore (*Gibberella zeae*) inocula on FHB severity (a) and DON concentration (b) in Stander (six-rowed) and CIho 5415 (two-rowed) barley under growth chamber conditions, n.s.=not significant ($P \leq 0.05$) based on LSD-test.

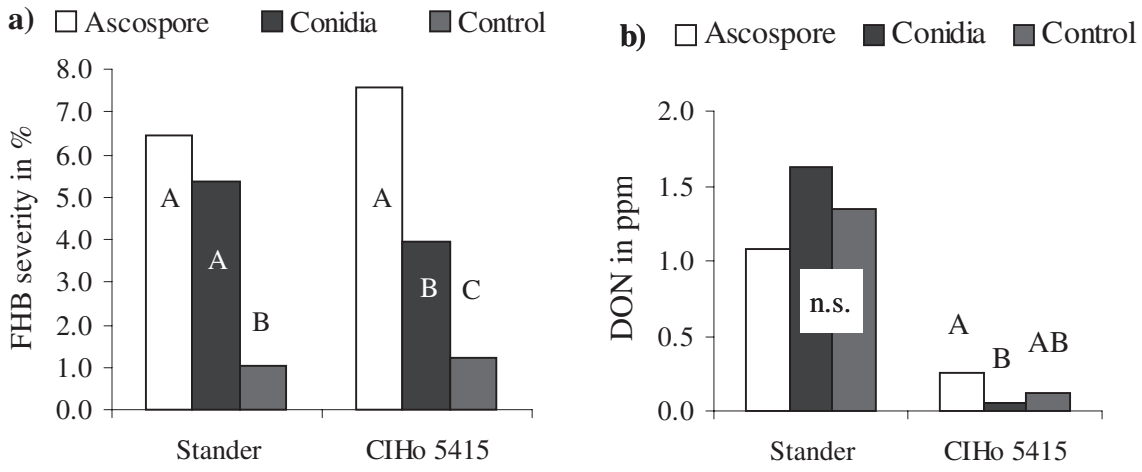

Fig. 3. Effect of conidia (*Fusarium graminearum*) and ascospore (*Gibberella zeae*) inocula on FHB severity (a) and DON concentration (b) in Stander (six-rowed) and CIho 5415 (two-rowed) barley under field conditions. Means followed by the same letter are not significantly different at P≤0.05 by the LSD-test, n.s.=not significant.

Table 1. Effect of conidia (*Fusarium graminearum*) and ascospore (*Gibberella zeae*) inocula on FHB severity and DON concentration in barley inoculated in growth chamber and field experiments, Fargo 2000.

	Growth chamber				Field			
Spore type	**FHB**		**DON**		**FHB**		**DON**	
Ascospore	33.8	n.s.[1]	2.13	n.s.	6.48	A	0.84	n.s.
Conidia	23.33	n.s.	1.95	n.s.	5.21	B	0.66	n.s.
Control	-----		------		1.14	C	0.74	n.s.

Means are based on six and twelve replicates in the growth chamber and field experiments, respectively. Means followed by the same letter are not significantly different at P≤0.05 by the LSD-test. The background infection level of the control in the field was significantly lower than the inoculated treatments.

[1]n.s. = not significant

Table 2. Effect of barley accessions on FHB severity and DON concentration in growth chamber and field experiments, Fargo 2000.

	Growth chamber				Field			
Spore type	**FHB**		**DON**		**FHB**		**DON**	
Stander	33.7	A	3.35	A	4.29	n.s. [1]	1.35	A
CIHo 5415	23.11	B	0.73	B	4.26	n.s.	0.14	B

Means are based on six and twelve replicates in the growth chamber and field experiments, respectively. Means followed by the same letter are not significantly different at $P \leq 0.05$ by the LSD-test.

[1]n.s. = not significant

CONCLUSION

The results from this study indicate that ascospores and conidia generally induce similar levels of FHB severity and DON concentration in inoculated barley under the more controlled conditions of the growth chamber. Thus, the choice of spore type for testing the resistance of barley to FHB may depend largely on the ease by which individual researchers can produce them. One other important consideration may be the genetic stability of the inoculum source. Propagation of sexual spores (ascospores) may result in greater genetic variability than with conidial inoculum.

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EFFECTS OF DEOXYNIVALENOL ON BARLEY LEAF PIGMENTATION

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OBJECTIVE

To determine how deoxynivalenol (DON) affects barley leaf tissues.

INTRODUCTION

Tricothecene toxins have been implicated as virulence factors in pathogenesis of *Fusarium graminearum* in wheat head blight. Mutant fungal strains lacking ability to synthesize trichothecenes were pathogenic but gave reduced incidence and severity of disease, less bleaching of heads, and less yield reduction compared to toxin-producing strains (Desjardins et al., 1996; Eudes et al., 1997; Mirocha et al., 1997; Proctor et al., 1995). DON is known to be a potent inhibitor of protein synthesis by animal ribosomes (Feinberg and McLaughlin, 1989). In plants, DON inhibited 3H-leucine incorporation into protein in maize and wheat tissues (Casale and Hart, 1998) and also inhibited in vitro protein synthesis by ribosomes isolated from wheat leaves (Miller and Ewen, 1997). In addition, several investigators have used growth inhibition as a way to compare DON sensitivity among wheat cultivars (Cutler and Jarvis, 1985; McLean, 1996). However, information is generally lacking on the cytological and physiological effects of DON on plant cells. As a first step in characterizing the role DON plays in pathogenesis of *Fusarium graminearum* in leaf and head tissues, we treated detached barley leaf tissues with DON and examined them daily for signs of injury or other alterations. As shown here, DON had pronounced and unexpected effects on leaf pigmentation at DON concentrations that were not usually lethal.

MATERIALS AND METHODS

Segments 1.0 cm long were cut from 7-day-old primary leaves of Robust barley plants after approximately 2/3 of the abaxial epidermis was stripped from the leaves. The stripped segments were floated (3/dish) on 1.5 ml of aqueous DON solution in glass dishes, 2.5 cm in diameter and 0.8 cm high. These dishes, in turn, were incubated in covered 9 cm Petri dishes in a plant growth chamber, usually with fluorescent light (110 µmol m-2s-1) and incandescent light (40 µmol m-2s-1) for 18 hr/day. DON was used at concentrations of 10-100 ppm at steps of 10 ppm.

RESULTS

Regardless of DON concentration, leaf segments usually remained alive for at least 5-6 days, exhibiting little or no water soaking or necrosis. Within 3-4 days, however, the segments changed color in three different ways depending on DON concentration:

1. At 10-30 ppm, tissues turned light reddish brown while retaining a green background comparable to the green of control segments floated on water. In darkness, the brown color did not develop.
2. At 50-70 ppm, most tissues turned white, losing all chlorophyll and carotenoid pigments. In darkness, this loss of pigment did not occur.
3. At 80-100 ppm, the leaf segments usually remained dark green, in stark contrast to the white segments at lower DON concentrations. At 4-5 days after treatment, the segments remained as green as they were at the time of treatment. The green color was retained in either darkness or light. Control segments floated on water generally became chlorotic by 4-5 days, the usual senescence response of barley leaf tissues to detachment from plants.

Color responses sometimes varied both among segments floated on a given DON concentration and in different portions of a given segment. For example, at 30-40 ppm, some segments were all brown or all white, while others were mottled brown and white. At 60-80 ppm, some segments had white spots within a dark green background. Although most segments appeared to remain alive after incubation on DON at 10-100 ppm, some segments became water soaked at the specific concentration of 50 ppm. Also, in separate trials at 200 ppm, about 50% of segments became water soaked by 5-6 days.

DISCUSSION

The results indicate that DON induced four distinct, but overlapping responses in detached barley leaf segments as follows:

1. Light dependent brown pigment formation at 10-30 ppm.
2. Light dependent loss of chlorophyll and carotenoid pigments at 50-70 ppm.
3. Light independent retention of chlorophyll at 80-100 ppm.
4. Cell death at 200 ppm and sometimes also at the specific concentration of 50 ppm.

These responses often were interrelated as, for example, the retention of chlorophyll at higher DON concentration counteracted chlorophyll loss seen at intermediate concentrations. However, the effects of DON on pigment formation, loss, or retention were not consistently related to the death of tissue. Most segments treated with DON did not die. Cells died at the very high concentration of 200 ppm, and, for unexplained reasons, at the intermediate concentration of 50 ppm.

In separate experiments (not described here), we determined that cytoplasmic streaming was not disturbed by 10-100 ppm DON in epidermal cells from barley coleoptiles. Thus, these epidermal cells, which lack chloroplasts, were relatively insensitive to DON.

The light intensity used in our experiments totaled 150 µmol m-2s-1, relatively low compared to intensities usually used to grow barley. In preliminary trials with light at 240 or 450 µmol m-2s-1, loss of chloroplast pigments occurred rapidly at all concentrations from 20-100 ppm DON. Chlorophyll was not retained at 80-100 ppm DON as it was earlier at 150 µmol m-2s-1.

We have not yet measured DON concentrations within treated tissues. Since DON inhibits protein synthesis by binding to ribosomes, we speculate that chloroplast ribosomes may be highly sensitive to DON, leading to inhibition of chlorophyll and carotene synthesis at concentrations that don't affect cytoplasmic ribosomes. In any case, the present results suggest that pigment alteration in barley heads infected by F*usarium graminearum* may be a consequence of pathogen-produced DON at concentrations which do not induce plant cell death.

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SPATIAL PATTERNS OF FUSARIUM HEAD BLIGHT IN NEW YORK WHEAT FIELDS IN 2000 AND 2001

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INTRODUCTION

Spatial pattern analysis is used in plant disease epidemiology for understanding the nature of inoculum sources (Madden and Hughes, 1999). We assessed the spatial patterns of the incidence of Fusarium head blight (FHB) in New York winter wheat fields in 2000 and 2001.

MATERIALS AND METHODS

The incidence of heads showing symptoms of FHB was assessed in 60 quadrats of size 0.093 m2 (1 ft2), 20 spikes per quadrat, during the late milk to dough stages. The BBD (Beta Binomial Distribution) program (Ver. 1.3) (Madden and Hughes, 1994) was used to calculate the index of dispersion and test whether the pattern of FHB incidence was random.

RESULTS AND DISCUSSION

In 2000, mean incidence of FHB was less than 10% in three fields, but was four to six times higher in field 4-00. Aggregation of heads symptomatic of FHB was significant in field 4-00 only (Table 1). In 2001, the incidence of FHB was less than 11% in all fields sampled. Tests of the index of dispersion indicated that the pattern of FHB was random in all six fields in 2001.

Corn residues were not observed in fields 1-00, 2-00 or 3-00. In field 4-00, a very few small remnants of corn stalks from a corn crop two years earlier were still visible on the soil. There was very little visible corn residue in fields 1-01, 2-01, 3-01, and 4-01. However, there was a fair amount of corn residue distributed on the soil surface in fields 5-01 and 6-01. The incidence of FHB was also highest in these two fields in 2001 (Table 1).

The random pattern of FHB observed in all fields (except 4-00) indicated that airborne inocula are important in contributing to FHB in New York. The observed aggregation of FHB in field 4-00, together with the observed corn residue, suggests that at least a portion of the inoculum for spike infection was derived from within-field sources. 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 higher incidences of FHB in fields 5-01 and 6-01 compared to the other fields sampled in 2001 indicated some role of within-field inoculum contribution to FHB. The absence of an aggregated pattern of FHB in those two fields suggests relatively low contributions of within field sources to inoculum levels.

These results present 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.

Further research is necessary to determine the relative contributions of external versus local sources of inoculum to FHB.

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Table 1. Incidence and spatial pattern of Fusarium Head Blight in New York winter wheat fields in 2000 and 2001.

Field[a]	Cultivar	Previous crop[b]		FHB incidence (%)[c]	Index of Dispersion[d]	
		Year-1	Year-2		*D*	*P*-value
1-00	Caledonia	oat	wheat	5.3	0.80	0.87
2-00	AC Ron	pea	corn	6.0	1.11	0.27
3-00	Caledonia	pea	corn	4.2	0.86	0.78
4-00	unknown	soybean	corn	23.8	1.77	<0.001
1-01	Caledonia	pea	corn	0.9	0.84	0.81
2-01	Caledonia	snap bean	corn	1.4	0.86	0.77
3-01	Harus	cabbage	corn	2.3	1.15	0.20
4-01	Geneva	pea	corn	2.7	1.01	0.45
5-01	Caledonia	corn	snap bean	7.8	0.79	0.88
6-01	Caledonia	corn	soybean	10.1	1.07	0.32

[a] Four fields were sampled in 2000 and six were sampled in 2001. The numbers after the dash indicate the year (-00 = 2000, -01 = 2001).
[b] Year-1 is one year previous to the current year. Year-2 is two years previous to the current year.
[c] The percentage of wheat heads showing symptoms of Fusarium Head Blight.
[d] $D > 1$ suggests aggregation of FHB incidence. *P*-values are for a test of whether *D* differs from its expected value for a random pattern of disease incidence.

ESTIMATION OF TYPE II RESISTANCE – A DILEMMA IN NEED OF A SOLUTION

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OBJECTIVE

The objective of this study is to develop a method of characterizing Type II resistance to Fusarium head blight of wheat that is accurate, precise, and reflects the smallest differences among genotypes.

INTRODUCTION

At the 2000 National Fusarium Head Blight Forum, Bushnell spoke about the need to distinguish clearly among the various types of resistance in cereals to Fusarium head blight, and to develop reliable methods of measuring resistance (Bushnell, 2000). Type II resistance to Fusarium head blight in wheat is resistance to spread of symptoms from an infection. It is the type of resistance most often investigated in genetic studies. Type II resistance is commonly measured as the percentage of spikelets blighted at one or more times after inoculation of a single floret. The progression of symptoms throughout the spike is measured either by visually estimating the percentage of spikelets blighted or by counting the blighted spikelets and expressing these as a percentage of total spikelets.

For foliar diseases, severity is commonly recorded as proportion (percent) of leaf area affected. This method compensates for differences in lesion size and leaf size among treatments, and it is faster than counting lesions. The assumption that underlies this method of severity assessment is that the density of inoculum landing per unit area of leaf is, on average, the same on all leaves being assessed. A leaf that is twice the area of another leaf will receive twice as many spores of a fungus, but the resulting percent severity of disease should be the same.

Is expression of head blight severity as the proportion of blighted spikelets the best way to characterize Type II resistance? It is commonly measured by observing the progression of blight throughout the spike from a single inoculated floret (point inoculation). The assumption of equal density of inoculum, which underlies the use of percent severity as a comparative measure of leaf disease severity, is not met by point inoculation. With point inoculation, spikes with different numbers of spikelets do not receive the same relative amount of inoculum. A spike with 20 spikelets and a spike with 10 spikelets both receive the same amount of inoculum (a certain number of conidia of the fungus applied to a single floret).

We would argue that the rate of invasion of a spikelet, as reflected by the number of spikelets blighted during some interval of time, does not depend on the total number of spikelets on a spike. Consider the example above: one plant with 10 spikelets per spike and another with 20 spikelets per spike. If, after single-floret inoculation, 2 spikelets on each plant be-

come blighted, proportional severity for the first plant would be 0.2 and for the second plant would be 0.1. The first plant would be considered twice as susceptible as the second. There seems no reason to suppose, however, that the total number of spikelets governs the rate at which the fungus invades the spike. If the 2 plants each had 2 spikelets blighted after single-floret inoculation, it seems logical to conclude that these plants have an equal degree of Type II resistance. By this reasoning, severity should be expressed as the number of spikelets blighted, not the proportion of spikelets blighted.

There is a problem with expressing severity at the upper end of the spectrum from complete resistance to full susceptibility, whether as number or proportion of blighted spikelets. Consider again the example of the 2 plants. If both are completely blighted after single-floret inoculation, then the first plant will have 10 blighted spikelets and the second will have 20 blighted spikelets. When severity is expressed as the number of spikelets blighted, the first would have only half the severity as the second, and would appear to have the same degree of Type II resistance as a plant with 20 spikelets that had 10 spikelets blighted. The number of spikelets per spike places an upper limit on severity, and it is not known whether the first plant, if it had 20 spikelets per spike, would have all of them blighted, still only 10 blighted, or some number between 10 and 20. This same uncertainty, however, would apply if severity were expressed as proportion. In that case, both plants would have a severity of 100%, but it is not known whether the plant with 10 spikelets is really as susceptible as the plant with 20. If at least one spikelet (normally at the base of the spike) remains unblighted after single-floret inoculation, then the number of spikelets blighted can be used as an estimate of Type II resistance. However, once all spikelets are blighted, there is uncertainty, but expression of severity as percent spikelets blighted does not eliminate this uncertainty.

METHODS

We used data from the 2001Uniform Winter Wheat Head Blight Nursery to compare these 2 ways of expressing severity (number of blighted spikelets versus proportion of blighted spikelets). To obtain these data, we inoculated a single floret of a well-developed spikelet near the tip of the head at the beginning of anthesis. Inoculated plants received a moist period of 48 hours after inoculation. At 10 and 20 days after inoculation, we counted the number of blighted spikelets. We also counted all the spikelets on the spike, so that counts could be converted to proportions. There were 6 replicate plants for most entries; a few had only 5.

RESULTS

Frequency distributions for individual plant severities at day 20 were similar for count and proportional data (Fig. 1A & B), except for a peak at a severity of 100% (Fig. 1B). Plants that comprised this group had a total number of spikelets that ranged from 12 to 22, and therefore in the frequency distribution for count data, these plants were distributed over this range.

The correlation between means of entries for number of blighted spikelets and percent severity was high (R=0.97). At the low end of the range of means, the association was close, but at higher severities there were some deviations between the 2 measures of severity (Fig.

2). For example, 2 lines had similar proportional severities, but differed by 2 spikelets when severity was expressed as counts (points labeled B and C in Fig. 2). Conversely, 2 lines that had similar severities expressed as counts differed considerably when severity was expressed as percent (points labeled A and B in Fig. 2).

The correlation of entry mean ranks was close for the 2 ways of expressing severity of head blight (Fig. 3). The 2 entries that deviated most from the trend line (KS96HW115 and VA98W0593; the points enclosed in a box) had the 2nd and 3rd fewest spikelets per spike (and 14.2 and 14.7). Thus, the susceptibility of these lines was inflated when severity was expressed as the proportion of spikelets blighted.

The average number of spikelets per spike for entries in the 2001 UWWFHBN ranged from 13.5 to 23.5. We investigated the possibility that entries with larger spikes would appear to have a greater degree of Type II resistance when severity is expressed as the number of blighted spikelets. The correlation between number of blighted spikelets and total spikelets per spike was 0.21, not significant. The correlation between ranks for these 2 variables was likewise not significant (R=0.178). The correlation between percent severity and total number of spikelets per spike was low, but significant (R=-0.377, P=0.008).

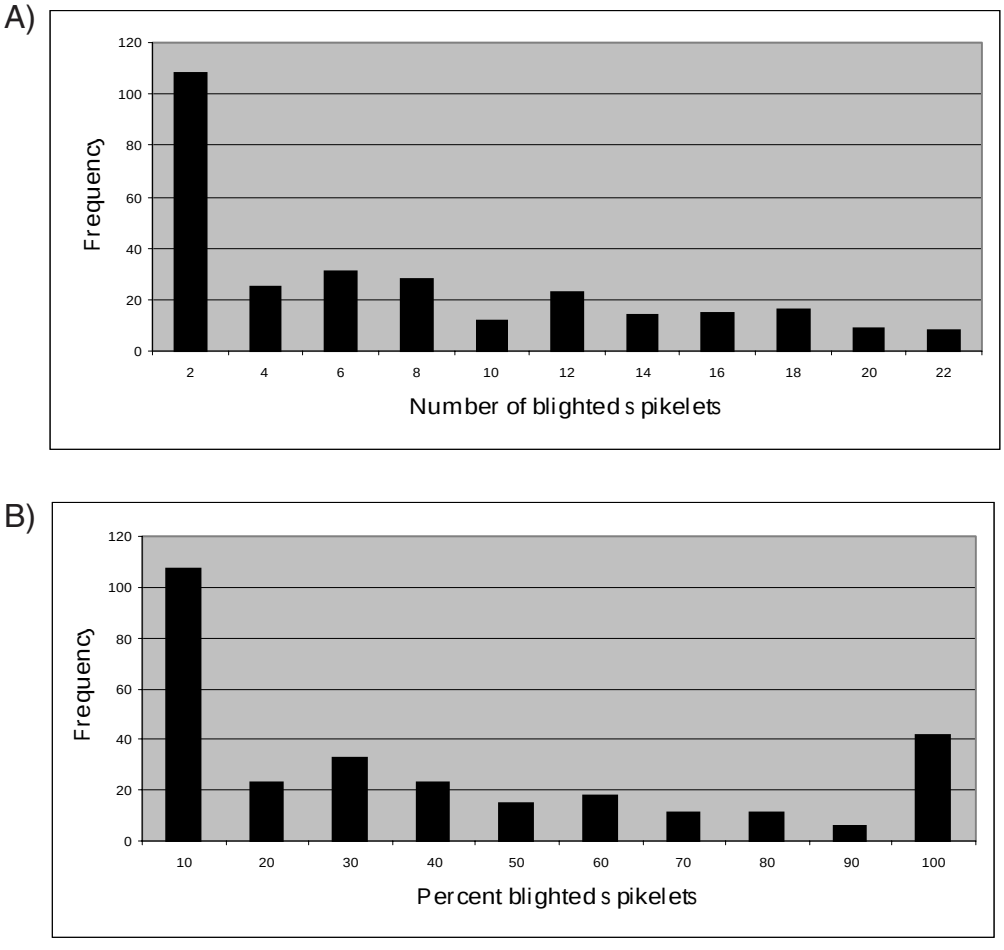

Figure 1. Frequency of Fusarium head blight severity values for entries in the 2001 Uniform Winter Wheat Fusarium Head Blight Nursery. Numbers on the x-axes are the upper limits of each interval. A. Frequency based on number of blighted spikelets per spike. B. Frequency based on percent of blighted spikelets per spike.

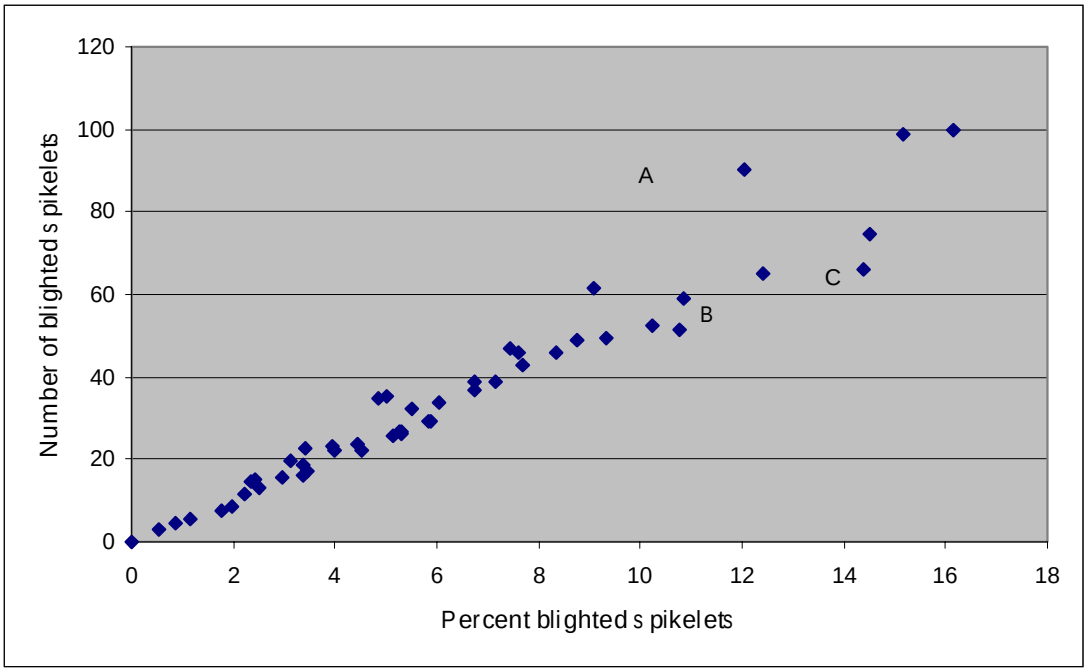

Fig. 2. Relation between head blight severity expressed as mean number of blighted spikelets per spike versus mean proportion of blighted spikelets for the 49 lines in the 2001 UWWFHBN.

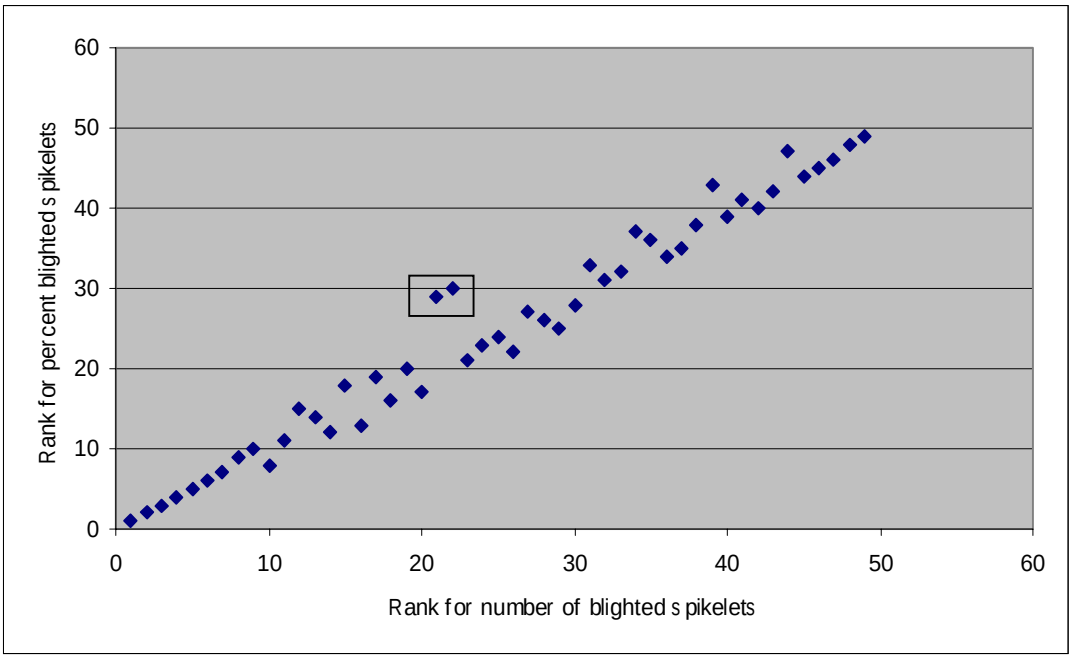

Fig. 3. Relation between rank of head blight severity expressed as number of spikelets blighted and rank expressed as percent of spikelets blighted for the 49 lines in the 2001 UWWFHBN.

DISCUSSION

It seems more logical to express severity of head blight, following single-floret inoculation, as the number of spikelets blighted rather than as the proportion of spikelets blighted. Expressing severity in absolute terms reflects absolute rate of blight development rather than a rate that is adjusted for the number of spikelets on the spike. Differences in spike size are not likely to influence the degree of Type II resistance. Data from genetic experiments are only ambiguous when severity has reached a maximum: all of the spikelets blighted. This ambiguity exists for relative (percent) severity as well.

The low, but significant correlation between percent severity and total spikelets per spike is not surprising because total spikelets is the denominator for the calculation of percent severity, and therefore these two variables are not independent of each other. This association suggests that selection for Type II resistance based on percent severity would favor lines with larger spikes. While this may be good for general improvement of plant type, it could result in failure to select for some genotypes with useful resistance because they happen to occur in a plant with a small head.

The conclusions from this study need to be tested by examining a larger set of data. We are in the process of conducting analyses with data from genetic studies, involving both advanced-generation recombinant inbred lines and early generation segregating populations. It appears that use of percent severity to characterize Type II resistance will not lead to gross errors in evaluating wheat lines, but it could lead to failure to select lines with potentially useful partial resistance and could be an additional source of error in genetic studies. Because greater resistance is the goal of germplasm enhancement and breeding programs, this is the end of the spectrum of reaction that is of greater interest. It appears that severity of head blight expressed as the number of blighted spikelets rather than the percentage of blighted spikelets distinguishes differences among genotypes better toward the resistance end of the spectrum.

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PLANT RESIDUE MANAGEMENT AND FUSARIUM HEAD BLIGHT

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ABSTRACT

The research presented in this review is part of an ongoing investigation to establish the correlation between residue management and the survivability of *Fusarium*. Residue decomposition and *Fusarium* survival are quantified when wheat, barley and corn plant residues are placed on and below the soil surface. Cover crop and nitrogen (N) fertilizer treatments are included as variables. Parameters related to decomposition such as soil temperature and water and carbon to nitrogen ratio of the residue are monitored. If *Fusarium graminearum* survival is related to residue decomposition, then residue management strategies which enhance displacement of *Fusarium* might be developed.

Wheat, barley, and corn residues, infested with *Fusarium graminearum*, were collected at crop harvest. On September 16-17, 1999 the prepared bags were placed in a field of wheat stubble. One-half of the replicated test plots were fertilized with nitrogen. In the spring of 2000 following standard crop rotation practices, a soybean cover crop was planted on one-half the plots to establish plant canopy and soil water variables. Decomposition rates (weight loss) and *Fusarium* populations were determined at 30 day sampling intervals throughout the study period (Fall 1999-Fall 2001). Nitrogen analyses were completed on composite residue samples for each residue type. Soil samples were collected at the research site to quantify chemical and physical properties. Populations of *Fusarium graminearum* on the residue were determined by quantitative plating techniques.

In the case of all three substrates, buried residue decomposed faster than residue left on the surface. Corn residue was lost at a faster rate than either the wheat or barley residues. Nitrogen fertilizer did not enhanced the decomposition rate. *Fusarium* populations appear consistent with the level of residue present. In the Project's final report, residue decomposition rates, fusarium survivability, soil water availability, soil temperature and residue nutrient status will be correlated based on residue placement, N fertility and cover crop.

This poster was presented at the 2001 Annual Meeting of the Soil Science Society of America, Charlotte, NC, October 21-25. Support for this research has been made available from the United States Wheat and Barley Scab Initiative, Grant 59-0790-9-070.

DEVELOPMENT OF PERITHECIA FROM *GIBBERELLA ZEAE* ON WHEAT RESIDUE

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) is the major causal organism of Fusarium head blight (FHB) in the United States. This disease can affect all classes of wheat causing reduction in yield, poor seed quality and mycotoxin contamination. *G. zeae* over-winters in wheat residue as hyphae. Very little is understood about the formation of perithecia from hyphae in wheat residues. Our objectives are to characterize the early stages of perithecial development and investigate whether colonization of specific wheat tissues is important to development of perithecia. Preliminary findings on perithecium development in debris will be presented. Implications of this work towards control of FHB will be discussed.

COMPARISON OF POPULATIONS OF *GIBBERELLA ZEAE* FROM KOREA AND NORTH AND SOUTH AMERICA

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ABSTRACT

We isolated populations of *Gibberella zeae* (*Fusarium graminearum*) from field samples of wheat, barley, maize or sorghum from North and South America, and from South Korea. We compared the phylogenetic lineage composition from these sources using AFLP markers produced by three standard primer combinations. United States populations of *G. zeae* from wheat are composed of a single phylogenetic lineage (lineage VII) and are diverse but relatively homogeneous across the country. South Korean populations from barley were dominated by a single lineage (lineage VI). South Korean populations from maize are dominated by lineage VII, but lineage III is a relatively common component. Populations of *G. zeae* from wheat in Brazil also appear to be dominated by lineage VII, but at least one other lineage is present. We have also examined *G. zeae* populations from wheat and sorghum in Uruguay.