INFLUENCE OF CROP ROTATION AND COVER CROP ON FUSARIUM HEAD BLIGHT OF WHEAT

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INTRODUCTION

Fusarium head blight (FHB) is a devastating fungal disease affecting the wheat industry world-wide (Bai and Shaner, 1994, Gilbert and Tekauz, 2002 and McMullen *et al*., 1997). FHB reduces yield and grade and may also contaminate the grain with fungal toxins (vomitoxins) which make grain unfit as food and feed (Gilbert and Tekauz, 2002). Host plant resistance has been considered as the most economical and environmentally-friendly means of disease management. Wheat varieties with sufficient resistance to FHB are not available for cultivation. Fungicide application is the common practice for the control of FHB. Resistance to some fungicides in the populations of FHB pathogen has been reported (Zhou *et al*, 1994). Of concern too, is the fact that chemicals have long-term environmental consequences. Therefore, alternative disease management options are needed to meet the immediate need of wheat growers against FHB. The FHB pathogen overwinters mainly on wheat stubble. We often recommend manipulations of cultivation practices, including crop rotation with non-host crops for disease management, as a good rotation would allow enough time for infested residue to decompose before the next cereal crop is seeded. There is little information available on long-term research data and the benefits of crop rotation with non-host crops on FHB disease management. FHB disease initiation starts with landing of ascospores originating from infected crop residue left on soil. Therefore, a cover-crop would likely act as a barrier to ascospore dispersal onto wheat heads. With these objectives in mind, two long-term experiments were initiated to determine the effect of crop rotation and cover crop on FHB disease management in wheat.

MATERIALS AND METHODS

Effect of crop rotation

The experiment was conducted at Carman Field Research Station, Carman, MB where FHB is known to be endemic. It was initiated in 2001 with the establishment of four foundation crops: canola, wheat, oats and peas on four plots of 10 X 60 M (main plot). The four main plots were separated by a 30 M strip of fall rye. In 2002, the main plots were divided into four sub-plots (10 X 15 M) where canola, wheat, oats and peas were grown. The crops included were canola Liberty Link variety 2663 (transgenic for regular herbicide), oats Riel, peas Carnival and spring wheat CDC Teal. The crops in the rotation were seeded under zero tillage conditions on the stubbles of previous years' foundation crops. In 2002, in the center of the 30 X 60 M barrier strip of fall rye planted in 2001, canola, wheat, oats and peas were seeded as foundation crops in 10 X 60 M plot for 2003, creating 10 M-wide barrier strips between the main plots to avoid inter-plot interference. There are 16 crop rotations in this trial to be conducted over the

years, allowing us to obtain two identical replicates for statistical analysis. The crops in the rotation were treated with appropriate herbicides for weed management. At maturity, the crops were combined and harvested and the stubbles were spread back into plots.

To estimate daily release of ascospores and macroconidia of *Fusarium graminearum*, one rotorod spore sampler (Aerobiology, Nepean, ON) was set up in the center of each main plot. Two other traps were placed outside of the crop plots to determine the background inoculum. The rods were changed every 24 hrs. The rods were stored in the cold room until further analysis.

Three 1-meter row wheat head samples were collected from each wheat plot in the rotation, and the samples were frozen until disease assessment. Percent disease incidence and disease severity were determined. FHB disease index was calculated as: % incidence X % severity/ 100. Before combining the harvest, three samples of six 2.5-meter rows were also hand harvested for yield, FDK and DON analysis.

Effect of cover crop on FHB

This experiment was conducted at the Point Field Research Station, Winnipeg, Manitoba following a randomized complete block design with four replications. The treatments were i) no *Fusarium* inoculum and no cover crop, ii) *Fusarium* inoculum but no cover crop, iii) no *Fusarium* inoculum but cover crop and iv) *Fusarium* inoculum and cover crop.

Trifolium pratense L. (red clover) was established as the cover crop treatment in plots about three weeks before wheat seeding. After seeding clover, all the plots including non-cover crop treatment plots were harrowed once. Usual agronomic practices were performed as and when necessary for crop management. *Fusarium* inoculum treatment plots were inoculated about two weeks before anthesis of wheat by spreading 100g *Fusarium*-infested corn inocula/ M^2 (corn spawn). The plots were irrigated with a boom sprayer in the evening for three days after inoculation to provide high humidity for perithecia development. In each plot, two rotorod spore samplers were set up at two levels, one just above the height of cover crop and the other at wheat head height. Four other spore traps were also placed at two said heights outside of the plots to monitor background inoculum levels.

Data on dry matter of wheat, cover crop and weeds were recoded four times during the period of spore trapping. Wheat yield and %FDK were also recorded.

RESULTS AND DISCUSSION

Crop rotation and Fusarium head blight

Overall disease incidence and severity of FHB on wheat was low in 2002. However, results indicated that percent disease incidence on wheat was the highest in pea-wheat crop sequence (19.25%) followed by canola-wheat (11.66%), wheat-wheat (10.33%) and oats-wheat (8.18%), while the percent disease severity was the highest in canola-wheat (43.09%) followed by oats-wheat (22.71%), wheat-wheat (18.35%), and peas-wheat (12.64%) crop rotations (Table 1). The FHB disease index on wheat was in the order canola-wheat (5.02), peas-wheat (2.43), wheat-wheat (1.89), and oats-wheat (1.85) crop sequences. *Fusarium* damaged kernel (FDK) or tombstone analysis yielded similar results. The FDK on canola-wheat, peas-wheat,

wheat-wheat and oats-wheat were 6.84%, 6.76%, 5.07 and 4.85%, respectively (Table 1). One would not expect more FHB disease when wheat was followed by non-host crops such as canola or peas. Our data corroborate results of other crop rotation trials in Brandon, Manitoba where FHB was also higher when wheat was followed by canola (personal communications with Debbie McLaren, AAFC, Brandon). We also observed higher FHB disease incidence and severity in a wheat plot few blocks away from this experimental plot where canola was grown in the preceding year (personal observation). In Manitoba, *Fusarium graminearum* is the dominant species associated with FHB. It is likely that canola and peas are better substrates to harbor and induce perithecium or ascospore production of *F. graminearum*, and this warrants investigation. Furthermore, canola and peas have a closed canopy and leaf defoliation that likely provided an advantageous environment for the establishment of the pathogen. These results are contrary to accepted theory that a three-year crop rotation with non-hosts including canola, pulses and forage legumes will reduce the risk of spreading and increasing the disease. However, Dill-Macky and Jones (2000) reported that FHB disease incidence was the greatest in corn-wheat rotation followed by wheat-wheat and the least in soybean-wheat rotation. Our one-year data is not enough to enable us to draw any conclusion on the benefit of the crop rotation with non-host crops like canola and peas.

As expected, yield was reduced when the same crop (i.e. wheat-wheat, or canola-canola) was planted in two consecutive years (Table 2). Wheat yield was significantly higher when wheat followed another crop. Bourgeois and Entz (1996) have studied the effect of crop rotation on wheat yields in Manitoba. They found 11% and 8% higher yield of wheat when wheat was grown after peas and canola, respectively. A similar report has been posted on the web by Manitoba Management Plus Program (source- Manitoba Crop Insurance Corporation) (www.mmpp.com/Crop_rotation_page.htm).

Cover crop and Fusarium head blight

Results of this experiment are being tabulated and analyzed at the present time. Yield, FDK and data on spore trapping will be presented at the meeting.

ACKNOWLEDGEMENTS

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Table 1. Fusarium head blight (FHB) disease incidence, severity and index on wheat following different foundation crops.

Foundation crop in 2001	Crop in 2002	%Incidence	%Severity	FHB index	%FDK
Canola	Wheat	11.66	43.09	5.02	6.84
Wheat	Wheat	10.33	18.35	1.89	5.07
Oats	Wheat	8.18	22.71	1.85	4.85
Peas	Wheat	19.25	12.64	2.43	6.76

Three samples of one meter row were randomly chosen from each wheat plot. Data are the mean of three samples. The samples included 67-120 wheat heads. One hundred grams of seed was used to determine percent FDK.

Table 2. Yield of canola, wheat, oats and peas following different foundation crops.

Foundation crops in 2001	Yield ton/ha			
	Crops in 2002			
	Canola	Wheat	Oats	Peas
Canola	**1.36**	1.47	3.19	2.47
Wheat	2.20	**1.01**	3.06	2.63
Oats	2.01	1.30	**2.39**	2.67
Peas	1.77	1.44	2.75	**1.59**

Figures are the mean of three samples from each plot, and the sample area was 1.22 X 2.5M (six rows of 2.5 meter long).

DETERMINATION OF WETNESS DURATION USING RADAR-DERIVED PRECIPITATION ESTIMATES

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ABSTRACT

Fusarium head blight (FHB) of small grains tends to be associated with certain environmental conditions, especially rain-induced wetness periods occurring near anthesis. A Geographic Information System-based model simulation which incorporates 4km resolution weather radar (NEXRAD)-derived precipitation estimates into a crop canopy energy balance-based scheme to estimate wetness duration periods for small grains on the 4 km spatial scale has been developed and initially tested, with promising results. Errors found in the NEXRAD precipitation estimates analyzed during the first and second years of this project with Michigan precipitation data were less pronounced than previous studies, with 96.3% of the precipitation-hours across the state of Michigan during the 1999 and 2000 growing seasons correctly classified and an overall mean bias and mean absolute precipitation differences of -1.6mm and 2.3mm respectively. An initial validation of simulated leaf wetness duration in 6 wheat field sites in Lower Michigan at head height during June and July of the 2002 growing season resulted in mean differences of -0.2 hours and mean absolute differences of 3.4 hours over 116 separate events associated with dew, precipitation, or both. Mean differences and absolute differences for events associated with precipitation only or with precipitation and dew were +1.5 hours and 3.7 hours, respectively, indicating a slight tendency for overprediction. In an effort to better parameterize the wetness duration simulation including evaporation rates of dew and total intercepted precipitation, a field study began in April, 2002 with greenhouse flats planted with spring wheat in individual 10cm pots. Following heading, the flats were monitored with a weighing lysimeter over time, providing estimates of plant evapotranspiration, dewfall, and interception of precipitation. Preliminary results from these data suggest a total nightly dewfall ranging from 0.0-0.3mm. To study rainfall interception, wheat heads at the flowering stage were cut and collected from extra plants in the flats and mounted on 30 cm long, 0.1mm diameter steel wires. The heads and steel 'stems' were in turn mounted on a heavy steel wire frame which held the mounted wheat heads and wire in a fashion similar to that grown in the field. Rainfall interception totals on the order of 0.1mm to 0.3mm were recorded for 11 events. The canopy interception was observed to be associated with the drop diameter of precipitation, with less canopy interception occurring with large droplet diameters and vice versa.

A SECOND GENETIC MAP OF *GIBBERELLA ZEAE*

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ABSTRACT

We recently reported the construction of a genetic map of *Gibberella zeae* made by crossing nitrate non-utilizing (*nit*) mutants of strains R-5470 (lineage 6 from Japan) and Z-3639 (lineage 7 from Kansas). This genetic map is based on 1048 AFLP markers that have been assigned to nine linkage groups. The map contains numerous loci with distorted segregation ratios and two possible chromosome rearrangements between the parental strains. The high degree of polymorphism and high marker density in this linkage map make it very useful for gene mapping studies. It has been used to map several genes related to trichothecene toxin biosynthesis and can also be used for QTL analysis. However, the segregation distortion in this wide cross may limit certain uses. Therefore, we constructed a second genetic map by making a narrow cross between two lineage 7 strains (Z-3639 and PH-1 from Michigan). The Z-3639 strain had a deletion in the *MAT*2 gene, which made it heterothallic. This avoided the segregation distortion associated with *nit* markers. In addition to AFLP markers, we also mapped some nuclear genes using RFLP-PCR. Segregation in the cross is normal, but marker polymorphism is low so more AFLP primer pairs will be needed to saturate the map. Loci common to the two genetic maps will allow identification of the linkage groups and elucidation of the segregation distortion and putative chromosome rearrangements in the original map.

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WHAT PART DOES PROGRAMMED CELL DEATH PLAY IN FUSARIUM HEAD BLIGHT?

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ABSTRACT

Deoxynivalenol (DON) is a strong inhibitor of protein synthesis and also induces programmed cell death (PCD) in animal cells (where it is termed "apoptosis") (Yang *et al*., 2000). Results of genetic manipulation of toxin production by the FHB pathogen, *Fusarium graminearum*, indicate that DON or other trichothecene toxins contribute to pathogen virulence in diseased wheat spikes (Desjardins *et al*., 1996.). DON is known to be toxic to plant cells but the processes leading to cell death have been little investigated. In studying the effects of DON in detached leaves of barley (Bushnell *et al*., 2002), we obtained preliminary results that support the hypothesis that DON induces PCD: 1) DON induced a gradual dissolution of chloroplasts (with concomitant loss of carotenoid and chlorophyll pigments) extending over three to five days before cells collapsed. Mitochondria likewise became degenerate. The tissues also suffered significant electrolyte loss over the 3-5 day period. Thus, cells underwent an ordered sequence of autolytic events leading to death, typical of PCD. Furthermore, the bleached tissues resulting from loss of chlorophyll pigments mimicked lesions of FHB in host spikes; 2) Like DON, cycloheximide and chloramphenicol, well known inhibitors of protein synthesis, induced gradual loss of chloroplast pigmentation and of electrolytes preceding cell collapse in the detached leaf tissues. Cycloheximide and several other inhibitors of protein synthesis have been reported to induce PCD in animal cells (Kochi & Collier 1993); 3) Ca ++ ions, known to be essential for PCD in plant cells (Groover & Jones, 1999), markedly accelerated DON-induced loss of both chloroplast pigments and electrolytes from leaf tissues. Together, these results indicate that DON induces PCD in leaf tissues and, therefore, may do likewise in FHB-infected spike tissues. We are following up these experiments by applying treatments to DON-treated leaves that are known to enhance or inhibit PCD. Further, we will extend these treatments to FHB- infected tissues to obtain cytological and physiological evidence for a possible role of PCD in FHB pathogenesis.

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INFLUENCE OF IRRIGATION FOLLOWING DISEASE ASSESSMENT ON DEOXYNIVALENOL ACCUMULATION IN *FUSARIUM*-INFECTED WHEAT

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ABSTRACT

A field trial was established in 2002 to evaluate the effect of moisture on deoxynivalenol (DON) accumulation in *Fusarium*-infected wheat. The trial was a split-split plot design with four replicates. Main plots were irrigation levels, subplots were wheat cultivar and sub-subplots were inoculation concentrations. Plots of the spring wheat cultivars, Wheaton (susceptible), Pioneer 2375 (moderately resistant) and Alsen (resistant), were inoculated with *Fusarium graminearum* at anthesis (Zadoks growth stage [GS] 61). Two inoculum concentrations (25,000 macroconidia/ml and 100,000 macroconidia/ml) were used to generate different Fusarium head blight (FHB) severities. Mist-irrigation (3.8 mm/day) was applied uniformly to all plots from anthesis until 15 days after inoculation (DAI) (GS 83). Then, the different irrigation treatments were imposed. Half of the plots continued receiving irrigation at the initial rate until harvest (35 DAI) and half received no irrigation. FHB severity was determined 16 DAI as a percentage of symptomatic spikelets for 20 spikes per plot. Sixty heads per subplot in each of two severity categories (FHB < 50%, FHB $\geq$ 50%) were tagged 16 DAI. Tagged heads (10/severity category) were harvested for each variety at GS 83 (early dough), GS 87 (hard dough), GS 91 (caryopsis hard) and GS 94 (harvest ripe). Kernels were dissected from collected spikes and assayed for DON concentration using gas chromatography / mass spectrometry. FHB severities among inoculation concentration treatments were significantly different ($P < 0.001$). Mean FHB severities were 30% and 70% for Wheaton; 29% and 50% for Pioneer 2375; and 26% and 51% for Alsen at the low and high inoculum treatments, respectively. Data from this experiment should provide an insight into aspects of DON accumulation in wheat. Sequential sampling following disease assessment may help characterize the timing of DON accumulation during an epidemic. The influence of irrigation treatments could aid in the prediction of the DON concentration in grain based on post anthesis weather variables.

SPATIAL PATTERNS OF FUSARIUM HEAD BLIGHT IN NEW YORK WHEAT FIELDS IN 2002

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ABSTRACT

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae*, is a disease of worldwide occurrence that severely reduces the yield, quality, and marketability of wheat. The spatial pattern of FHB incidence was studied in 60 arbitrarily selected winter wheat fields in central and western New York at kernel soft dough stages in June 2002. The fields varied in wheat cultivar, preceding crop, presence of corn residue, and intensity of FHB epidemic. Incidence of FHB was randomly distributed among 60 sampling quadrats in 55 of the 60 fields. Fields with random FHB ranged from trace to 23% in average incidence of FHB and followed bean, corn, oat, pea, sorghum, and soybean. The five fields with aggregated FHB ranged from trace to 27 % in average incidence of FHB and followed bean, corn, oat, and pea. Mean incidence of FHB was not significantly different between fields with and without corn residue, though incidence of FHB and aggregation was highest in two fields sown into standing corn residue without tillage. For eight fields that had corn residue from a corn crop 2 or more years before wheat, there was no evidence of aggregation among all quadrats in a field or among quadrats with corn stubble; also there was no difference in the mean incidence of FHB between quadrats with and without corn residue. Spatial patterns do not supply direct proof of inoculum source, but they suggest likely origins of inoculum that can be confirmed by other observations and experimentation. Based on the predominantly random patterns of FHB in 2002, we suggest that FHB epidemics in rotational wheat fields of New York may be initiated by deposition of spores from diffuse atmospheric inoculum. Over-wintered corn residues are the most prevalent and likely regional source of atmospheric inoculum for FHB in New York.

INFLUENCE OF CORN RESIDUE AND CULTIVAR SUSCEPTIBILITY ON THE ACCURACY OF FUSARIUM HEAD BLIGHT RISK ASSESSMENT MODELS

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OBJECTIVES

To evaluate the performance of forecasting models for Fusarium head blight of wheat in the United States

INTRODUCTION

Disease forecasting models for Fusarium head blight (FHB) of wheat were developed by a cooperative effort among researchers in OH, PA, ND, IN, SD, MO, and KS (De Wolf *et al*. 2000). These forecasting models were based on logistic regression analysis of 50 location-years of disease observations and predict the probability of a FHB epidemic based on environmental variables. Members of the cooperative effort are currently evaluating two models for delivering FHB forecasts at a regional level. For convenience, we will refer to these models as Model 1 and Model 2.

Model 1 uses weather variables observed during a 7-day period prior to flowering. More specifically, these models use duration of time (h) that temperature is between 15 and 30°C and the duration (h) of precipitation (De Wolf *et al*. 2001). This model correctly predicted 70% of 50 cases used to develop the models. Model 1 correctly predicted 78% of non-epidemic years (FHB severity greater than 10%), but correctly classified only 56% of the epidemic years.

Model 2 uses environmental variables observed during the 7-days period prior to flowering and a 10-day period beginning at flower initiation (De Wolf *et al*. 2001). Variables used by this model are the duration (h) of temperature between 15 and 30°C for the 7-day period prior to flowering, and the duration (h) in which temperature is between 15 and 30°C and relative humidity is greater than 90% during the flowering-time period. Model 2 correctly classified 84% of the 50 cases used to develop the model with near equal accuracy for both epidemic and non-epidemic cases.

MATERIALS AND METHODS

Researchers in PA, OH, ND, SD, and IN provided crop growth stage and disease observations from replicated research plots, and this information was combined with hourly measurements of temperature, relative humidity, and precipitation. The presence or absence of corn residue within the plots was noted at each location. The total data set consisted of 23 location years not used in model development.

Models 1 and 2 were evaluated for prediction accuracy with the new data, and model accuracy was compared with previous estimates. Model errors were evaluated for trends that should facilitate application of present models and development of the next generation of forecasting models.

RESULTS AND DISCUSSION

The total number of cases provided for this project from each state included three cases from IN, four from ND, six from OH and five from both PA and SD. Disease severity at these sites ranged from 0 to 74%. Nine of the 23 cases were considered to be epidemics when converted to the binary scale used by the models (FHB severity greater than 10% = 1). Seven of 23 cases had significant levels of corn residue within the plots.

Model 1 correctly classified 15 of the total 23 validation cases correctly (Table 1). All eight errors made by Model 1 were false negatives (incorrectly predicting low disease). In comparison, Model 2 correctly predicted 17 of the 23 cases. Five of the six errors made by Model 2 were false negatives. These prediction accuracies were lower than previous estimates of model accuracy (De Wolf *et al*. 2001). The high rate of false negative errors was of particular concern. However, nearly all of these errors were associated with sites that had high levels of corn residue, or the highly susceptible spring wheat cultivar 'Norm'.

Corn residue

When the models were evaluated with sites with little or no corn residue, Model 1 correctly predicted 11 of the 16 cases, and Model 2 correctly classified 13 of the 16 cases (Table 1). In contrast, both Model 1 and 2 correctly classified only four out of the seven sites that had high levels of corn residue within the plots. The reduction in model accuracy in association with corn residue may, in part, be explained by the high levels of inoculum often associated with this type of residue (Francl *et al*. 1999).

Cultivar susceptibility

Cultivar susceptibility also appeared to affect model accuracy. In this analysis, the highly susceptible cultivar Norm was associated with three of the five errors made by Model 1 for the low residue data set. All three errors were false negative predictions (incorrectly predicting low disease). Similarly, two of the three errors made by Model 2 for the same data set involved Norm, and both errors were false negative predictions. The number of errors that correspond to Norm suggest that highly susceptible cultivars may have an increased likelihood of severe disease that is not considered by the prediction models.

Verification of the prediction accuracy of Models 1 and 2 supports continued deployment in disease forecasting efforts. However, these results indicate that the models may be less accurate when wheat is produced in fields with high levels of corn-residue, or when highly susceptible cultivars are grown. Future modeling efforts will attempt to incorporate potential inoculum source and cultivar susceptibility into the forecast models.

Table 1. Prediction accuracy of FHB forecasting models for 23 location-years not used in model development.

	Model Prediction Accuracy (%)	
Data set	**Model 1**	**Model 2**
Full data set (n=23)	65	74
Location years with low level of corn residue (n=16)	69	81
Location years with high level of corn residue (n=7)	57	57

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EFFECT OF CEREAL RESIDUE BURNING ON THE INCIDENCE AND STRATIFIED DISTRIBUTION OF *FUSARIUM GRAMINEARUM* AND *COCHLIOBOLUS SATIVUS* IN WHEAT AND BARLEY PLANTS

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ABSTRACT

The effect of residue burning on the stratified incidence of *Fusarium graminearum*, *Cochliobolus sativus*, and other pathogens was studied in barley and wheat planted at three locations in Minnesota in 2001. Cereal residues were burned 1-5 days after planting using a propane-powered flame thrower. Subcrown internodes, crowns, nodes, and kernels were excised from 30 plants collected from each plot at maturity. Tissue segments were surface-sterilized, plated onto half strength PDA (pH=5.5), incubated at 20-24°C under flourescent lights (12:12 light:dark) for 6-7 days. The observed colonization of tissues showed that regardless of the host, *F. graminearum* was mostly associated with kernels, whereas *C. sativus* was mostly associated with crowns and the first node. In contrast, *Pyrenophora teres* in barley was mostly associated with the third node. Burning significantly reduced cereal residues ($P<0.01$), and also significantly reduced the survival of *F. graminearum* and *C. sativus* ($P<0.01$). The overall incidence of *F. graminearum* was significantly less ($P=0.05$) in wheat plants collected from burned plots (3.3%) in comparison with those collected from the non-burned plots (5.3%). The effect of residue burning on the incidence of *C. sativus* and *P. teres* was not significant. Our data shows that *F. graminearum*, *C. sativus* and *P. teres* preferentially colonize certain plant parts and that residue burning may provide an option in the management of cereal diseases such as Fusarium head blight.

IDENTIFICATION OF ENVIRONMENTAL VARIABLES THAT AFFECT PERITHECIAL DEVELOPMENT OF *GIBBERELLA ZEAE*

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ABSTRACT

Perithecial development of *Gibberella zeae* and the severity of Fusarium head blight are dependent upon favorable environmental conditions. Information about the conditions favorable to *G. zeae* perithecial development could be useful in predicting wheat head blight epidemics. The development of *G. zeae* perithecia on corn stalk residue was monitored in replicated plots in wheat fields near Wooster, OH (2000) and State College, PA (2001-02). Environmental variables including temperature, relative humidity and rainfall were recorded directly within the plots with an automated datalogger. The moisture levels of the stalks were monitored with electrical resistance sensors, and the duration of stalk wetness (DSW) was recorded. Observations of perithecial development were made every five to seven days, and paired with environmental variables to identify those variables associated with perithecial development. The rate of perithecial production was the greatest in 2000 and the lowest in 2001. An extended period of DSW was associated with an increase in perithecial development at all locations over in the three years of this study. During this increase in perithecial development, the 2000 and 2002 years had 14 and 18 days respectively with average temperatures greater than 15°C. In comparison, only eight days with average temperature greater than 15°C occurred in 2001. Both the 2000 and 2002 locations received a more than 100 mm of rain during the period of rapid perithecial increase. However, only 60 mm of rain were recorded in 2001. In 2002, a decrease in the rate of perithecial production was associated with a six-day period of average temperature less than 7°C but the rate increased again when temperatures increased to greater than 15°C. These results suggest that the number of perithecia produced and their rate of development are influenced by temperature and moisture in a wheat field environment. In the future, information relating weather conditions with critical periods for perithecial developmental may improve the accuracy of wheat head blight forecasting systems.

RELATIONSHIP OF TEMPERATURE AND MOISTURE TO *GIBBERELLA ZEAE* PERITHECIAL DEVELOPMENT IN A CONTROLLED ENVIRONMENT

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OBJECTIVES

To examine the relationship between temperature and moisture on *Gibberella zeae* development in a controlled environment on corn stalk residue.

INTRODUCTION

Fusarium graminearum (*G. zeae*) survives in association with debris of corn, wheat, barley and many cultivated grasses (Parry, 1995). These residues are major sources of inoculum for epidemics of Fusarium head scab of wheat in North America (Francl *et al*. 1999). A better understanding of factors affecting survival and reproduction of *G. zeae* on these residues is important to the development of new management strategies for head scab of wheat and barley.

Past research has shown that temperature and osmotic water potential are two important factors in stimulating growth, reproduction, and sporulation of *G. zeae* (Sung, 1981; Tschanz, 1976). This research, however, has only examined temperature and moisture independently, and has not used crop residues as a substrate. Our objectives were to further examine the effects of temperature and moisture on the development of *G. zeae*, and examine these two important factors together using crop residues.

MATERIALS AND METHODS

Inoculation of stalks – Corn stalks collected near State College, PA once the plants had reached physiological maturity. These stalks were cut into ~30 cm sections, disinfested twice and placed into cold storage (-10°C) until inoculation. At the time of inoculation, the stalks were removed from the cold storage, placed into stainless steel trays, covered with aluminum foil, and disinfested for a third time. A 3mm^2 section of *G. zeae* infested carnation leaf was placed on the stalks, and stalks were incubated for approximately 14 days at 25°C in continuous darkness.

Calibration of Sensors with Stalks – After the stalks were infested, six stalks were arbitrarily selected and paired with an electrical resistance sensor. The relationship between electrical resistance and water content was individually calibrated for each stalk. These calibrations were done by wetting the stalks and taking repeated measurements of electrical resistance and stalk water content as the stalks dried. Regression analysis was used to

develop a calibration curve for each stalk-sensor combination. Sensors were used to monitor and adjust moisture levels within a humidity chamber.

Controlled Environment Chambers – A three-compartment humidity chamber was used to control the three moisture treatments of dry (< 40% RH), moderate (40-80% RH) and wet (>80% RH). A layer of disinfested sand, two stalk-sensor pairs, six infested stalks, and a temperature/humidity probe were placed into each compartment of the humidity chamber. The temperature for each run was held constant at 15, 25, or 30°C. The number of perithecia at previously selected points on the six infested stalks were counted every 5 days for a 20-day period. Developmental stages of the perithecia were also recorded at this time. All treatments were repeated at least 3 times and analysis of variance used to identify differences among treatments.

RESULTS AND DISCUSSION

After 11 to 16 days of incubation, the number of perithicia produced at 15 or 25°C was significantly greater (p = 0.01) than the number produced at 30°C, but there was no significant difference detected between the 15 and 25°C treatments. The number of perithecia was significantly (p = 0.01) increased at the high moisture level compared to the low moisture level treatments. There were no perithecia produced with treatments that included 30°C or the low moisture level (Figure 1). The results indicate the perithecial development of *G. zeae* maybe limited by extended periods of residue dryness or temperatures above 30°C.

In these experiments, perithecia with ascospores were produced at 15°C and 25°C (Figure 2). These results do not agree with previous reports that suggest that perithecial development was limited or did not occur at 15°C (Tschanz 1976). Further experiments are underway to further evaluate temperatures that may limit *G. zeae* perithecial production.

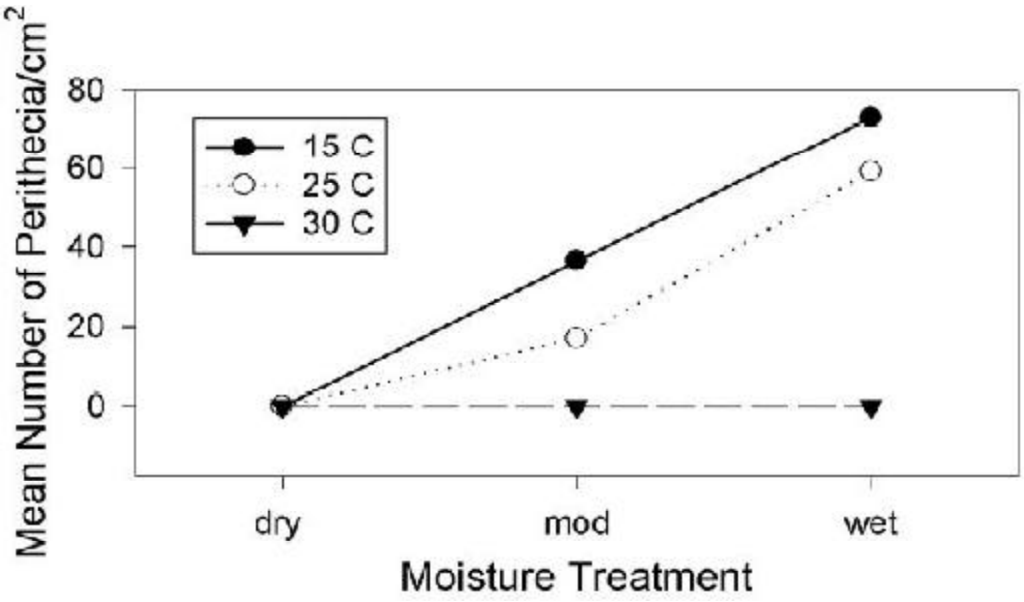
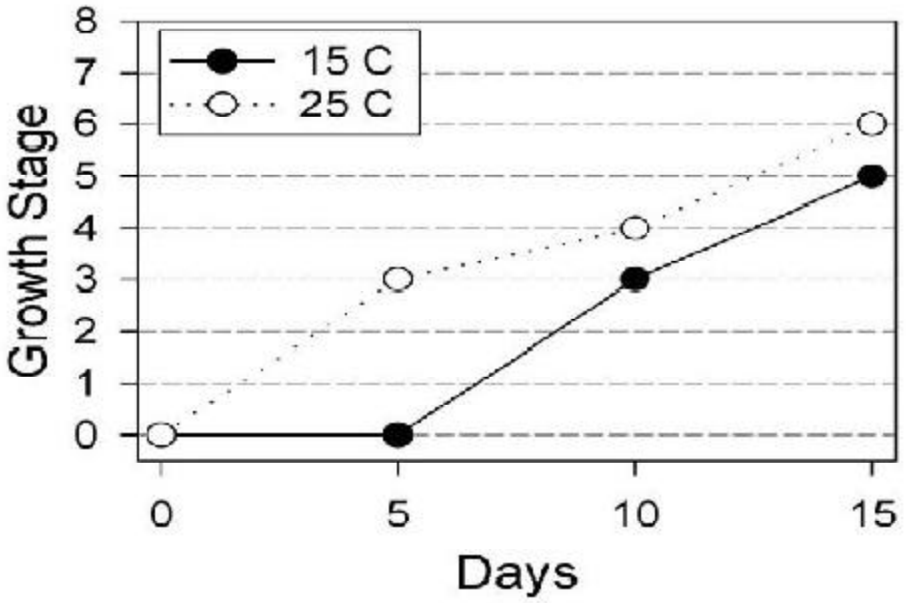


Figure 1. Mean number of *Gibberella zeae* perithecia produced on infested sections of corn stalk incubated at different combinations of temperature and moisture level



Perithecia growth stages: 2 = perithecia just pigmented; 4 = perithecia beginning to form; 6 = asci formed but spore development incomplete; 8 = ascospore development complete

Figure 2. Developmental stages of *Gibberella zeae* perithecia produced at 15 or 25°C at similar moisture levels

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INCIDENCE-SEVERITY RELATIONSHIPS FOR FUSARIUM HEAD BLIGHT ON WHEAT

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ABSTRACT

The relationship between incidence (proportion of plant units diseased) and severity (the amount of plant tissue affected by disease) is a valuable tool that is useful for making disease surveys, assessments, evaluating host resistance, and for determining action thresholds for management decisions. The assessment of severity is tedious and time consuming and may be prone to bias and large experimental error. Therefore, the existence of a quantifiable relationship between incidence and severity greatly facilitates evaluation of disease intensity for estimates of crop damage. These benefits arise because incidence is determined easily, with more accuracy and precision than severity, and with lower cost. Thus, the intent of this study was to determine the relationship between incidence (*I*; percent heads infected) and severity (*S*; percent infected spikelets within infected head) of Fusarium head blight, and determine if severity could be predicted reliably from incidence data. Disease assessment for both incidence and severity were made visually at several sample sites (ranged from 45 to 100 sites per field) in artificially and naturally inoculated research plots and production fields over four years. At each sample site, at least 20 heads were evaluated for incidence and severity. Incidence of infected heads and the average percentage of spikelets with disease on each date for each field in each year were analyzed using linear regression analysis. Ten different, but interrelated, models were fitted to the data and models were compared based on R^2, mean square error, and residual plots. Mean disease incidence and severity varied among data sets, ranging from 28.0 to 75.4% for incidence, and from 9.1 to 28.2% for severity.

The best fitting model was CLL(S) = α + ß CLL(I), in which CLL is the complementary log - log transformation. R^2 values ranged from 0.69 to 0.91. Although there was considerable variability of *S* for a given *I* in some years, there was a highly significant relationship between *S* and *I* in each year, and the functional relationship was very consistent between years.

SPATIAL ASPECTS OF FUSARIUM HEAD BLIGHT EPIDEMICS ON WHEAT IN OHIO

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OBJECTIVE

To quantify the spatial pattern of Fusarium head blight incidence in wheat fields.

INTRODUCTION

Fusarium head blight of wheat (*Triticum aestivum* L.) caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a limiting factor in wheat and barley 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. Disease pattern is a useful ecological characteristic that helps define a population such as diseased wheat heads (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 help us determine efficient sampling procedures that result in precise estimates of mean disease intensity and help determine the proper statistical analysis for comparing treatments.

MATERIALS AND METHODS

Disease Assessments.
Epidemics of Fusarium head blight of wheat were monitored in four fields in 2001 and in six fields in 2002. In each field, three transects with 15 sample points per transect, spaced at 1-m intervals, for a total of N = 45 sample points per field, or 10 transects with 10 sample points per transect, spaced at 1-m intervals, for a total of N = 100 sample points per field were established. Each sample point was marked with a flag that remained in the field throughout the assessment period. At each sample point, the incidence of scab was recorded for a 1-ft subtransect 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 **Ö**, a measure of the variation (heterogenity or aggregation) in disease incidence per sample unit. Values of 2 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(a) 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 in 2001(Table 1), and from 0.137 to 0.687,with a median of 0.250 in 2002 (Table 2). As anticipated, *p* increased over time within all fields.

The program BBD successfully calculated maximum likelihood estimates of *p* and 2 for all the data sets in both years. 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% in 2001 and over 60%of the data sets in 2002, and by the binomial distribution in 58% and 40% of the data sets in 2001 and 2002, respectively.

The values of 2 ranged from 0.00 to 0.073, with a median of 0.011 in 2001, and from 0.00 to 0.039, with a median of 0.019 in 2002. Estimated 2 in over 90% of data sets were greater than 0 (Tables 1 and 2) indicating overdispersion.

The index of dispersion *D*, ranged from 0.88 to 4.50, with a median of 2.22, and from 0.89 to 2.80, with a median of 1.83 in 2001 and 2002, respectively. *D* and 2 were both positively correlated with the estimated parameter *p.*

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

In conclusion, it was found that heads of wheat infected with scab were aggregated within the wheat fields. Moreover, the degree of aggregation was moderate 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(a) test[b]	
		p	se(p)	?	se(?)	*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	0.0090	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; ?, aggregation parameter; se(?), standard error of designated estimated parameter.

[b] *z* , standard normal statistic of the C(a) test; P(*z*): significance level of *z* .

[c] se not defined when ? = 0.

Table 2. Statistics for describing the spatial pattern of the incidence of Fusarium head blight in six wheat fields in Ohio in 2002.

	Disease Assessment	Estimated beta-binomial parameters[a]				C(a) test[b]	
Field	date	*p*	se(*p*)	?	se(?)	z	P(z)
F 1 (Wooster)	06/10	0.419	0.0121	0.007	0.0058	1.69	0.045
	06/12	0.523	0.0143	0.017	0.0079	3.99	<0.001
	06/14	0.590	0.0155	0.025	0.0097	5.91	<0.001
	06/17	0.656	0.0165	0.036	0.0120	8.32	<0.001
	06/19	0.687	0.0152	0.039	0.0106	6.83	<0.001
F 2 (Wooster)	06/26	0.320	0.0121	0.000	-c	-0.85	1.000
F 3 (Wooster	06/26	0.363	0.0080	0.008	0.0040	2.65	0.004
F 4 (Wooster)	06/15	0.137	0.0072	0.029	0.0066	8.89	<0.001
	06/18	0.176	0.0082	0.031	0.0069	9.85	<0.001
	06/21	0.253	0.0072	0.008	0.0040	2.66	0.004
	06/24	0.342	0.0084	0.012	0.0045	4.02	<0.001
	06/28	0.426	0.0108	0.029	0.0071	9.97	<0.001
F 5 (Hoytville)	06/27	0.285	0.0078	0.010	0.0043	3.44	<0.001
F 6 (Hoytiville)	06/27	0.265	0.0085	0.017	0.0054	6.07	<0.001

[a] *p*, expected probability of a head being diseased, estimated as the mean incidence; ?, aggregation parameter; se(*), standard error of designated estimated parameter.

[b] *z*, standard normal statistic of the C(a) test; P(z), significance level of z.

[c] se not defined when ? = 0.

EFFECT OF WHEAT FLORAL STRUCTURE EXTRACTS AND ENDOGENOUS COMPOUNDS ON THE GROWTH OF *FUSARIUM GRAMINEARUM*

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INTRODUCTION

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum,* has become a wide spread problem in the United States with the increased use of reduced tillage practices (2). Infection of the florets causes sterility, poor seed fill, reduced seed quality and contamination of grain with mycotoxins (2). Since FHB severity levels are higher when wet weather coincides with wheat anthesis in the field and when anthers, rather than emasculated spikelets, are inoculated in the greenhouse, it is presumed that anthers are the common route of entry into the plant (2,7). These findings led to the theory that there may be compounds in anthers that stimulate growth of *F. graminearum*. The compounds thought to be responsible for stimulation were choline acetate and glycinebetaine (4,7-9). *F. graminearum* has been shown to possess separate constitutive high-affinity transport system that is specific for both choline and betaine, indicating that choline and betaine may be specifically utilized by *F. graminearum* (5,6). These compounds may play a role in pathogenesis of *F. graminearum* or reaction of wheat cultivars to the pathogen.

OBJECTIVES

The first objective of this study was to examine the effect of choline and betaine on spore germination and hyphal elongation of *F. graminearum*. The second objective was to determine the relationship between fungal hyphal growth and extracts of different floral structures from nine wheat genotypes with varying reactions to *F. graminearum*.

MATERIALS AND METHODS

Rate of hyphal radial growth of three *F. graminearum* isolates was measured on water agar and 2% dextrose agar amended with 10nM to 1000µM stock solutions of choline chloride or betaine hydrochloride in two separate experiments. The control was unamended water agar and dextrose agar.

Ascospore or macroconidia germination was evaluated on glass slides covered with a 10µM, 100µM, and 1000µM layer of choline, betaine, or an equal molar mixture amended agar. Unamended agar was the control.

Nine genotypes were selected based on differences in mean FHB severity and incidence from the 1999 Uniform Winter Wheat FHB Screening Nursery (1), (Table 1). Mean incidence and severity were based on seven field locations across six states in the United States and one

nursery in Ontario, Canada. Plants were grown in the greenhouse and spikes were collected when one floret had extruded anthers. Extracts from anthers, paleas or lemmas were combined with water agar and rate of hyphal growth of two *F. graminearum* isolates were measured.

The percentage of increased growth compared to the unamended control was calculated for the floral part extracts. Analysis of variance (ANOVA) was conducted using the general linear model in MINITAB software package for rate of hyphal extension, percentage of germinated spores and percentage of increased growth compared to the unamended contol.

RESULTS

The three *F. graminearum* isolates had significantly different ($P = 0.05$) growth rates, but growth rate of an isolate was constant across repeats of experiments, with an average radial growth rate of 0.35 - 0.64 mm/hr on water agar and 0.35 - 0.54 mm/hr on dextrose agar. Choline had a significant ($P = 0.05$), but relatively small, effect on radial growth on water agar, but not on dextrose agar at concentrations from 10nM to 1000nM compared to the unamended control by 72 hours after plating in the first experiment. However, this small effect was not observed in the second experiment at concentrations ranging from 10µM to 1000µM.

Betaine had a significant ($P = 0.05$), but relatively small, effect on radial growth on water agar, but betaine did not affect growth on dextrose agar at concentrations from 10nM to 1000nM compared to the unamended control by 72 hours after plateing in the first experiment. In the second experiment, betaine did not significantly affect radial growth on the 10µM to 100µM amended agar, although there was inhibition of hyphal growth of all isolates at the 1000µM concentration compared to the unamended control of both agars in the second experiment.

Likewise, the equal molar mixture of choline and betaine significantly ($P = 0.05$), although only slightly, affected the radial growth in concentrations ranging from 10nM to 1000nM in the first experiment. In the second experiment, the equal molar concentrations of choline and betaine significantly ($P = 0.0001$) inhibited hyphal growth at the 1000µM concentration compared to the unamended control of both agars, but had little effect on hyphal growth with concentrations ranging from 10µM to 100µM.

Ascospores and macroconidia germinated readily on unamended water and dextrose agar with 99% germination 24 hours after plating. Germination of ascospores and macroconidia were not significantly ($P = 0.05$) affected by 10, 100, and 1000µM concentrations of choline, betaine, or an equal molar mixture when compared to the unamended control plates over a 24 hour period (data not presented).

Hyphal growth was not significantly affected ($P = 0.05$) by anther, palea, or lemma extracts from the resistant or susceptible genotypes when compared to the unamended control (data not presented), or when growth rate was expressed as a percentage of the control.

CONCLUSIONS

Macroconidial germination has not been shown to be enhanced by choline or betaine (3,7). This study agrees with these findings and also shows that ascospore germination was unaffected. Germination of ascospores or macroconidia appears to be unaffected by the presence of choline or betaine.

Various *in vitro* studies have found that choline, betaine and equal molar mixtures of concentrations ranging from 0.1µM to 1mM stimulated, inhibited, decreased hyphal branching, increased hyphal extension and had no affect on specific growth rate of hyphea of *F. graminearum* (3,7,9,11). In the current study, radial growth was found to be enhanced by low concentrations of choline on water agar, which agrees with previous findings (10), but this enhancement is not believed to be biologically significant. Regardless of the different isolates of *F. graminearum* or different protocols used, results of this study are in agreement with the findings of previous studies (3,11) in that levels of choline or betaine in floral parts probably have little if any stimulatory effect on growth of *F. graminearum*.

Results of this study did not show a correlation between FHB resistance reaction of nine wheat genotypes and rate of radial growth of two *F. graminearum* isolates on agar amended with anther, palea or lemma extracts. These findings are in partial agreement with previous findings (3). Therefore, endogenous compounds of wheat floral structures are not thought to be important in resistance reactions of wheat genotypes.

Our results indicate that endogenous compounds in wheat floral structures do not enhance their colonization by *F. graminearum* and that putative compounds in floral structures have no substantial role in resistance to *F. graminearum*.

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A PHENOLOGY-BASED PREDICTIVE MODEL FOR FUSARIUM HEAD BLIGHT OF WHEAT

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OBJECTIVES

Our goal was to establish a model to account for anther extrusion period that could be used to calculate probabilities of Fusarium head blight incidence as the window of opportunity for infection advances from beginning of anther extrusion to complete anther fall. The model combines several elements of meteorology, biology of *G. zeae* and wheat phenology.

INTRODUCTION

Fusarium head blight (FHB), incited by a fungus (*Gibberella zeae* (Schwein.) Petch), is an important disease affecting wheat. Fusarium head blight fungus survives in crop debris and windborne or splashed spores infects the heads during flowering. Humid weather and moderate temperatures are favorable for infection (Sutton, 1982). Fusarium head blight can be devastating to yields if a large proportion of plants are infected. The fungus also can produce harmful mycotoxins which depreciate grain value (McMullen *et al*., 1997).

Despite the absence of reliable data for comparing FHB intensity amongst different years in southern Brazil, it is generally accepted that the disease has been more severe in the last decade. It is believed that a combination of 'El Niño' years and of abundant sources of inoculum are the cause for such high intensity of FHB in the wheat fields in southern part of Brazil (Fernandes, 1997).

The relative narrow susceptible phase of wheat and the strong dependence on climatic requirements for infection success makes the pathosystem suitable for modeling. A realistic approach should account for availability of susceptible tissue besides weather driven pathogen dynamics.

Process-based models of crop growth and development are exciting tools emerging from the on-going information technology. Models can improve our understanding of the complex processes underlying wheat production including Fusarium head blight management. Their analytical power can help deal with difficult tasks such as predicting the incidence of Fusarium head blight on wheat.

MATERIAL AND METHODS

Brief Model Description

Model Framework. To develop a wheat simulation model into an Object Oriented environment we started with a small generic crop model. This model is available at www.icasanet.org/

modular. The model contains three main modules: Soil, Plant and Weather (Jones, *et al*., 2001). The model originally written in FORTRAN was converted to JAVA and followed the principles of Object Oriented approach. The modular structure was used to depict classes and provide them with the right data behavior. One of key features of a modular approach is that models should relate to the real world components or processes.

Wheat Simulation Model. Wheat simulation, a process oriented model which is based on daily time-steps considers 1 m^2 area of wheat crop. It simulates the dynamics of wheat biomass through inputs of historical records of weather data, cultivar coefficients, and soil properties. The wheat simulation model includes growth, phenology and water balance routines.

The plant growth module computes crop growth and development based on daily values of maximum and minimum temperatures, radiation and the daily value of two soil water stress factors, deficit and surplus. This module also simulates leaf area index (LAI), which is used in the soil water module to compute evapotranspiration.

Crop development is simulated based on thermal time required to reach specific growth stages. The model also accounts for simulating the dynamics of heading emergence including extrusion of anthers (flowering). State variables and simulated processes allow accounting for incidence of Fusarium head blight.

The water budget in the model includes precipitation, irrigation, runoff, water infiltration in the soil profile, crop transpiration, and evaporation. Crop evapotranspiration is determined from leaf area index.

Fusarium head blight Simulation Model. A module was developed to simulate head infection through inputs of local weather data. The first anthers were empirically set to be extruded on day five after heading emergence. Flowering dynamics was handled as a cohort of heads exhibiting anthers resulting from simulation and assumed to be a potential infection site.

Predictive modeling tries to match the rules (models) for guessing (predicting) the Fusarium head blight incidence from weather variables. Stepwise multiple regression procedures were used to determine the prediction rules. The weather variables examined were solar radiation, maximum temperature, minimum temperature and precipitation.

Model Inputs

Input data such as location, soil, crop and management files are required to run the model. An advanced user-friendly interface allows users to easily manipulate input files, create simulations, execute single and batch run simulations and produce text and graphical reports. The data base was implemented using PostgreSql and Interbase for remote and local access, respectively.

RESULTS AND DISCUSSION

The model predicted reasonable well the phenological stages of the wheat cultivar BR23, especially at the flowering stage, except at very early or very late sowing dates. In general, the

date for heading stage (50 % heads emerged) was predicted within an interval of two-three days around the observed date.

Findings from field experiments revealed that daily number of anthers per head varied significantly. In general, in a single head flowering lasts from five to eight days. As a contrast, in a group of heads the course of anther extrusion last from 14 to 18 days. The peak of number of extruded anthers was observed at six to eight days after the beginning of flowering.

Growth chamber experiments showed that anther extrusion was responsive to temperature. Rate of extrusion increased proportionally to temperature increments (Vargas *et al*., 2001). This conceptual model was translated to the predictive model.

The model attempts to predict the probability of Fusarium head blight based on the weather variables occurring around flowering. The weather variables inserted in the model are rain greater than 1mm and maximum temperature. An ascospore cloud is formed every day rain is greater than 1 mm. The ascospore maturation rate is reduced at temperatures lower than 20 °C. Daily ascospore cloud values are summed in simple 4-day moving periods.

If anthers are present infection occurs during a rain event greater than 1 mm. The proportion of infected heads depends on the time course of anther extrusion and the size of the ascospore cloud.

In the field, the level of Fusarium head blight varied among experiments. The disease intensity was dependent on weather conditions during the flowering stage. Sowing date could alter flowering date of a cultivar in a particular year causing great differences in disease levels. As a consequence, fields with distinct sowing date can have a different level of disease (Figure 1). Thus, to predict Fusarium head blight incidence the simulator first needs to be very accurate in predicting growth stages of wheat. Any slight deviation from the target (susceptibility window) may cause a considerable error in predicting Fusarium head blight incidence. Further studies on wheat phenology are being planned. Hopefully, as more data becomes available it will be possible to improve the model performance.

The Fusarium head blight predictive model predicts the probability of disease occurrence; it does not predict level of disease severity. The predictive Fusarium head blight model predicted moderate to high levels of incidence for a majority of simulated wheat fields with sowing dates in the period of 1998 to 2002, at Passo Fundo, RS, Brazil. This moderate to high incidence was probably due to the high frequency of rainy days during the flowering stage of wheat.

In the year 2002, for example, a “El Niño” event occurred. In southern Brazil a “El Niño” year means precipitation above normal at spring time coinciding with heading stage of wheat. Thus, diseases such as tan spot, glume blotch and Fusarium head blight are usually severe in “El Niño” years. As a consequence during such years wheat yields are degraded (Cunha, *et al*., 2001). Besides, rainfall around harvest time may contribute to a lower test weight of wheat which penalizes profits.

Plans for the future

"As is" the predictive model is a convenient tool for researchers, teachers and students to use in the study of wheat development and incidence of Fusarium head blight. The model was developed using up to date technology for Web deployment. Therefore, it can be shared over the Web with a wide variety of potential users.

So far, this predictive Fusarium head blight model has been developed and tested using the Brazilian wheat cultivar BR23 and historic weather data from Passo Fundo, RS, Brazil. Thus, model outputs should be interpreted cautiously avoiding extrapolation to other cultivars and regions before further testing and validation. Nevertheless, the model is suitable for general research and educational purposes. Hopefully, as more data becomes available, it can be easily modified to accommodate different cultivars and regions.

In the meantime, aiming to reduce the error in estimating the "window of susceptibility" model is being modified so that the user can enter the date(s) for any growth stage(s) before flowering. Finally, the modular structure adopted in the model construction should facilitate adding new components, as they become available, to expand model capability.

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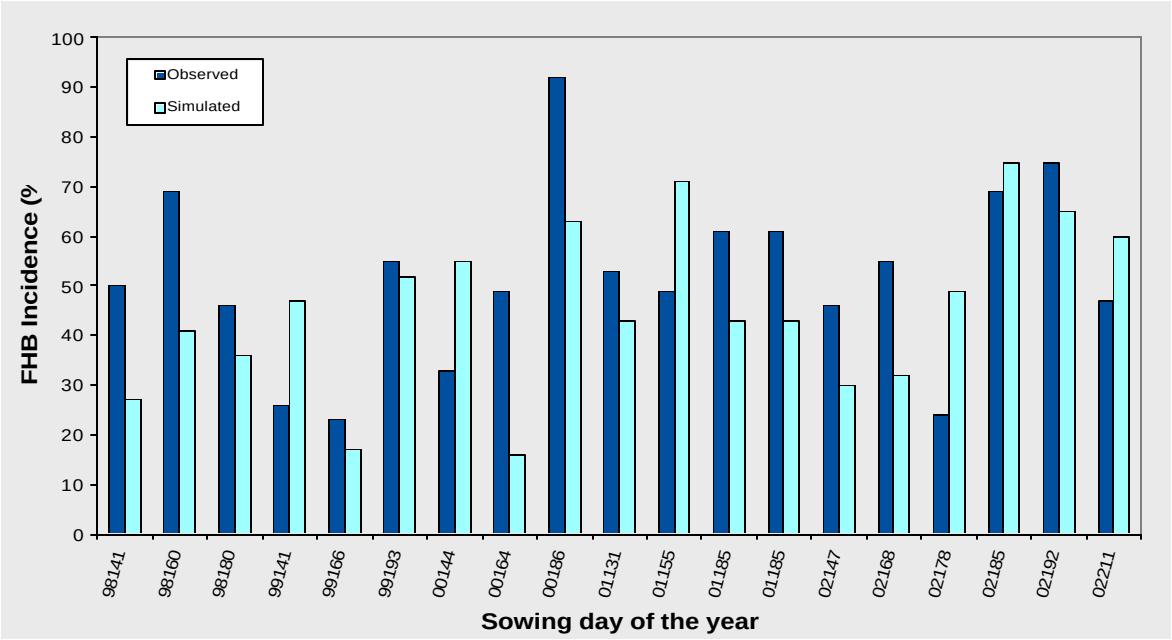

Figure 1. Simulated and observed FHB incidence for the wheat cultivar BR23 at Passo Fundo, RS, Brazil.

AFLP-ASSISTED GENETIC CHARACTERIZATION OF *FUSARIUM GRAMINEARUM* ISOLATES FROM CANADA

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INTRODUCTION AND OBJECTIVES

Fusarium graminearum (Teleomorph - *Gibberella zeae*) is one of the most important plant pathogens, which causes fusarium head blight (FHB) in economically important cereals such as wheat, barley and corn. FHB has caused high losses in yield and grain quality, thus affecting every aspect of the grain industry (Gilbert *et al*., 2001. Mycopathologia 153: 209 – 215). Hence, to combat the disease, chemical and biological control, and breeding for resistance have been the methods of control. But, in the process of developing the various control measures, we should also bear in mind the natural ability of the pathogen to evolve over these control strategies. For example in China, resistance of *F. graminearum* to benzimidazole and cabendiazime fungicides has been reported (Zhou *et al*. 1994. Journal of Nanjing Agricultural University 17(3): 33 - 41). Most of our current resistance to FHB has been traced back to only a few sources (Van Ginkel *et al*., 1996. Plant Dis. 80: 863 – 867) and hence, it is possible that *F. graminearum* could adapt to resistant varieties. Also, this adaptation of the pathogen could extend to chemical and biological control. A better understanding of the genetic structure would shed more light on the biology of the pathogen, which would be the key in controlling the disease. DNA-based molecular genetic characterization of the pathogen with molecular marker techniques is better suited for such studies. Among the various marker techniques available, AFLP fingerprinting is more accurate and produces a larger number of polymorphic bands with high reproducibility, than the other techniques such as the slow and laborious RFLP and the less reproducible RAPD. The objectives of this study were to determine: 1. the genetic diversity of the *F. graminearum* isolates; 2. the geographical and host specificity of the isolates; 3. the correlation between genetic structure and toxin production.

MATERIALS AND METHODS

Fifteen isolates of *F. graminearum*, isolated from different geographical locations (Alberta, Manitoba, Ontario and Saskatchewan) and hosts (barley, corn, weed and wheat), were used for the AFLP analysis. The origin, vegetative compatibility group, aggressiveness and levels of toxin production of the isolates are presented in Table 1. The mycelia grown in potato dextrose broth were frozen and ground in liquid nitrogen, and the genomic DNA isolated with the help of the CTAB method. The standard protocol, (Vos *et al*., 1995. Nucleic Acid Res. 23: 4407 – 4414), with a few modifications, was used for the AFLP analysis. The genomic DNA was cut with *Eco RI* and Mse I restriction enzymes, ligated with E*co RI* and *Mse I* adapters, preamplified and amplified with five set of selective primers during the AFLP analysis (Table 2). The polymorphic bands were viewed with the help of silver nitrate staining (Promega, Madison, WI). The scored polymorphic bands were analyzed using unweighted pair group

mean analysis (UPGMA), in SAHN program of NTSYS- pc 2.1 software package (version 2.1; Exeter Software, Setauket, NY), which was used for the cluster analysis and the construction of the dendrogram.

RESULTS AND DISCUSSION

The AFLP analysis of the 15 isolates of *F. graminearum* yielded 105 polymorphic bands from the five primer sets used. Two isolates, FG8 and FG14, both isolated from corn in Ontario and belonging to the same VCG-E (Table 1), showed great similarity and the least genetic distance (Fig 1). FG7, an isolate from corn in Ontario, with significant aggressiveness, produced four toxins, namely DON, 3-ADON, 15-ADON and NIV, and was seen as a distinct sub-branch in the dendrogram (Fig 1). The geographic location and the hosts from which the isolates were obtained seem to have had a mild influence on the clustering of the isolates, as they seem to form small clusters based on either their originating geographic location or host (Fig 1). Among the isolates that were all isolated from wheat, FG2 isolated from winter wheat was distinct from the other two isolates, FG1 and FG4, isolated from red spring wheat, which shared higher genetic similarity (Fig 1). It is interesting to note that isolates FG1 and FG4 came from Alberta and Manitoba (Table 1), respectively. Among the isolates from Ontario that formed small clusters in the dendrogram, isolates FG5 and FG10, isolated from winter wheat, were distinct from the other isolates from corn and barley (Fig 1 and Table 1). Isolate FG13, which was isolated from a weed in Saskatchewan, formed a distinct sub-branch and thus was distinct from the isolates from Ontario in the other branch of the cluster (Fig 1).

AFLP analysis showed genotypic diversity between the *F. graminearum* isolates with reference to their vegetative compatibility groups, and this supported tan earlier work (Bowden and Leslie, 1992. Exp. Mycol. 16: 308 – 315), which showed genotypic diversity among G. zeae isolates based on their VCG. The branching of the isolates in the tree was more influenced by the VCG and the levels of toxin production of the isolates (Fig 1). This was clearly seen in the analysis, as isolates FG8 and FG14 belonging to the same VCG- E showed the least genetic distance, and isolate FG7 with the highest levels of all the four toxins, formed a distinct branch in the large upper cluster of the tree (Fig 1). The analysis showed a weak host or geographic specificity among the isolates, as observed by the weak clustering of the isolates in the tree based on their geographic location or the host from which they were isolated. This supported an earlier work (Van Eeuwijk *et al*., 1995. Theor. Appl. Genet. 90: 221 – 228), which showed non-specificity of resistance in wheat with European strains of *F. culmorum, F. graminearum and F. nivale.* The low geographic specificity of the data, as indicated by isolates FG1 and FG4 isolated from wheat in Alberta and Manitoba, respectively clustering together, seems to suggest the movement of the pathogen to new areas. The distinct branching of FG2 (from winter wheat) from the cluster of FG1 and FG4 (from spring wheat), seems to suggest that they produce and release spores at different times in a season to coincide with the availability of the susceptible growth stage of the respective host (winter or spring wheat) for infection and colonization.

It would be interesting to find the lineage of these 15 isolates from Canada that come from different geographical locations and different hosts. The *F. graminearum* clade includes seven distinct lineages (O'Donnell 2000. Proc. Natl. Acad. Sci. 97: 7905 – 7910), with the isolates

from the USA falling within lineage 7. Therefore, it would be useful to find the lineage of the Canadian isolates and to check whether they shared any similarity with their US counterparts. Isolates of *F. graminearum* that produce the FHB toxins belong either to the DON chemotype or the NIV chemotype (Marasas *et al*. 1984. The Pennsylvania State University Press, University Park, PA.). Most of the isolates from the USA belong to the DON chemotype (Anne Desjardins - Presentation at the CPS Annual Meeting, Waterton, Alberta, 2002). They produce small amounts of 3ADON and 15ADON. The NIV chemotype produce DON at only less than one percent of NIV (Anne Desjardins- personal communication). But two of our isolates, FG7 and FG10, produced significantly very high levels of DON when compared to NIV, especially FG7, which produced 249 ppm of DON and 1 ppm of NIV. It would be very interesting to genetically characterize these isolates, which would throw more light on the biochemistry of toxin production and also help us to understand whether the pathogen is going through a process of evolution to become a more aggressive form!

ACKNOWLEDGEMENTS

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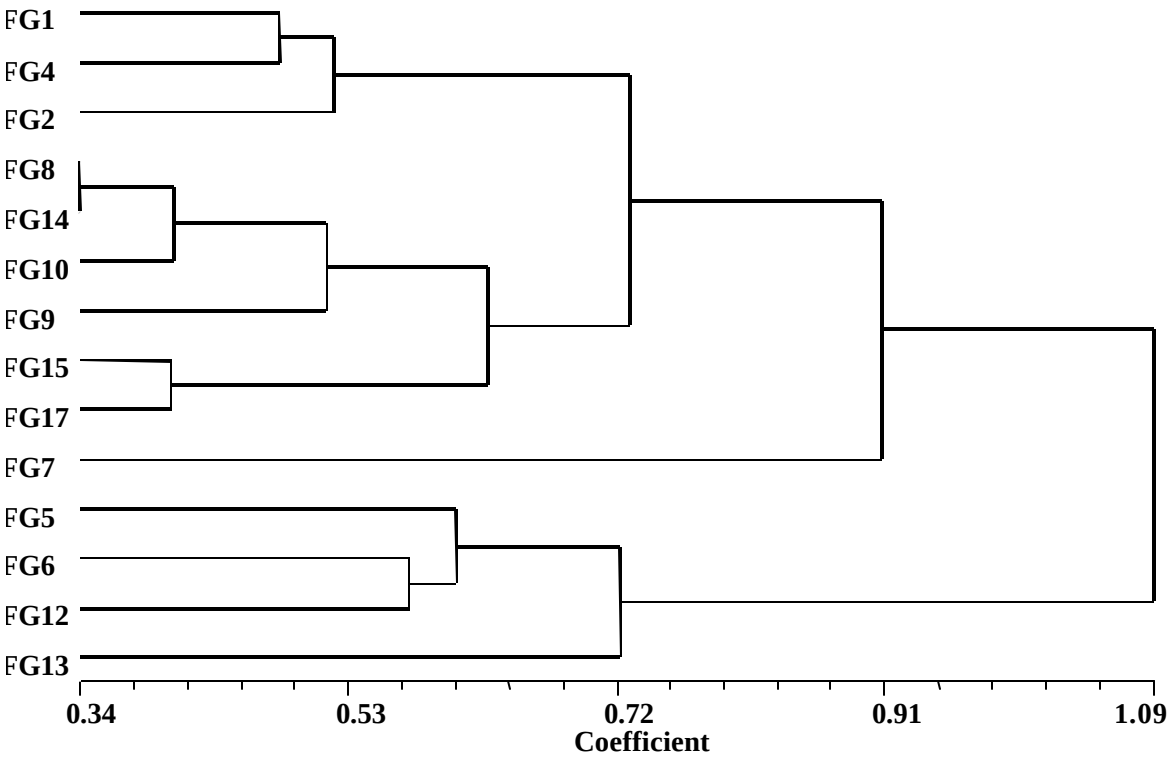

Figure 1. Dendrogram from UPGMA of AFLP data of *F. graminearum*

Table 1. Origin, VCG, aggressiveness and toxin production of *F. graminearum* isolates.

DAOM #	*Host*	*Location*[$]	*VCG*[†]	*Aggressiveness*		*T1**	*T2**	*T3**	*T4**
				SFI[‡]	*Spray*[£]				
213295 (FG1)	Wheat	AB	J	37	64.9	0.2	0	0.5	0
177409 (FG2)	Wheat	ON	C	17.2	56.4	88.3	8.6	0	0
192132 (FG4)	Wheat	MB	H	32.8	69	2.3	0	5.9	0
177406 (FG5)	Wheat	ON	A	33.2	56.1	10	0	13.1	0
178149 (FG6)	Barley	ON	L	33.4	56.2	27.8	0	24.7	0
170785 (FG7)	Corn	ON	K	31.5	43.6	249	11.7	3.1	1
180378 (FG8)	Corn	ON	E	30.5	45.9	20.5	0	16.2	0
180379 (FG9)	Corn	ON	F	36.3	60.2	80.9	2.8	0.4	0
177408 (FG10)	Wheat	ON	B	29	53.4	53.1	0	44.6	0.3
180377 (FG12)	Corn	ON	M	26.8	39.1	35.3	0	26.9	0
213384 (FG13)	Weed	SK	I	24.8	51	12.1	0	15.4	0
180376 (FG14)	Corn	ON	E	36.9	67.6	21.7	0	17.3	0
192130 (FG15)	Wheat	MB	D	32.2	52.2	6	0	11.6	0

$Location: AB- Alberta; MB- Manitoba; ON- Ontario; SK- Saskatchewan

†Vegetative Compatibility Group

‡Single Floret Inoculation with macroconidia (10µl of 50,000spores/ml)

£Spray inoculation with macroconidia (2 – 3 ml of 50,000 spores/ml)

*T1-DON- Deoxynivalenol; T2- 3ADON- 3-acetyl DON; T3- 15ADON- 15-acetylDON; T4- NIV- Nivalenol (in ppm)

(Modified from Gilbert et al., 2001. Mycopathologia 153: 209)

Table 2. List of selective primers used for the AFLP analysis.

Primer Set	*Eco* RI end	*MSe* I end
1	AC	A
2	AC	T
3	AA	T
4	AA	AT
5	TG	TT

ASSESSMENT OF THE DIFFERENTIAL ABILITY OF *FUSARIUM* STRAINS TO SPREAD ON WHEAT AND RICE

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ABSTRACT

In order to understand gene function related to pathogenicity we are evaluating the abilities of different strains of *Fusarium* to spread on the hosts and conducting genomic analysis of these interactions. Several strains selected from each of eight known phylogenetically distinct lineages of *Fusarium graminearum* were tested for their ability to spread on Norm, a susceptible cultivar of wheat, after inoculation of a single central floret. Similar studies were also conducted using strains belonging to other *Fusarium* species namely, *F. cerealis, F. pseudograminearum, F. culmorum* and *F. lunulosoporum*. All these strains were found to differ significantly in both their ability to spread within the wheat head as well as the type and amount of mycotoxins they produce. A few of the *F. graminearum* strains were also tested for their ability to infect rice panicles. These strains caused necrosis in rice, but mycotoxin production was not detected in infected rice florets. Symptom expression, the presence of fungus in each spikelet, as determined by culturing, and mycotoxin concentrations were recorded from inoculated wheat heads and rice panicles 14 days after inoculation. Based on these pathogenicity tests one highly aggressive and one less aggressive strain were chosen for studies conducted with the aim of understanding these variations at the genomic level. cDNA libraries were created by subtractive hybridization to compare mRNA populations from wheat heads inoculated with the two strains in order to identify genes specific to each interaction. Marked differences in the transcript profile of these two interactions was revealed during the initial infection phase.

DEVELOPMENT OF *GIBBERELLA ZEAE* ON WHEAT TISSUE

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of cereal grains worldwide. In the United States this disease is mostly attributed to infections by the fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). The disease cycle of FHB is a valuable resource when considering control of *G. zeae*. The development of perithecia on wheat residues and the inoculum produced by perithecia have important impact on disease in reduced tillage systems. Our objective is to characterize the colonization of vegetative tissue and the subsequent development of perithecia. All plant tissue and cell types are susceptible to ramification by hyphae of *G. zeae.* However, the colonization of tissues adjacent to cells supporting perithecium formation is especially significant to the development of perithecia. Chlorenchyma tissue of the internodes and parenchyma tissue of the stem nodes are tissues found to directly underlie cells that support perithecium development. Perithecia form through stomates above chlorenchyma of the stem internode and from epidermal cells above the parenchyma of the stem node region. We are also interested in determining whether head infections proceed down the stem or if stem tissue is colonized from independent stem infections. Development of strategies for limiting infection of vegetative tissue is contingent upon understanding the mode of infection. The results of this study will give insights into the disease cycle as well as an understanding of infection pathways.

THE DONCAST MODEL: USING WEATHER VARIABLES PRE- AND POST-HEADING TO PREDICT DEOXYNIVALENOL CONTENT IN WINTER WHEAT

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ABSTRACT

Accurate predictions of deoxynivalenol (DON) concentrations in mature wheat grain are needed at heading for decisions on whether a fungicide application is necessary to control fusarium head blight, *Fusarium graminearum* Scwabe. Our model, now named "DONcast", was developed using weather and DON data from 399 farm fields across southern Ontario, Canada, from 1996 to 2000 (Hooker *et al*., 2002). A web site was launched in 2000 for providing DON predictions (in µg g^{-1} of mature wheat grain) to growers across Ontario http://www.ownweb.ca/models/public/fusarium/default.cfm?location=none. From 2000 to 2002, DONcast was validated on 121 wheat fields on private farms across Ontario. All parameters of the first DONcast model were reviewed and other variables were considered with the addition of both weather and DON data from 2000 and 2002. DONcast was refined further by considering agronomic influences such as wheat variety, previous crop, and tillage system from all 520 fields between 1996 and 2002. In the refined DONcast model, weather was still important between 7 days before heading and 10 days after heading. In the first period 4 to 7 days before heading, DON generally increased with the number of days with >5 mm of rain, and decreased with the number of days of <10°C. In the second period 3 to 6 days after heading, DON increased with the number of days of rain >3 mm, and decreased with days >32°C. In the third period 7 to 10 days after heading, DON increased with the number of days with >3 mm of rain. Using multiple regression procedures, the refined model accounted for lower concentrations of DON when cool temperatures (mean daily temperatures < 15°C) occurred between 3 and 10 days after heading. Wheat variety susceptibility coefficients from inoculated misting trials were also included in the refined model, along with a variable for the presence of host crop residue on the soil surface at wheat planting (Schaafsma *et al*. 2000). While only one equation is used in each case to forecast DON, the equation is different depending on the situation. In fields where the previous crop was not wheat or corn, the refined model explains 78% of the variation in DON using the equation if rain occurred between 3 and 6 days after heading. If no rain occurred between 3 and 6 days after heading, then another equation is used, which explains 63% of the variation in DON. In fields where the previous crop was corn or wheat, another prediction equation explains 86% of the variability in DON. Using the refined DONcast model, DON concentrations of < 1 µg g^{-1} were predicted correctly on 46 of 52 fields in 2001 and on all 34 fields surveyed around weather stations in 2002. Concentrations of > 1 µg g^{-1} were predicted correctly on 9 of 14 fields in 2001, and on 5 of 11 fields in 2002. Accurate predictions of < 1.0 µg g^{-1} suggests that control strategies may not be warranted, while predictions of 1 to 2 µg g^{-1} suggests that control strategies may be warranted to improve the grade and marketability of wheat.

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FUSARIUM HEAD SCAB RISK FORECASTING FOR OHIO, 2002

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ABSTRACT

During the 2002 wheat growing season, head scab risk assessment models were used to predict the risk of Fusarium head scab in Ohio. This was the second year for testing these models in the state. Head scab risk assessment probabilities were derived from logistic models previously developed from hourly weather, crop growth and disease observations from 50 location-years representing three wheat production regions in the US. Hourly weather data from 14 weather stations located in Ohio, Indiana and Michigan were used to determine duration of weather events for the pre- and post-anthesis time periods examined by the models. Disease risk probabilities were calculated using logistic equations determined by two models representing the critical weather conditions during the time period 7 days prior to anthesis (Model I) and the time period inclusive of the 7 day pre-anthesis plus 10 additional days post anthesis (Model II). Weather conditions in early April were relatively dry and warm providing conditions for rapid and early development of the crop. Anthesis dates for wheat fields from south to north in the state varied by more than four weeks (10 May to 9 June) due to cool weather that slowed plant development in May. Precipitation events became more frequent during late April and throughout May across the state with most locations reporting up to 32 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 when most of the wheat was in anthesis. Scab risk probabilities were calculated for early, mid and late anthesis dates for each weather station location. Calculated risk probabilities ranged from 0.00 to 0.81 for Model I and from 0.02 to 0.69 for Model II. Of 42 location-anthesis date scab risk probabilities calculated, Model I predicted 31 location-anthesis dates with low to moderately low risk and Model II predicted 40 location-anthesis dates with low or moderately low risk. Only one location (Ft. Wayne, IN) had a moderately high risk prediction for Model I and Model II and another site (Oxford, OH) had a moderately high risk prediction for Model II. Based on these results, the head scab risk prediction was reported to be low to moderately low for the majority of locations in the state. Head scab risk predictions were posted on the Ohio State University Ohio Field Crop Disease web page (www.oardc.ohio-state.edu/ohiofieldcropdisease/) during the critical time of disease development through harvest. Approximately 14 to 18 days after anthesis 159 fields in 30 counties were surveyed for scab incidence by the OSU Extension Agents. From 1 to 10 fields were surveyed per county. Disease surveys indicated the average incidence of head scab was 4.1% with a range of 0% to 48.6%. Over 75% of the surveyed fields had scab incidence levels below 5%, and only 4% of the surveyed fields had incidence levels above 15.1%. Results of the Scab Risk Assessment Models indicated that they generally predicted the risk of scab correctly for the majority of locations in the state.

PRACTICAL APPLICATION OF FUSARIUM HEAD BLIGHT RISK PREDICTIONS

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Fusarium head blight (FHB), primarily caused by the residue-borne fungus *Fusarium graminearum*, continues to be an important economic problem in the more humid, temperate regions of the US and Canada (Bai and Shaner 1994, McMullen *et al*. 1997). Control of this disease has been difficult, but progress has been made by scientists funded by the U.S. Wheat and Barley Scab Initiative (USWBSI) toward the management of FHB. An important objective of the USWBSI has been the development of adequate disease forecasting systems for FHB.

The most significant purpose of a head scab forecasting system would be to function as an early warning system. If accurate disease predictions could be made prior to floret infection at anthesis, then growers could use preventative disease control options, such as chemical or biological agents, to avert disease and yield loss. Secondly, a timely disease warning would also provide valuable time for farmers, grain handlers and food processors to deal with the prognosis of disease and the potential for mycotoxin contaminated grain by establishing the necessary infrastructure to appropriately test for, and manage, damaged grain.

Last year in a presentation at the 2001 National Fusrium Head Blight Forum, Dr. Len Francl reviewed the various types of forecasting systems available for small grain diseases (Francl 2001). He discussed two FHB forecasting systems that are now being tested. In Ontario, Canada, Hooker *et al* (2002) have developed a model that utilizes weather data pre anthesis and weather forecasts post anthesis to predict deoxynivalenol levels in harvested grain. In Ohio, De Wolf *et al*, (2001) have developed a disease forecasting system based on risk assessment. Generally, risk assessment models estimate the probability (i.e., risk) of an undesirable event occurring at a given location and time (Teng and Yang, 1993). FHB appears well suited for risk assessment modeling because of the severity of epidemics, compound losses from mycotoxin contamination and yield loss, and the relatively narrow time periods of pathogen sporulation, inoculum dispersal, and host infection (De Wolf *et al*. 1999, Francl *et al*. 1999).

Our main objectives were to develop relatively simple models using readily accessible weather variables that would be applicable over a large geographic area including spring and winter wheat areas. Secondly, to meet the immediate need of farmers we needed to develop a forecasting system in as short a time as possible. To meet this goal we used historic disease data and weather records for model development. Dr. De Wolf presented a description of the initial FHB risk models at the National Fusarium Head Blight Forum meeting in 2000 (De Wolf *et al* 2000). We have been testing the models in Ohio during 2001 and 2002 (Lipps and Mills, 2002) and other states (ND, SD, MO, MI and PA) have tested them during 2002.

The risk predictions models were developed using historic disease and weather data obtained from cooperators in ND, OH, MO and KS (De Wolf *et al*, 2000, De Wolf *et al*, 2001). Logistic regression models were developed from hourly weather, crop growth and disease level observations from 50 site-years representing three wheat production regions in the US. Correlation analysis identified combinations of temperature, relative humidity and rainfall across time periods 7 days prior to and 10 days after anthesis as significant independent variables. Of several logistic regression models developed the following two models were adopted for further testing because of their relatively high prediction accuracies.

Model I predicts the probability of head scab based on the weather that occurs prior to anthesis. This is the time when fungal inoculum develops. Model I utilizes the duration of precipitation in hours and the number of hours when the air temperature is between 15 and 30°C for 7 days prior to flowering. Cross validation prediction accuracy for this model was 78% for determining when disease will not be severe (severity $\leq$ 10%). Its accuracy for predicting when an epidemic will occur (severity $\geq$ 10%) was 56%.

Model II predicts the probability of scab based on the weather that occurs 7 days before and 10 days after anthesis. This model addresses the time when the fungus is developing spores, when infection occurs and when disease develops. Model II utilizes the number of hours when the air temperature is between 15 and 30°C for 7 days prior to flowering and the number of hours when the relative humidity is 90% or above and the air temperature is between 15 and 30°C for 10 days after flowering. Cross validation prediction accuracy for this model was 83% in determining when disease will be severe (severity $\geq$10%).

In order to make the FHB risk forecasting models more user-friendly, Dr. De Wolf and Mr. Mills developed a Microsoft Excel workbook that contains the various logistic equations. Probabilities are automatically calculated when the appropriate weather data is entered and the anthesis date is designated. The Excel file not only calculates the scab risk probability values, but also graphs the weather variable coordinates and plots them in relation to a risk threshold curve (logistic regression equation where predicted FHB severity is $\geq$ 10%). The distance of weather variable coordinates from the threshold curve defines the relative risk probability for the weather station location.

There is considerable flexibility for using FHB risk models, especially for processing weather data and presenting the prediction information to the growers. In Ohio during the 2002 season, hourly weather data from 14 weather stations were used to make risk probability calculations for three anthesis dates (early, mid and late anthesis) for each weather station location. Actual risk probabilities (as a percentage) were not presented directly to the public, but numerical probabilities were classified into 'Risk Levels' (low, moderately low, moderately high, and high) based on logistic regression thresholds in order to help growers better interpret the risk of scab in their area. To facilitate the timeliness of reporting information during the critical period of scab development, a web page (www.oardc.ohio-state.edu/ohiofieldcropdisease/) was used to deliver scab risk assessments.

Michigan and Pennsylvania, took a similar approaches to providing public access to FHB risk forecasts and managing the problem of obtaining accurate anthesis date information. Both reported prediction results on a web site (URL for MI was http://www.cips.msu.edu/cips/

headblight/index.htm and for PA was http://www.wheatscab.psu.edu/) and used weather data from multiple locations (18 location in MI and 33 locations in PA). Additionally, both provided daily risk probabilities for each of the weather locations and presented these as contour maps of the state with risk probabilities as color-coded areas between contour lines. This map-based presentation style required the farmer to choose the appropriate anthesis date for his fields to obtain the FHB risk probability map for that date.

Validation of the FHB risk assessment models in the field is a problem because of the large area, and consequently large number of fields, for which predictions are made. In Ohio, thirty County Extension Agents assessed the incidence of head scab in 159 fields by counting the number of diseased and non-diseased heads per foot of row in 10 locations per field. The FHB risk models predicted low to moderately low scab risk for most of the state in 2002. The FHB survey indicated that over 75% of the fields had incidence levels below 5% (average for all fields = 4.1%). In Michigan, risk assessment Model II predicted low to moderately low scab risk for 17 of the 18 weather station locations. A survey of fields in southern Michigan indicated that FHB incidence was low to moderately low and ranged from 0 to 25% in individual fields. Of 50 grain samples submitted from fields throughout the state all but one had DON levels between 0 and 0.5 ppm. Risk assessment models were not developed to predict DON levels in grain. The low DON levels detected in MI were probably due to the low level of disease and the dry conditions during the grain filling period.

Disease forecasting systems are never 100% accurate because they are mathematical predictions representing a multitude of variables that determine disease progress. Each of the variables can have a wide range of values. Problems that limit the accuracy of FHB forecasting models include variables associated with the pathogen (variation in inoculum levels in the field due to differences in residue management systems, variation in crop rotation sequences among fields, wind and rain splash dispersal gradients, pathogen species composition); host (anthesis date differences, susceptibility level, duration of anther retention, head height); and weather (variation in rain and RH duration across an area, temperature variation due to topography). Although models are designed to be robust, they will not accurately describe all possible situations. Risk predictions may be improved by using weather data from many sites, obtaining more accurate anthesis dates for an area, averaging risk probabilities over a multiple-day anthesis periods for locations and monitoring inoculum levels. Currently, cooperative research is being conducted in ND, SD, IN, OH and PA to develop a database of weather, crop development and pathogen inoculum level information to further validate and improve FHB risk assessment models.

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EPIDEMIOLOGICAL STUDIES ON FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTH DAKOTA FOR 2002

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INTRODUCTION AND OBJECTIVES

South Dakota State University is part of a multi-state 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 risk advisory/forecast system. Primary objectives include: 1) monitoring inoculum dynamics and disease development in relation to specific environmental parameters; and 2) to evaluate currently proposed forecast models.

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. By collecting disease and environmental data for multiple plantings of susceptible wheat across several locations, we might better characterize both epidemic and non-epidemic conditions for FHB 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 (26 April, 6 May, and 22 May, 2002), 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*. (1998), with some modification (sampled spikes were not covered prior to sam-

pling). 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 data were collected from each replicate within each planting date three to four times between late anthesis (Zadoks 67) and soft dough stage (Zadoks 85). In each replicate, 150 spikes from primary tillers were visually rated for FHB. Severity of FHB for each spike was rated on a 0-9 scale roughly based on percent of the spike visually blighted (0 to 90+%). Incidence rate was calculated by: number of infected spikes divided by total spikes counted per replicate. Severity was calculated for infected spikes by: (sum of spike severity ratings) divided by the number of infected spikes per replicate.

Data from the 2002 FHB monitoring plots will be entered into two FHB risk assessment/ disease forecast models made available by Ohio State University (Ohio I and Ohio II; De Wolf, *et al*, 2000). Ohio model I is used to predict risk of a FHB epidemic based on temperature and precipitation variables prior to anthesis. Ohio model II is intended to predict disease risk based on temperature and humidity before and after flowering begins. Model I is intended to predict epidemics before infection, while Model II is intended to estimate disease risk after infection may have occurred.

RESULTS AND DISCUSSION

Major environmental parameters for each planting date are summarized in Table 1. Generally, dry conditions with warm temperatures were experienced throughout the growing season in 2002. A short period of three to four days of wet weather was experienced just prior to flowering of PD 1, but was followed by very warm, dry conditions for several days.

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

PD	Time period (susceptible)	Avg. air temp (°C)	[a]Avg. e_a (kPa)	Precip. (mm) / duration (hrs)	15°C < T < 30°C (hours, max=168)	RH > 90% (hours)	[b]T*RH (hours)
1	DOY 177-183	25.7	2.00	0 / 0	107	10.5	7.5
2	DOY 180-186	26.4	2.15	0 / 0	108	9.5	9.5
3	DOY 190-196	20.5	1.75	2.0 / 2	123	46	27.5

a. vapor pressure of the air
b. hours temp is between 15°C and 30°C **and** RH > 90%

Inoculum level estimates for 2002 are presented in Table 2, and disease levels are presented in Table 3. Inoculum was considered to be moderate as estimated by both the Burkard spore trap and by the spike-wash method. Disease incidence was much higher than expected, ranging from 10 to 45% of head affected by FHB. Severity however was very low in all cases, ranging from 1 to 8% blight on infected spikes, on average.

Table 2. Inoculum level estimates over susceptible periods in each planting date.

PD	Time period (susceptible)	Burkard Spore Trap (cfu / day)	[a]Spike-wash (cfu / spike)
1	DOY 177-183	335	75
2	DOY 180-186	321	105
3	DOY 190-196	215	122

a. average of 3 reps, 10 spikes per rep.

Table 3. Final disease ratings.

	Plant Date 1		Plant Date 2		Plant Date 3	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Rep 1	44.7	8.2	43.3	6.1	21.3	2.4
Rep 2	46.7	7.2	38.7	5.0	14.7	1.6
Rep 3	26.7	7.3	37.3	5.6	10.7	1.2
PD Mean	39.3	7.6	39.8	5.6	15.6	1.7
Overall:	Disease Incidence = 20%		Disease Severity = 5%			

The high incidence levels, coupled with the moderate levels of airborne and spike-borne spores suggest that inoculum levels were present at a level conducive to disease, however environmental conditions experienced during anthesis and after were not considered to be conducive to FHB development beyond the initial infections.

The results of model validation of Ohio models I and II are given in Table 4. FHB disease index for all plantings is given as an indicator of overall disease level and is the product of incidence and severity for each planting date. The probability of an epidemic occurring based on the two models is also given. Based on this set of validation runs, Ohio model I was consistent across plantings in relative rank and appear to correlate well to FHB incidence, however, it suggests that an epidemic is likely for PD 1, which had only 2.5% disease. Ohio model II suggests that no epidemic levels would be reached, which corresponds to the final disease estimates. It is believed that the parameter for precipitation (hours of precipitation duration) incorporated into Ohio I does not account for precipitation patterns of the Great Plains, which typically receive large quantities of precipitation in relatively short periods of time.

Table 4. Validation of Risk Assessment / Disease Prediction Models (Ohio I and II)

Planting Date	FHB Index (Inc*Sev, %)	FHB Incidence (% infected spikes)	Ohio Model I (risk probability)[a]	Ohio Model II (risk probability)[b]
1	2.5	39	0.53	0.08
2	2.2	40	0.31	0.14
3	0.3	16	0.19	0.33

a. epidemic threshold = 0.5
b. epidemic threshold = 0.44

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FHB INOCULUM DISTRIBUTION ON WHEAT PLANTS WITHIN THE CANOPY

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ABSTRACT

Fusarium head blight (FHB) of wheat is a potentially devastating disease in many of the wheat growing regions of the U.S. and Canada. The primary inoculum for this disease is generally considered to be ascospores of the fungus *Gibberella zeae* (ana: *Fusarium graminearum*). Perithecia of the fungus develop on infected crop residues, especially corn stalk pieces, remaining on field surfaces. The perithecia forcibly eject ascospores, but their fate is not certain. A large proportion of ascospores may not be able to contact susceptible host tissues because the infection window is quite narrow. Instead, these spores may land on non-susceptible tissues (leaf, stem, etc.). These spores may germinate and reproduce epiphytically. A study was initiated in the 2002 field season to investigate the types (conidia or ascospores) and distribution of spores of the FHB pathogen. Wheat plants were collected from 10 sites (3 groups per site) around the state and subsequently dissected and processed to enumerate conidia (of *F. graminearum*) and ascospores (of *G. zeae*) on individual leaves at specific leaf positions on the plants, as well as on the spikes. Ascospores and conidia were recovered at levels from 0 to 1500 spores per leaf. Relative ratios of ascospores to conidia varied greatly from 7:1 down to 1:4. Generally, ascospores outnumbered conidia at all leaf positions across most locations, with some notable exceptions. The results of the sampling show a distinct bimodal distribution pattern for ascospore counts with higher concentrations (50 to 200% greater) at the upper-most leaf position and the lowermost leaf position within the canopy than at the center leaf position. It is also noted that conidial distribution among leaves varied widely across locations. In some locations, few conidia were identified, while at other locations, all leaves were found to hold large numbers (up to 1500 spores) per leaf. This suggests that the fungus may undergo epiphytic growth and reproduction, resulting in increased inoculum load within the canopy of a wheat crop.

SOUTH DAKOTA FUSARIUM HEAD BLIGHT RISK ADVISORY FOR 2002

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ABSTRACT

In 2002, the small grains pathology project at South Dakota State University launched a web-delivered, weather-based risk advisory for Fusarium head blight (FHB) in northeastern South Dakota. A thirteen-county area comprising the majority of the spring wheat region in the state was selected for intensive inoculum, disease and environment monitoring. This area was selected for a FHB risk advisory to be issued on a county by county basis. Advisory information was to be posted to the internet every one to two days detailing potential risk of disease to wheat crops in each of the 13 counties. Experimental risk assessment models (Ohio I and Ohio II) were utilized to provide risk probability based on a few selected environmental parameters. Model output was considered as part of the overall risk assessment upon which advisories were based. An advisory of 'high-risk" was issued for the entire thirteen county region for a three-day period near the end of June, but was downgraded as weather conditions became unfavorable for disease development. Disease levels were low in nearly all counties, with levels approaching 5% incidence for small sections of two counties in extreme north and northeast SD. Following the 2002 season, much of the environmental and disease data from the past three years were incorporated into a model development phase resulting in several linear models for the prediction of inoculum, infection and disease.

INCIDENCE OF *FUSARIUM GRAMINEARUM* AND *COCHLIOBOLUS SATIVUS* IN WHEAT AND BARLEY CULTIVARS AT THREE LOCATIONS IN MINNESOTA

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ABSTRACT

Wheat and barley entries in the 2002 Red River Valley on Farm Yield Trials grown at Perley, East Grand Forks and Humboldt, MN, were assayed for the colonization of kernels by *Fusarium graminearum* and *Cochliobolus sativus*. The trial consisted of 24 wheat and 8 barley lines-grown in commercial fields in a randomized complete block design with two replications. At maturity, 100 spikes per plot were arbitrarily collected and threshed. Kernels (200-400 per treatment) were surface sterilized, plated onto half strength PDA (pH=5.5) and incubated at 20-24ºC, under fluorescent lights (12:12 light:dark) for 5-6 days. The incidence of kernels colonized by *F. graminearum* was highest at Humboldt (18.4%, wheat; 22.2%, barley). The incidence of *C. sativus* colonized kernels was highest in wheat at Perley (30.6%), and in barley at East Grand Forks (23.1%). Ranking of wheat cultivars for kernel colonization by *F. graminearum* and *C. sativus* was significantly affected by the interaction of cultivar by location, however at all locations, the wheat cultivars Alsen and Gunner had low levels of *F. graminearum* and Dandy, Norpro, Pioneer 2375, Oxen and AC Vista were more highly colonized. Oxen and Gunner generally had low levels of kernel colonized by *C. sativus*, while AC Vista and MN97803 showed higher kernel colonization across locations. The six-rowed barley lines MN109, MN110 and Lacey generally had greater kernel colonization by *F. graminearum* than Robust, Drummond, Foster and Legacy. The incidence of *C. sativus* colonized kernels was similar in all barley entries except Conlon. Kernels of Conlon, a two-rowed barley, had the lowest incidence of *F. graminearum* but the highest incidence of *C. sativus*. The data suggests that the colonization of wheat kernels by *F. graminearum* and *C. sativus* may be influenced by differences in inoculum availability in a particular location, and site-specific environmental conditions.

AIRBORNE POPULATIONS OF *GIBBERELLA ZEAE*: SPATIAL AND TEMPORAL DYNAMICS OF SPORE DEPOSITION IN A LOCALIZED FUSARIUM HEAD BLIGHT EPIDEMIC

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ABSTRACT

Viable propagules of *Gibberella zeae* (anamorph *Fusarium graminearum)* were collected from the air over two wheat fields (spaced 0.5 km apart) in Aurora, New York in May/June 2002. Corn kernels inoculated with a clonal isolate of *G. zeae* were placed in one of the fields. Petri plates with *Fusarium* selective medium were suspended 30 cm above the wheat canopy. Fields were sampled a total of 20 days before, during, and after wheat anthesis. Ninety six plates were exposed continuously during each day (sunrise to sunset) and another 96 plates were exposed continuously during each night (sunset to sunrise). Significantly more colonies were collected during the night than during the day. Seven major deposition events were apparent during the sampling period, and three of these were coincident with rainfall. Three major deposition events occurred during flowering; the largest occurred two days after anther extrusion. The field bearing the clonal source of *G. zeae* was exposed to more colonies than the other field. DNA fingerprinting analyses are being conducted to assess the genetic diversity of airborne populations of the pathogen and contributions from local and regional sources of inoculum.

DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN INDIANA, 2002

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ABSTRACT

We are participating in a multi-state cooperative study on the epidemiology of Fusarium head blight (FHB) of wheat. We monitored weather, inoculum production, and disease development in order to obtain data that can be used to quantify the effects of weather on inoculum production and disease development. We planted 3 winter wheat cultivars at 3 dates at the Purdue Agronomy Research Center. Corn residue in the plots served as a source of inoculum. An automated Campbell station recorded weather data. The dates of flowering initiation among treatments ranged from 22 to 30 May. There were several days of unusually warm weather during the third week of April, and then several weeks of cooler than normal weather. During the 2 wk prior to 22 May, rain fell on 8 days, but mean daily temperature was above 15 °C on only 16 May. Daily mean temperatures began rising after 26 May, but by then there was little rainfall. Daily airborne spore concentrations estimated from a Burkard sampler ranged from 0 to 164 cfu·10 m^{-3} d^{-1}. A second sampler was located in a field 1.6 km away, but also with corn residue on the surface. There was close agreement between the numbers of spores collected each day at the 2 sites (r=0.95). Daily variation in number of airborne spores was large. On only one occasion, 26 and 27 May, were there 2 consecutive days with high counts. Each day we also collected heads at both sites for direct assay of spores of *G. zeae*. Spores recovered per head ranged from 0 to 750 d^{-1}. The higher values occurred later in the season, when wheat was in the grain filling stage. Numbers of spores recovered from heads at the 2 sites were in general agreement (r=0.75) except for 5-7 June, when substantially more spores were recovered at the main site. Based on estimates of the volume of air intercepted by a wheat head during 24 h, the Burkard samplers and the head washing assays gave similar estimates of the number of spores that impact a head each day, although correlations between daily values were low. Detailed assessment of incidence and severity of FHB were made at 2- to 3-day intervals in the 2nd planting of cultivar Elkhart. Incidence and severity both increased linearly from 5 June, when symptoms first appeared, through 21 June. Incidence increased from 3 to 16% (0.8% per day) and severity increased from 30 to 83% (3.5% per day). We assessed incidence and severity in all plots on 21 June. Among the cultivar-planting date treatments, mean FHB incidence ranged from 1.4 to 9.2%. The effects of cultivar, planting date and their interaction were all highly significant. For the various cultivar-planting date combinations, there was a significant correlation between incidence of FHB and the number of spores detected with the Burkard sampler on the 4th or 5th day after beginning of anthesis (r=0.81 and r=0.91, respectively). The correlation between incidence and the sum of spore densities on these 2 days was 0.97. We used data from this study to evaluate 2 weather-based forecast models developed by DeWolf *et al*. These models predicted a low probability of a “severe” epidemic, defined as an incidence of greater than 10%, for all cultivar-planting date combinations, consistent with what we observed.

COMPARISON OF SPRAY, POINT INOCULATION METHODS, AND FDK TO FACILITATE EARLY GENERATION SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WINTER WHEAT

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INTRODUCTION

Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) is an important disease of wheat (*Triticum aestivum* L.) in Canada, and worldwide (Sutton, 1982). Different types of wheat resistance to FHB have been reported, but in the breeding programs Type 1, or resistance to initial infection, and Type 2, or resistance to spread of symptoms within the head are used most often (Schroeder and Christensen, 1963, Mesterhazy, 1995). The most frequently used source of FHB Type 2 resistance worldwide is Sumai 3.

Several authors reported that Type 1 and Type 2 resistance varied independently among cultivars (Schroeder and Christensen, 1963; McKendry *et al*., 2001; Desmeules *et al*., 2001), and that selection of genotypes with resistance to FHB depends on the inoculation technique used (Engle *et al*., 2001; Bockus *et al*., 2001). Shaner and Buechley (2001) proposed the expression of severity as the number of spikelets blighted, not the proportion of spikelets blighted, in order to avoid the influence of spike size on the degree of Type 2 resistance. Van Saford *et al*., (1999), and Hall *et al*., (2000) reported that some genotypes have a lower kernel infection rate then expected from their spikelets infection rate, and also the opposite.

In order to increase the comprehensive resistant level to FHB, different resistance types to FHB should be pyramided into improved varieties (Xu *et al*., 2001).

Even though spray and point inoculation methods are used most frequently as the ways to estimate wheat resistance or susceptibility to FHB, these two methods are not often directly compared using segregating populations with known type of FHB resistance.

The objectives of this study are:

-to directly compare FHB severity after spray and point inoculation method using a segregating population with type 2 FHB resistance,
-to compare Type 1 and Type 2 resistance with Type 4, or resistance to kernel infection,
-to compare expression of FHB severity as the number of spikelets infected with expression of FHB severity as the proportion of spikelets infected, in order to examine the influence of spike size on the degree of FHB resistance.

MATERIALS AND METHODS

The influence of different methods of inoculation on number or proportion of FHB infected spikelets, and percent of Fusarium damage kernels (FDK), were studied using an F_3 population carrying Type 2 FHB resistance. The progeny (n=85) was derived from a cross of resistant (WEKO60DH3 - a Sumai 3 derivative) and susceptible (AC RON) parents. The segregating generations, and parents, were planted on October 20, 2000, in 2-m long single rows, spaced 17.8 cm apart, at Ridgetown, Ontario.

In order to obtain uniform growth stage at the time of inoculation, individual heads, rather than whole plants or plots, were inoculated at 50 % of anthesis (Zadoks growth stage 60-69) (Zadoks, 1974). The heads were spray inoculated with 2 mL of the suspension sprayed onto individual heads, and point inoculated with 10 µl of suspension injected into single florets. The suspension of macroconidia, including three isolates of *F. graminearum,* was produced in liquid shake culture using modified Bilay's medium, and used at a concentration of 50,000 spores/mL.

Between 10 to 20 plants from each progeny and the parents were inoculated using both methods of inoculation. Clear plastic bags were placed over the inoculated heads, and left for 48 hr to maintain humidity. The plots were misted daily with an overhead mister that delivered about 7.5 mm of water each day. The plots were fertilized and maintained using provincial recommendations.

The number of diseased spikelets and the total number of spikelets were recorded for each inoculated wheat head. The spikelet infection rate was calculated as the number of diseased spikelets, or percentage of diseased spikelets of the total number of spikelets. The average infection rate from each row was calculated. The inoculated heads were hand harvested separately. Heads from each row, with the same inoculation method, were threshed together using a single head thresher, retaining all light kernels.

The number of healthy and *Fusarium* damaged kernels were counted, and percentage of FDK was calculated for each line and parents, after both methods of inoculation. In order to avoid the influence of inoculation method on % of FDK, % of FDK after both methods of inoculation was also averaged for each line, and their parents. FDK were identified as shriveled kernels, with chalky, pink or white color. The F_3 progeny was assigned to phenotypic classes on the basis of their position in the distribution of the proportion of FHB infected spikelets, and % of FDK.

For the proportion of FHB infected spikelets the following classes were used: 1=0-2.5, 2=2.51-5, 3=5.01-7.5, 4=7.51-10, 5=10.01-12.5, 6=12.51-15, 7=15.01-17.5, 8=17.51-20, 9=20.01-22.5, 10=22.51-25, 11=25.01-27.5, 12=27.51-30, 13=30.01-32.5, 14=32.51-35, 15=35.01-37.5, 16>37.51.

For % of FDK, there were the following classes: 1=0-2, 2=2.01-4, 3=4.01-6, 4=6.01-8, 5=8.01-10, 6=10.01-12, 7=12.01-14, 8=14.01-16, 9=16.01-18, 10=18.01-20, 11=20.01-22, 12>22.01.

Data management and all statistical procedures were completed using SAS v. 6.0. (SAS Institute Inc, 2001).

RESULTS AND DISCUSSION

Transgressive segregants, with higher levels of resistance than the parents, were found using visual symptoms and % FDK after both methods of inoculation (Fig. 1-2). Minimum, maximum, and mean values for % of infected spikelets after point inoculation were 3.7, 32.7, and 11.4, and lower than after spray inoculation where they were 4.8, 42.0, and 15.4, respectively. This result was expected because this population carrying Type 2 resistance from Sumai-3. Overall correlation between % of diseased spikelets after spray and point inoculation was positive, but low ($r=0.38$, $P<0.001$).

Values for minimum, maximum, and mean percent of FDK after point inoculation were 0, 31.0, and 9.0, and these were higher than % FDK after spray inoculation (0, 20.6, and 6.2, respectively). AC RON, a FHB susceptible cultivar, had lower scores than WEKO609H3 for % FDK after spray inoculation with *F. graminearum* (Fig. 2 B). Correlation between % FDK and % FHB infected spikelets after the spray inoculation method was significant ($r=0.28$, $P<0.05$), while correlation between % FDK and % FHB infected spikelets after the point inoculation method was not. When % FDK after both methods of inoculation was averaged for each line, and correlated with % FHB infected spikelets, the results showed again that % FDK correlated weakly with % FHB infected spikelets after the spray inoculation method ($r=0.24$, $P<0.05$), but not after the point inoculation method in this population (even while carrying Type 2 resistance). According to our results, % FDK can be estimated more accurately after spray, than after point inoculation method. It was unexpected that there was no significant correlation between % FDK after spray, and % FDK after point inoculation method in this population. When lines were ranked, according to % FDK after different methods of inoculation, just 2 of the 10 top lines were the same.

The number of infected spikelets correlated well with proportion of infected spikelet ($r=0.82$, 0.87, $P<0.001$), after point (Fig. 3A), and spray (Fig. 3B), inoculation method, respectively, even when several outliers were also identified (Fig. 3). There was a good segregation for total number of spikelets within the spike in this population, and ranged from 15 to 22. When lines were ranked, according to proportion of FHB infected spikelets, or number of FHB infected spikelets, 7 of 10 lines with lowest % of FHB infected spikelets were the same, confirming that there is no influence of spike size on degree of FHB resistance. We concluded that visual estimate of the proportion of FHB infected spikelets can be recommended for selection in early generations, rather than the more time and labor intensive counting of FHB infected spikelets.

This study showed that there is an advantage of using both methods of inoculation, because progeny lines with higher levels of Type 1, Type 2, and Type 4 were identified, and they would not have been identified if only one of the methods was used. When these Types of resistance are identified in an early segregating generation, they should be pyramided sooner in the improved FHB varieties.

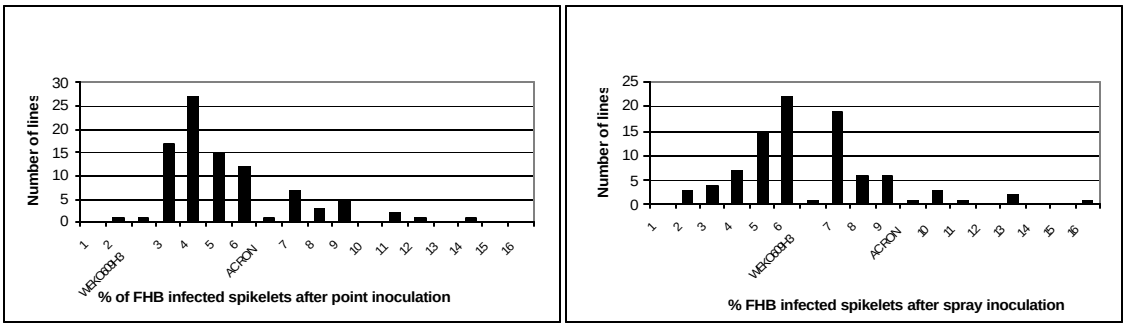

Figure 1. Frequency distribution of proportion of infected spikelets in F_3 generation after point (A), and spray (B) inoculation with *F. graminearum* .

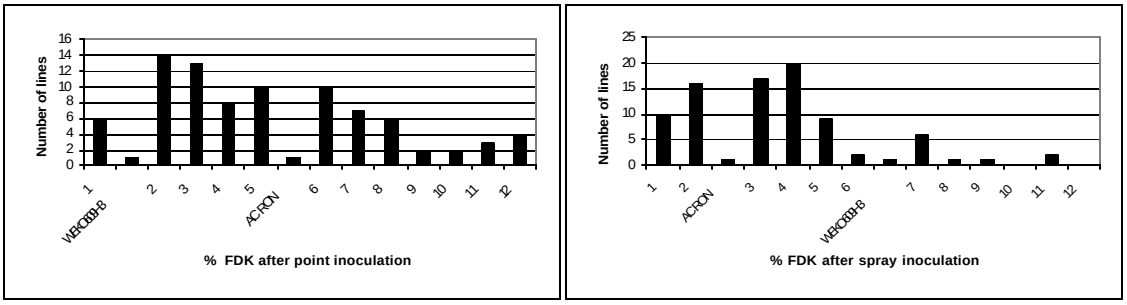

Figure 2. Frequency distribution of % FDK in F_3 generation after point (A), and spray (B) inoculation with *F. graminearum*.

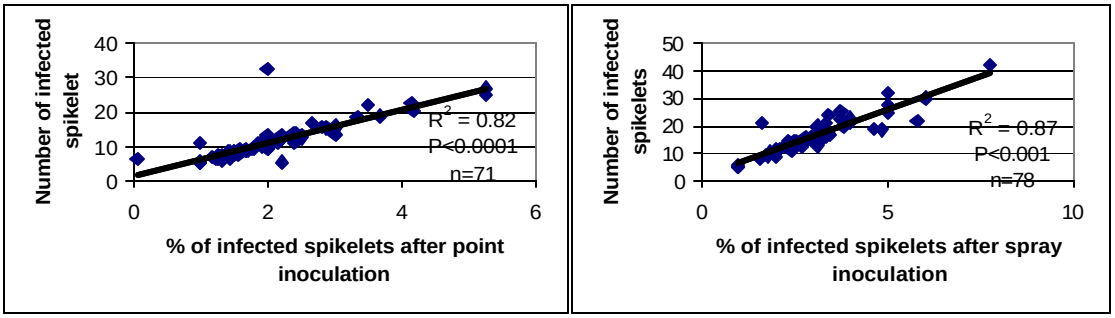

Figure 3. Relationship between number and proportion of infected spikelets in F_3 generation after point (A), and spray (B) inoculation with *F. graminearum* .

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REMI MUTAGENESIS IN THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium graminearum is an important pathogen of small grains and maize in many areas of the world. Infected grains are often contaminated with mycotoxins harmful to humans and animals. In the past decade, wheat head blight (scab), primarily caused by *F. graminearum* in North America, has emerged as a major threat in wheat production. To better understand the molecular mechanism of plant infection and virulence of *F. graminearum*, we used the REMI (Restriction-Enzyme Mediated Integration) approach to generate random targeted mutants. Over 7000 hygromycin-resistant transformants have been generated by transforming pCB1003 or pCX12 into *F. graminearum PH-1*. A corn-silk infection assay was devised to screen for mutants with reduced virulence. Many of the REMI pathogenicity mutants identified in corn-silk assays were dramatically reduced in their ability to infect and colonize flowering wheat heads. Genetic analysis and plasmid rescue are underway to identify and characterize genes disrupted in these mutants.

THE *FUSARIUM GRAMINEARUM* GENOMICS PROJECT

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ABSTRACT

*Fusarium graminearum (*teleomorph *Gibberella zeae)* is a broad host range plant pathogen that infects many crop plants worldwide. We have taken a genomics approach to better understand pathogenicity in this fungus. We have generated a collection of ESTs derived from cDNA libraries generated from cultures grown under several culture conditions and from infected wheat plants. Sequences were initially assembled into contigs and singletons, based on sequence comparisons, and a putative single gene set was identified. These sequences were compared to a yeast protein sequence reference set and to the GenBank non-redundant database using BLASTX. These results can be observed on the web (see link from www.scabusa.org). Based on presumptive gene function identified by this process, we were able to compare patterns of gene expression among cDNA libraries. Homologues of some known fungal virulence and pathogenicity factors and developmentally important genes were identified by this analysis. Funding for the complete genome sequence has been obtained. A discussion of the availability of tools for genomics and their potential uses will be presented.

COMPARATIVE VIRULENCE OF ISOLATES OF *FUSARIUM* SPECIES CAUSING HEAD BLIGHT IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) is an important disease of wheat in Canada. To supplement the development of FHB-resistant cultivars, the virulence of *Fusarium* isolates representing eight pathogenic species was investigated. Six wheat genotypes were artificially inoculated with 12 isolates of *Fusarium graminearum* and six isolates each of *F. acuminatum, F. avenacium, F. crookwellense, F. culmorum, F. equiseti, F. poae*, and *F. sporotrichiodes*. The pathogens were isolated from naturally infected wheat, barley, and oat heads collected from cross Canada from 1965 to 2001. A single spore culture was established for each isolate, from which spore suspension was produced. Inoculation was performed by spraying spores over spikes at the 50% anthesis stage. Symptoms of FHB were rated as disease severity using a 0-9 scale at 4, 7, 14, 21, and 28 days after inoculation; and as percent infected spikelets after 21 days. All isolates caused visible infections to the six wheat genotypes but only those of *F. graminearum, F. crookwellense,* and *F. culmorum* resulted in severe disease development and were considered highly pathogenic. Significant differences ($P < 0.05$) were also observed among isolates and from genotype x isolate interactions for the three highly pathogenic species. However, the genotype x isolate interactions were low (< 15%) compared to differences between isolates or genotypes and did not suggest the occurrence of pathogenic races. The presesence of different virulence among isolates suggests that screening for resistance to FHB require a mixture of several isolates of these pathogens to be included. Wheat genotypes differed significantly ($P < 0.001$) in susceptibility, and responses of the genotypes to isolates of the highly pathogenic species were generally similar. AC Foremost, CIMMYT11, and Quantum were the most susceptible; FHB37 and HY664 were intermediate; and Sumai 3 was resistant. These results indicate that selection for resistance to one species in wheat may also confer resistance to the others.

POPULATION GENETIC DIFFERENTIATION AND LINEAGE COMPOSITION AMONG *GIBBERELLA ZEAE* (*FUSARIUM GRAMINEARUM*) IN NORTH AND SOUTH AMERICA

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ABSTRACT

Gibberella zeae (*Fusarium graminearum*) causes Fusarium head blight (FHB) of wheat and barley, and has been responsible for severe economic losses worldwide. Sequence analyses of *G. zeae* have been interpreted to mean that populations of *G. zeae* are composed of eight potential phylogenetic lineages, with a phylogeographic structure among these lineages. We used AFLP polymorphisms to compare populations of *G. zeae* from the United States, Mexico, Brazil, and Uruguay. We have also examined populations of *G. zeae* isolated from sorghum seed in Uruguay. Populations of *G. zeae* causing FHB in the United States include only a single phylogenetic lineage (Lineage 7). Subpopulations from throughout the United States have high genotypic diversity, do not deviate from expectations of random mating, and are interconnected by extensive gene-flow. South American populations of *G. zeae* from both wheat and from sorghum include a minority component of isolates that cluster with other phylogenetic lineages (Lineages 1, 2, and 6), but are dominated by genotypically diverse populations of isolates from Lineage 7. Populations of *G. zeae* causing FHB on wheat from two locations in Mexico are dominated by isolates from Lineage 3. Population genetic comparisons of Lineage 7 isolates from North and South America indicate that while intercontinental gene flow may occur, the amount of gene flow between the continents is much less than that which occurs within each continent.