SESSION 3:

FHB MANAGEMENT

Co-Chairpersons: Marcia McMullen
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AGGRESSIVENESS OF *FUSARIUM GRAMINEARUM* 3ADON AND 15ADON POPULATIONS AS AFFECTED BY HARD RED SPRING CULTIVAR RESISTANCE AND FUNGICIDE TREATMENT, UNDER FIELD CONDITIONS IN NORTH DAKOTA

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INTRODUCTION

Trichothecene mycotoxins produced by *Fusarium graminearum* include deoxynivalenol (DON) and its derivatives 3-acetyl nivalenol (3ADON), 15-acetyl nivalenol (15ADON), and nivalenol (NIV). Based on the profile of trichothecenes produced, the fungal isolates can be identified and grouped as one of the chemotypes, 3ADON, 15ADON, and NIV. In recent years, the *F. graminearum* 3ADON isolates have increased dramatically in the Northern Great Plains of the US and Canadian prairie provinces, based on several molecular studies (Burlakoti et al. 2008; Ward et al. 2008; Gale et al. 2007; Guo et al. 2008; Puri and Zhong 2010). Puri and Zhong (2010) tested thirteen 3ADON and twelve 15ADON isolates collected from North Dakota for their aggressiveness on three wheat genotypes with different level of FHB resistance. Their results showed that the 3ADON population is more aggressive than the 15ADON population, based on disease development and DON production. However, Ward et al. (2008) in their greenhouse study did not find significant difference between the two populations in disease development although the 3ADON isolates produced higher DON concentration than the 15ADON isolates. More recently, van der Ohe et al. (2010) showed that no significant difference was observed between 3ADON and 15ADON populations in FHB disease development in susceptible genotypes. Their results also indicated that 3ADON isolates produced more DON as compared to 15ADON isolates in both susceptible and resistant wheat genotypes with one exception, although resistant cultivars exhibited resistance regardless of the pathogen chemotypes used as inoculum.

At present, Fusarium head blight is managed primarily through using a combination of moderately resistant wheat cultivars and a triazole fungicide application. A majority of the FHB moderately resistant cultivars recently released in the Northern Plains region contain a single source of resistance from a Chinese wheat cultivar Sumai3 (fhb1gene). However, little information is available on the interaction between wheat genotypes and the newly emerged 3ADON population in comparison with the 15ADON population for FHB development and DON production, and for interaction with fungicides. *The objectives of this study were to:* 1) compare 3ADON and 15ADON populations for FHB development and DON production on a FHB susceptible and a moderately resistant spring wheat cultivar; 2) determine if one population competes over the other when mix-inoculated on both cultivars; 3) obtain information on the effectiveness of fungicide application in disease management in field plots inoculated with individual and mixed isolate populations.

MATERIALS AND METHODS

Plant materials, Inoculations, and FHB Disease Rating: Two wheat cultivars, Briggs (FHB susceptible) and Alsen (FHB moderately resistant with fhb1 gene from Sumai 3), were planted in a randomly complete block design with a split plot arrangement with three replications, at the NDSU Agricultural Research Station at Fargo on April 20, 2010. Wheat cultivars served as the main plot, and inoculum type and fungicide application were treated as the subplots. The test plots were planted on 2009 soybean ground, to minimize the chances of a previous year's inoculum effect. The plot size was 10 x10 feet. Each field plot was separated with a 20 feet strip planted with Alsen to minimize the chance of inoculum interference from one treatment to the other. Three plots of each cultivar with and without fungicide treatment were spray-inoculated with a mixture of ten 3ADON isolates (A), or a mixture of ten 15ADON isolates (B), or an equal mixture of A and B at 100,000 spores/ml, when the plants were at the flowering stage (Feekes GS 10.52). Non-inoculated and non-sprayed plots of each cultivar were used as checks. All twenty isolates used in this study were recovered from FHB infected heads collected from various locations of North Dakota in 2008 and characterized for chemotype and aggressiveness in the greenhouse (Puri and Zhong, 2010; unpublished).

For the fungicide treated plots, Prosaro (prothioconazole + tebuconazole, 6.5 fl oz/acre) fungicide was sprayed 12 hrs prior to evening inoculations. FHB disease incidence and severity data were collected when the plants were at late milk stage to early dough stage (Feekes GS 11.1) by using the FHB disease rating scale developed by Stack and McMullen (1995). One hundred heads from each plot were rated. Fifty diseased heads from each treatment were tagged and harvested separately at maturity (Feekes GS 11.4) for fungal isolation and DON analysis. Fungal isolates from both cultivars Briggs (n = 117) and Alsen (n = 78) inoculated with a mixture of 3ADON and 15ADON populations were recovered by plating scabby grains on ½ PDA. One isolate from each scabby grain was recovered. All tagged disease heads from each plot were hand-thrashed, ground separately and submitted to the NDSU Veterinary Diagnostic Lab for DON analysis.

DNA extraction and Isolates Genotyping: All 195 *F. graminearum* isolates recovered from both cultivars were plated individually on ½ PDA plates containing sterile cellophane membrane on the medium surface. The plates were incubated for 4 days under 12 hrs light and dark cycles. The mycelia of each isolate were scraped using a flamed spatula and stored in 2.0 ml centrifuged tubes. DNA was extracted using the FastDNA® Kit along with the **FastPrep**® Instrument (MP Biomedicals, Solon, OH) according to the manufacturer's instruction. Trichothecene chemotype was determined using the trichothecene specific multiplex primers (3CON, 3NA, 3D15A, and 3D3A) (Starkey et al., 2007; Ward et al., 2002).

RESULTS AND DISCUSSSION

All inoculated field plots of both cultivars developed certain levels of FHB, whereas, non-inoculated and non-sprayed plots (checks) of both cultivars were free of disease, except for a few heads with less than 7% disease severity. The weather was dry and warm most of the time between inoculation and disease ratings. In the susceptible cultivar Briggs, the 3ADON population alone and the mixture of 3ADON and 15ADON isolates caused significantly higher FHB severity (mean values = 58.8% and 54.4% respectively), as compared to the 15ADON population (mean value = 35.0%) (Table 1). Similarly, grain samples collected from Briggs inoculated with the 3ADON isolates had significantly greater levels of DON (36.4 ppm) as compared to those inoculated with the 15ADON isolates (18.8 ppm) (Table 1). In contrast, no significant differences in FHB severity and DON accumulation were observed between the two isolate populations on the resistant cultivar Alsen. All three inoculum treatments were not significantly different in causing FHB incidence for either cultivar. It was expected that a similar level of disease incidence might occur in all inoculations, because the same concentration and volume of spores were used.

Inoculated plots treated with Prosaro fungicide had significantly less disease incidence and severity as compared to the non-sprayed plots (Table 1). The results showed that fungicide application was effective in reducing FHB regardless of inoculum sources used (Table 1). Grain samples from fungicide treated, inoculated plots had significantly lower DON accumulation as compared to the plots without fungicide treatment. No significant differences were observed between the 3ADON isolates and the 15ADON isolates in both susceptible and moderately resistant cultivars, when fungicide was applied.

A total of 117 isolates were recovered from Briggs inoculated with a mixture of 3ADON and 15ADON isolates. PCR analysis indicated that 64 of the isolates were of 15ADON chemotype and 53 were of 3ADON chemotype. Among 78 isolates recovered from Alsen inoculated with the mixed isolate population, 37 were 15ADON chemotype and 41 were 3ADON chemotype (Table 2). Chi-square tests showed that the recovery rates of the two chemotypes from both cultivars inoculated with the mixed population were not significantly different, suggesting that both types of isolates had a similar infection and survival rate under the conditions used in the study.

In conclusion, the results indicated that the newly emerged 3ADON population of *Fusarium graminearum* is more aggressive than the prevalent 15ADON population in FHB development and DON production in the susceptible cultivar, but not in the moderately resistant cultivar. Although the FHB resistance gene fhb1 is capable of providing resistance to both populations, the higher DON potential produced by the newly emerged population may pose a bigger challenge for sourcing low DON grain when very high disease pressure occurs. Our results indicated that deployment of resistance cultivars combined with fungicide application is an effective strategy in FHB disease management.

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Table1. Effect of *F. graminearum* 3ADON and 15ADON populations and fungicide treatment on FHB incidence, severity, and DON production in two hard red spring wheat cultivars, under field conditions in North Dakota.

Treatments	FHB[a] % incidence		FHB[a] % severity		DON[b] (ug/kg)	
	Briggs[c]	Alsen[c]	Briggs[c]	Alsen[c]	Briggs[c]	Alsen[c]
3ADON population (A)	42.3a	30.7a	58.8a	17.2a	36.4a	12.6a
15ADON population (B)	32.0a	46.0a	35.0b	16.2a	18.8b	11.4a
Mixture of A+B (C)	36.7a	44.3a	54.5a	18.6a	32.7a	10.5a
A + fungicide[d]	2.2b	8.0b	11.7c	9.7b	7.6c	4.6b
B + fungicide[d]	1.3b	7.0b	9.3c	7.9b	3.7c	3.4b
C + fungicide[d]	2.7b	4.7b	15.7c	8.2b	3.3c	4.1b
Non-inoculated, non-fungicide sprayed plots	1.3b	0.3b	7.0d	2.3b	0.3c	0.3b
LSD (P = 0.05)	NS		8.0		5.8	

Mean values followed by the same letter in each column are not statistically significantly at $P< =0.05$ by the LSD test. NS = not significant

[a]FHB = Fusarium head blight; [b]DON = deoxynivalenol; [c]Briggs = FHB susceptible hard red spring cultivar; Alsen = moderately FHB resistant hard red spring wheat cultivar (Sumai 3 source); [d]Fungicide = Prosaro (prothioconazole + tebuconazole) applied at 6.5 fl oz/Acre at flowering

Table 2. Recovery of 3ADON and 15ADON isolates from FHB resistant cultivar Alsen and susceptible cultivar Briggs inoculated with mixed population under field conditions

Cultivar	Isolate tested	3ADON	15ADON	Chi-square (P = 0.05)
Alsen	78	41	37	Non-significant
Briggs	117	53	64	Non-significant

EFFECTS OF WITHIN-FIELD CORN DEBRIS IN MICROPLOTS ON FHB AND DON IN ELEVEN U.S. WHEAT ENVIRONMENTS IN 2010

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ABSTRACT

Our experimental objective was to quantify the relative contribution of within-field corn debris as an inoculum source of *Gibberella zeae* for Fusarium head blight and DON contamination in eleven variable wheat environments in 2010, all in regions where corn is the predominant crop in the agricultural landscape and corn debris is left on the land surface over large areas. Our research is based on the hypothesis that spores of *Gibberella zeae* that are deposited on wheat spikes and that result in Fusarium head blight come primarily from well-mixed, atmospheric populations in an area. The research was conducted in commercial-scale wheat fields in Illinois, Missouri, Nebraska, New York, and Virginia, each following a non-susceptible crop. Locally overwintered, natural corn stalks were collected in spring from each locale by placing a 33 inch diameter plastic 'Hoola Hoop' onto six arbitrarily selected areas in a corn stubble field, and then removing all of the stubble within the hoop and placing it in a paper bag. Replicated (six) microplots containing corn debris or no added debris were set out in each field and were separated by a minimum of 100 ft in each dimension. Debris was secured within the source circles by using cages fashioned of 2 ft high hardware cloth and shaped with the same 33 inch diameter plastic 'Hoola Hoop', fastened with plastic zip-ties, and secured to the soil with metal ground staples. Wheat spikes above each microplot were rated at soft dough stage for FHB incidence, severity, and index. At grain maturity, at least 100 spikes from each microplot were harvested, dried and shipped to Cornell where grain was threshed from a subsample of spikes and sent to the assigned USWBSI Testing Lab for DON analysis. Mature spikes from each microplot were also suface-disinfested and plated on Fusarium selective media to determine the incidence of spikes infected by *G. zeae*.

Characterization of epidemics over the 11 environments differed through the lenses of visual symptom development, incidence of mature spike infection, and toxin contamination. At every location except Chatham, VA, more than 20% of mature spikes were infected by *G. zeae*, regardless of the degree of symptom development at soft dough stage or the level of DON observed. This suggests that post-anthesis infection was quite common across environments in 2010. Based strictly on FHB index at soft dough, we observed one severe epidemic (in Nebraska), five moderate epidemics (in Illinois, Missouri, and Nebraska), and five mild epidemics (in New York and Virginia). However, the high FHB incidence observed in Wilbur, NE was associated with low DON concentrations. On the other hand, three of the moderate epidemics, based on symptoms, were associated with toxin levels above 2 ppm. Mean DON levels in the no-debris microplots were 2.9 ppm in Urbana, IL, 4.4 ppm in Columbia, MO, and 12.2 ppm in Novelty, MO, and there was detectable DON at every site except Chatham, VA. Across the 11 environments, there was significantly (P=0.05) higher DON in grain from corn debris microplots (1.8 ppm) than from no-debris microplots (0.2 ppm) only in Bath, NY. It is especially noteworthy that DON levels were not significantly

higher in corn debris microplots than no-debris microplots in any of the high DON locations, suggesting the predominance of regional atmospheric inoculum in those locations. FHB incidence, severity, or index was not significantly (P=0.05) higher in corn debris-containing than no-debris microplots in any of the 11 fields at soft dough stage. And only at Wilbur, NE did mature wheat spikes from microplots containing locally overwintered corn debris show a statistically significant increase in infection incidence by *G. zeae* over those from microplots with no corn debris.

By inference of our results over two years and 21 winter wheat environments, it appears that elimination of corn debris from single wheat fields in major corn-producing regions may have rather limited benefits in terms of reducing FHB and especially of reducing DON contamination of grain. One caveat regarding this interim conclusion is that the microplot experimental design (small area sources of corn debris) we used may have resulted in an underestimation of the contribution of large area sources of corn debris to wheat infection and DON contamination. Much larger replicated plots will be necessary to definitively assess the quantitative contribution of corn debris to local wheat infection and DON accumulation on an agricultural field scale.

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VALIDATION OF BARLEY DON RISK PREDICTION MODEL

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INTRODUCTION

Fusarium head blight (FHB) of barley is a devastating disease in the U.S. Northern Great Plains and elsewhere. It is caused by the fungus *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe). Losses occur through the blighting of florets, disruption of grain fill, and most importantly through the contamination of grain with trichothecene mycotoxins, primarily deoxynivalenol (DON). Scabby kernels are associated with gushing in beer and therefore DON concentration in grain is used to estimate this risk and crop rejection or severe discounts can be implemented if the level detected exceeds 0.5 parts per million (ppm).

Management of FHB in high-risk regions is currently accomplished with agronomic practices that limit in-field inoculum (e.g. rotation) and fungicide application after spike emergence. While not completely effective, fungicides usually reduce both disease and DON. The application of fungicide is most effective if sprayed prior to infection. Therefore, a need exists for implementing and evaluating an advisory system that predicts the risk of an economic level of DON occurring in a malt barley crop.

OBJECTIVE

Develop and validate a weather-driven disease model predictive of economic DON accumulation in malting barley.

MATERIALS AND METHODS

Field experiments were conducted during the 2005-10 growing seasons using a set of regionally adapted malting barley varieties. At least three varieties, namely 'Conlon' (2-row), 'Robust' and 'Tradition' (both 6-row), were common at all locations. The years 2005-8 were used in model development and 2009 was used in validation. Data from 2010 is pending and will be evaluated at a later date. The incidence (number of diseased spikes/total), severity (number of diseased spikelets/total) of FHB, and deoxynivalenol (DON) concentration (ppm) were determined for each variety at each location*year. A binary response variable, eDON, was created based on whether the mean DON concentration for each variety at every location*year met or exceeded 0.5 ppm. Pearson correlation coefficient was calculated between incidence, index and DON concentration to assess the association between the disease severity and mycotoxin production.

Development of an infection model for FHB. The effect of temperature (*t*) and duration of wetness (*w*) on disease development was modeled using Duthie's modified Weibull function (Eq. 3.2 in Duthie, 1997). Since the disease data in controlled environment was not available for this pathogen in barley, similar data from wheat (Andersen, 1948) were used estimate the parameters in the Weibull function. The Marquardt iterative method of the NLIN procedure in SAS was used to perform the analysis.

Extending this infection model to the field. The infection model developed in the controlled environment based on the disease data from wheat was extended under field conditions. The measures of temperature and relative humidity (RH) during the 10-day interval prior and including the heading day (day at which the crop was at 50% Feekes 10.5) were used. The average hourly temperature (AVGTEMP) and a weighted duration of hours with RH≥90% (WRH90) over the 10-day period were assumed as alternatives to *t* and *w*. The predictor WRH90 was calculated using the formula:

$$\text{WRH90} = \sum_{i} x_i \left[1 + \frac{W_i}{\sum_i W_i} \right]; \quad W_i = \begin{cases} x_i - 8 \text{ if } x_i > 8; \\ 0 \text{ otherwise} \end{cases}$$

where x_i is an instance of uninterrupted duration (h) when RH≥90% and *i* is an indicator to represent such uninterrupted durations in the 10-d interval. In particular, WRH90 prioritizes longer uninterrupted humidity run (RH≥90%) during the 10-d interval.

For each event, Weibull function was calculated in response to AVGTEMP (average hourly temperature) and WRH90 (weighted duration of hours with RH ≥90%) over the 10-d period. The variable obtained was hereafter referred as WEIB_WRH90. The Pearson correlation coefficient was calculated between disease metrics and WEIB_WRH90 to assess the association between disease metrics and weather conditions.

Regression model to predict eDON. A logistic regression model was developed to predict the risk of DON accumulation greater ≥0.5 ppm using the predictor WEIB_WRH90 (De Wolf, et al, 2003). Total prediction accuracy, sensitivity and specificity were calculated. The probability of being a positive eDON (p*) was selected at which the sum of sensitivity and specificity was highest. The model was validated using 11 locations from the year 2009.

RESULTS AND DISCUSSION

Development of an infection model for FHB. The parameter estimates obtained from the PROC NLIN procedure based on controlled environment disease data for wheat-FHB, were significant with narrow confidence intervals (data not shown). The response surface generated by the Weibull function was given in Figure 1.

Extending the infection model to the field. The predictor WEIB_WRH90 obtained from the weather data had a correlation coefficient of 0.58 with FHB incidence, DON concentration and eDON (*p<0.001*). This indicated that the weather conditions prior to heading influenced the disease development as well as DON concentration.

Regression model to predict eDON. The total prediction accuracy, sensitivity and specificity of the regression model to predict eDON were 86%, 80% and 87%, respectively. The cut-off probability (p*) was 0.35 in order to consider the event as positive eDON. This probability is equivalent to a WEIB_WRH90 value of 0.63. In other words, a WEIB_WRH90 of 0.63 indicates the risk of there being ≥ 0.5ppm DON for a barley crop which was heading at that location. The model performed equally efficient on the validation data set obtained from three varieties at 11 locations in the year 2009.

Estimation of barley DON risk in North Dakota for 2010. In general, the locations in the western ND were dry and had low risk during the growing season. In contrast, locations in eastern ND were always at higher risk of economic DON levels. For example, see the comparison between Williston (western North Dakota region) vs. Langdon (eastern North Dakota region) in Figure 2. The risk on adjacent days was generally correlated, indicated the influence of the interval on calculated daily risk.

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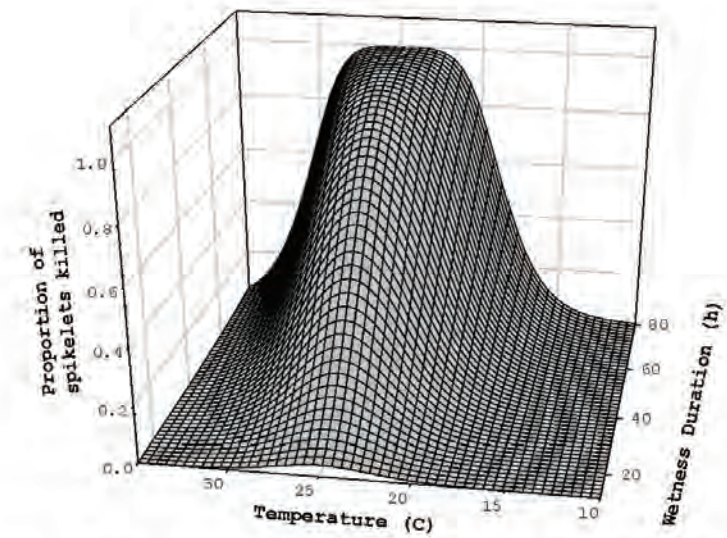

Figure 1: Response surface generated the Weibull function given in Eq. 1. This models FHB incidence in wheat following inoculation and incubation at different combinations of temperature and wetness duration.

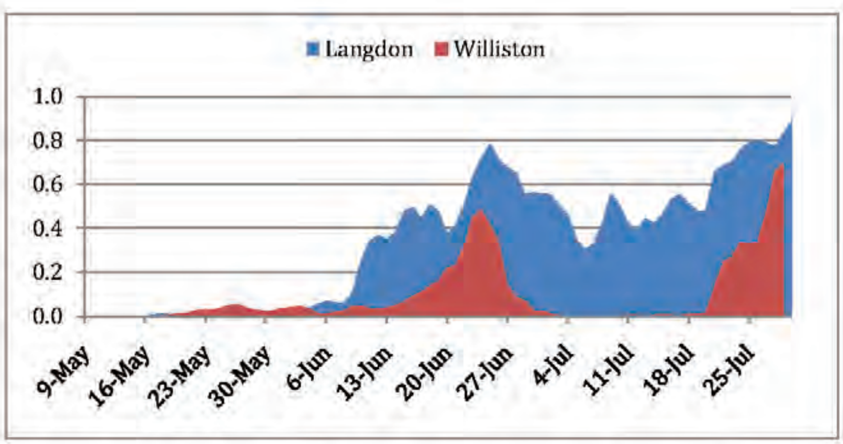

Figure 2: The distribution of the WEIB_WRH90 at two locations in North Dakota between May 10, 2010 and July 31, 2010. The cut-off threshold to classify the day as risk is 0.63.

MULTI-STATE UNIFORM FUNGICIDE EVALUATIONS FOR CONTROL OF FUSARIUM HEAD BLIGHT AND ASSOCIATED MYCOTOXINS

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ABSTRACT

A multi-state research project was conducted across seven states (Arkansas, Illinois, Maryland, Michigan, Minnesota, North Dakota, and South Dakota) and five wheat market classes (durum, hard red spring, hard red winter, soft red winter, and soft white winter) to evaluate: i) experimental, non-registered fungicides for efficacy on Fusarium head blight (FHB) and the associated mycotoxin deoxynivalenol (DON); ii) three application timings of Caramba (metconazole; BASF Corp.) and Prosaro (prothioconazole + tebuconazole; Bayer CropScience) fungicides for control of FHB and DON; and iii) the effect of Headline (pyraclostrobin; BASF Corp.) fungicide on DON. The two experimental fungicides that were evaluated were LEM17 (DuPont Crop Protection) and A9232D (Syngenta Crop Protection). These fungicides were applied at Feeke's Growth Stage (FGS) 10.5.1 and compared to the non-treated control and the standard fungicide treatments Caramba and Prosaro applied at FGS 10.5.1. Out of 17 trials, significant ($P \leq 0.05$) *F*-tests for FHB index were observed in seven trials. The fungicides LEM17 and A9232D significantly reduced the FHB index compared to the non-treated control in five and six of these seven trials, respectively. Out of ten trials in which DON results were available, significant *F*-tests for DON were observed in nine trials. The fungicides LEM17 and A9232D significantly reduced DON compared to the non-treated control in one of nine and three of these nine trials, respectively. Neither LEM17 nor A9232D achieved significantly better control of FHB or DON than Caramba or Prosaro in any of the trials. Caramba or Prosaro fungicides achieved significantly better reduction of FHB index than LEM17 or A9232D in five of seven trials and two of seven trials, respectively. Caramba or Prosaro fungicides achieved significantly better reduction of DON than LEM17 or A9232D in six of nine trials and three of nine trials, respectively. To better understand the width of application window to achieve the best control of FHB and DON, Caramba and Prosaro fungicides were applied at the approximate timings of FGS 10.5, 10.5.1, and five days following 10.5.1. In general, applications of Caramba or Prosaro at FGS 10.5.1 provided the best reductions of FHB and DON; however, in some cases, applications at FGS 10.5 or five days following FGS 10.5.1 provided similar results. When applied at FGS 9, 10, or 10.5, Headline fungicide significantly increased DON over the non-treated control in two of nine trials, two of eight trials, and one of five trials, respectively.

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EVALUATION OF HOST PLANT RESISTANCE AND FUNGICIDE TREATMENT FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Recent fungicide technology has improved control of Fusarium head blight (FHB), caused by *Fusarium graminearum*, in wheat and barley. Fungicides in the demethylation inhibitor (DMI) class have proven to be the most effective in managing FHB, although results are variable. Caramba® (metconazole; BASF) and Prosaro® (tebuconazole + prothioconazole; Bayer CropScience) are currently the most effective fungicides available for reducing FHB and deoxynivalenol (DON) in the U.S.; however, they do not provide complete control. Planting a FHB-resistant cultivar is another management tool for producers. Our objective was to evaluate the effectiveness of two foliar applied fungicides and host plant resistance on suppression of FHB, DON accumulation, yield, and test weight. The experiment was a split-plot design with fungicide treatment as the main plot and variety as the sub-plot, blocked into four replications. An inoculated, mist-irrigated disease nursery was used to test two DMI fungicides, Caramba and Prosaro, and twelve wheat cultivars ranging from FHB susceptible to FHB resistant. Data were collected on FHB incidence, severity, Fusarium damaged kernels (FDK), DON, yield, and test weight. FHB index and incidence/severity/kernel quality index (ISK index) were also calculated. Data were analyzed using PROC MIXED in SAS 9.2. Both fungicide and cultivar had a significant effect on all measured variables. Significant interactions between fungicide and cultivar were detected for FDK and test weight. In individual non-treated plots, FHB incidence ranged from 10% to 100% thereby confirming high disease pressure and varying cultivar FHB resistance levels. Averaged over all cultivars, both Caramba and Prosaro significantly ($P < 0.05$) increased yield and test weight, and significantly lowered FHB index, FDK, and ISK index compared to the non-treated control. No significant differences were found between Caramba and Prosaro for all measured variables. When the cultivars were split into a resistant and a susceptible group, FHB-resistant cultivars significantly ($P < 0.001$) outperformed the FHB-susceptible cultivars, regardless of the fungicide treatment, for all parameters. In the non-treated plots, FHB–resistant cultivars had higher yield (11.2 bu/A) and test weight (5.7 lbs/bu.), and lowered FHB index by 45.2% and FDK by 79% when compared to the FHB-susceptible cultivars. Notably, in the non-treated plots, IL06-13708 yielded significantly ($P < 0.05$) more than Sisson when treated with either fungicide, and Pioneer 25R47 when treated with Prosaro. Also, the most FHB-resistant cultivar, IL02-18228, was the only cultivar to not realize a significant yield increase from the addition of fungicides. This is preliminary data from one year, but based on our results from previous tests, we can conclude that under severe FHB pressure, wheat producers can produce high yields of sound grain by using cultivars with high FHB resistance levels in combination with either Caramba or Prosaro fungicide.

DEFINING THE WINDOW OF SCAB SUSCEPTIBILITY IN MID-ATLANTIC WINTER WHEAT

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ABSTRACT

Previous research suggested that winter wheat is susceptible to FHB infection during the period from 0 to approximately 10 days after mid-anthesis, with the exact growth-stage when FHB susceptibility ends still uncertain. The present study, carried out with Dr. Ruth Dill-Macky of the University of Minnesota, aimed to more clearly establish when and how rapidly the window of wheat susceptibility to FHB infection closes. An inoculated, mist-irrigated field experiment was performed in Raleigh, NC. Plots of the susceptible cultivar P26R12 and the moderately resistant cultivar NC-Neuse, both soft red winter wheats, were inoculated at 0, 7, 9, 11, 13, 15, 17, 19, or 21 days after anthesis (daa) with a suspension of 5 x 10^5 macroconidia/ml of *Fusarium graminearum*. Only one inoculation-date treatment was applied to each plot. All cultivar*inoculation-date treatments were replicated three times. Mist-irrigation was provided for 28 daa to promote disease development. From each plot, spikes were sampled at 14, 21, 28, 35, 42 days after inoculation (dai), and spikes were sampled at harvest ripeness in all plots, in order to assess the effect of infection timing on visual kernel damage, *Fusarium* infestation, and DON contamination**.** Growth stage at each inoculation date was determined by dissecting other sampled spikes, and temperature and rainfall were monitored using a local weather station. A similar experiment was conducted in a greenhouse to compare the field results with those obtained under controlled conditions.

The 2010 field season was characterized by severe drought during the weeks critical to infection and disease development. The experiment is being repeated in another year. Preliminary results are as follows:

1. In the field, FHB visual symptoms were significant (index > 2%) in the susceptible cultivar P26R12 from inoculations at both 30-40% anthesis and kernel watery ripe (the 0- and 7-daa treatments). In the moderately resistant cultivar, NC-Neuse, visual symptoms were only significant (index >2%) from 0-daa inoculations. DON at harvest ripeness was above 2 ppm for both inoculation-date treatments in both cultivars, although barely so for NC-Neuse inoculated 7 daa. This suggests that the window of susceptibility may vary depending on cultivar resistance level, if measured by development of visual symptoms and DON > 2 ppm. It also suggests that in a dry spring, the period of susceptibility ends before 9 daa.

2. In plants inoculated at anthesis, DON reached its maximum level (on a per-seed basis) a week earlier in the S cultivar than in the MR cultivar (at 21 and 28 dai, respectively).

3. The greenhouse data suggest a longer window of potential susceptibility in both cultivars than the field results show. For example, in the greenhouse FDK percentages for both cultivars did not taper off until inoculations were later than medium milk. The prolonged drought during and after anthesis likely shortened the period of susceptibility in the field this year, despite misting, and the field results on duration of susceptibility might be different in a naturally wet spring.

ECOLOGY OF *BACILLUS AMYLOLIQUEFACIENS* ON WHEAT FLORETS IN RELATION TO BIOLOGICAL CONTROL OF FHB/DON

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ABSTRACT

The TrigoCor strain of *Bacillus amyloliquefaciens* is one of a handful of biological control agents (BCAs) that shows potential in the integrated management of FHB/DON. TrigoCor inhibits the growth of *Fusarium graminearum* in antibiosis assays, and has resulted in excellent and consistent reduction of FHB symptoms and DON accumulation in greenhouse experiments. Like other BCAs tested through the USWBSI, TrigoCor has shown inconsistent biocontrol in the field. The goal of our current USWBSI project is to identify strategies for enhancement of biocontrol by elucidating the ecology of interactions between *Bacillus* and *F. graminearum* on wheat florets under controlled conditions as well as under field conditions. Using TrigoCor as a model BCA, we are describing the dynamics of microbial populations and of *Bacillus*-generated antifungal metabolites relative to biological control. We examined populations of hand-sprayed *Bacillus* on wheat heads over critical infection periods in three greenhouse experiments and in two New York locations during the 2008, 2009, and 2010 field seasons. Using dilution plating, we quantified *Bacillus* populations on wheat heads at 0h, 24h, 72h, 7d, and 14d after *Bacillus* application. Although *Bacillus* population levels remained fairly stable on wheat heads in both the field and the greenhouse throughout the sampling period, the quantity of *Bacillus* on wheat heads was one or more orders of magnitude higher in the greenhouse (10^8 CFUs/head) than in both field locations in 2009 (10^6-10^7 CFUs per head), and 2008 (increased over the first week from 10^4 CFUs/head to a constant level 10^6 CFUs/head). In addition to these hand-sprayed field trials, we also quantified population levels on field plots commercially sprayed (20 gal/A, paired Twinjet nozzles facing front and back and aimed 30° from horizontal) with *Bacillus*. The commercially sprayed field plots also had *Bacillus* population levels per head that were two or more orders of magnitude lower than on wheat heads in the greenhouse (10^4-10^6 CFUs/head at 0h and 24h after *Bacillus* application from fields in New York, North Dakota, and Missouri in 2008, as well as throughout a 14d sampling period from a field trial in upstate New York in 2009). Treatment with TrigoCor did not provide significant reductions in FHB in any of the hand-sprayed or commercially sprayed trials in 2008 or 2009. To determine if raising the *Bacillus* population levels on wheat heads in the field would supply better disease control, in the 2010 field season we increased the inoculum concentration and volume applied per head to wheat heads in two NY locations. At 0 and 1d post-*Bacillus* application, the level of *Bacillus* recovered from wheat heads at both locations was comparable to the level recovered from heads in the greenhouse (10^8CFUs/mL), and at 3,7, and 14d post-application the level was lower (10^7CFUs/head) than populations in the greenhouse but still higher than in previous field seasons. At one field location there was a statistically significant decrease in FHB severity (p=0.014), but otherwise treatment with TrigoCor did not provide significant reductions in FHB or DON. The insufficient FHB control of TrigoCor in the 2010 field season, despite the *Bacillus* population numbers on wheat heads being similar to levels on greenhouse heads, suggests that population levels alone do not explain the ability of *Bacillus* to control FHB.

In addition to bacterial population dynamics, we are assessing the production and persistence of antifungal metabolites relative to disease control in greenhouse and field environments. Using a modification of an LC protocol initially developed for monitoring broth components, we monitored the levels of key antifungal compounds present on wheat heads in the greenhouse and in two NY winter wheat fields and one spring wheat field in 2010. In the greenhouse antifungal lipopeptides were present in detectable levels at 0 and 7d post-*Bacillus* application. Conversely, in all three field locations the level of antifungal metabolites on wheat heads decreased quickly by 3d post-application, and was barely detectible by 7d post-application. It is likely that the inadequate persistence of antifungal metabolites on wheat heads in the field is an important factor limiting disease control, particularly because these metabolites are believed to be the primary mode of action of *Bacillus* biocontrol agents.

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ADVANCES IN THE DEVELOPMENT AND APPLICATION OF PREDICTION MODELS FOR FHB AND DON

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ABSTRACT

A multi-state collaboration is progressing in efforts to develop the next generation of prediction models for Fusarium head blight (FHB) and the mycotoxin deoxynivalenol (DON). The next generation of predictive models is currently under development and will target both disease epidemics and unacceptable levels of DON contamination. The current versions of these prediction models range in accuracy from 75-78% based on the data used to develop and test the models. The prediction models are delivered for public use via the Fusarium Head Blight Prediction Center (www.wheatscab.psu.edu). This web-based tool delivers daily estimates of disease risk to 25 states where FHB has been a recurring problem. The risk of a FHB epidemic greater than 10% FHB index is presented as a risk map within the prediction tool. The risk maps are based on hourly weather inputs from the Real Time Mesoscale Analysis (RTMA), and have a spatial resolution of 5 km throughout the area covered by the prediction effort. The system also incorporates observations from weather stations associated with agricultural weather networks in cooperating states. This information provides an independent confirmation of the disease risk. A state commentary describing the risk of disease is provided along with the risk maps as an added feature of the web-base tool. This text commentary is intended to help growers of farm managers integrate multiple sources of information and accurately evaluate the local risk of FHB. In 2010, state commentaries were distributed by an FHB Alert System hosted by the USWBSI, which sends the information via e-mail and cellular phone text messages, notifying users of potential changes in FHB risk. Surveys evaluating the use and value of the prediction models and the FHB Alert System are underway.

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INTEGRATED MANAGEMENT FOR FUSARIUM HEAD BLIGHT IN WISCONSIN

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ABSTRACT

As part of the USWBSI coordinated integrated management trials, a field study was conducted in 2009-2010 at the Lancaster Agricultural Research Station (Lancaster, WI) to examine the effect of fungicide and wheat cultivar on development of Fusarium head blight (FHB), DON, and grain yield. The trial was established into previous crop wheat in a randomized complete block with a split-plot arrangement. Fusarium head blight was allowed to develop naturally. The whole plot was fungicide (UTC; Proline applied 6.5 oz/A at Feekes 10.5.1; and Proline applied at 3 oz/A + Folicur applied at 3 oz/A at Feekes 10.5.1) while the subplot was wheat cultivar. Kaskaskia and Truman represented moderately resistant cultivars, IL01-11934 and PIP720 represented moderately susceptible, and LW 860 and LW 863 represented susceptible cultivars. Data collection included: (i) pre-fungicide and post-fungicide spray application foliar disease assessments (0-10 scale), (ii) incidence and severity of FHB at soft dough, (iii) grain yield, and (iv) percentage Fusarium damaged kernels (FDK). Samples from each plot also had a grain sample submitted for DON testing. Data were subjected to ANOVA using PROC MIXED and mean separations were based on Fisher's protected LSD (5%). Effects of fungicide were noted for FDK percentage ($P = 0.0109$), with levels lower for either applications of Proline (7.8%) or Proline + Folicur (7.4%) compared to the UTC (11.5%). There were differences in cultivar for the post-fungicide application disease rating ($P = 0.0045$), with the lowest rating for Truman, and also FHB incidence ($P = 0.0109$), with the highest log-percentage for the two susceptible cultivars (LW 860 and LW 863). Lastly, there was a fungicide x cultivar interaction ($P = 0.0446$) on grain yield. Lowest yields were found for Kaskaskia-UTC (73 bu/A), Truman-UTC (78 bu/A), and IL01-11934-UTC (81 bu/A). Overall, these results indicate that the use of multiple management tactics, including cultivar and fungicide, are needed to effectively reduce the risk of FHB.

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RESULTS OF THE 2010 UNIFORM FUNGICIDE TRIAL ON BARLEY, FARGO, ND

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OBJECTIVES

To evaluate the disease, yield, and quality parameters achieved by: applying two registered triazole fungicides at three separate growth stage timings; applying two non-registered fungicides at early full head emergence; and applying a mid-season leaf application of a QoI (strobilurin) fungicide.

INTRODUCTION

North Dakota participates in the USWBSI sponsored uniform fungicide trials of the FHB Management research area, with evaluations on spring barley, as well as on hard red spring wheat and durum wheat. In barley, almost all malting approved cultivars are susceptible to Fusarium head blight (FHB), and deoxynivalenol (DON) levels must meet the <0.5ppm standard required by the US malting and brewing industries. Because cultivar resistance is not available in currently approved malting cultivars, fungicides have been examined as a tool to help reduce FHB and DON. Research in ND has shown the superior efficacy of certain triazole fungicides in barley in reducing DON levels (3,4). These products, prothioconazole (Proline), prothioconazole + tebuconazole (Prosaro), and metconazole (Caramba) were only recently labeled in the US (2). Although these three products have proven to have the greatest efficacy of registered products, they are all triazoles, and another chemistry or combination of products may also have high efficacy, if available. Also, previous work has shown the potential of DON levels rising above the untreated check with late season leaf and heading application of QoI (strobilurin) fungicides in wheat (1), and this effect needed to be further examined in barley. Uniform trials on barley in 2009 in ND indicated that application of a strobilurin fungicide at boot stage (Feekes 10) and at head half emerged (Feekes 10.3) resulted in DON levels equal to the untreated check (data not published).

MATERIALS AND METHODS

Plots of 'Tradition' spring barley were planted at the Fargo Agricultural Experiment Station on April 20, 2010, into barley stubble that had been chisel plowed twice prior to planting. Treatments in the plot area were in a randomized complete block design. The plot area was fertilized for an 80 bu/acre yield potential. Herbicides were applied at the five leaf stage, to control grassy and broadleaf weeds. Plants were grown to maturity and harvested with a Kincaid small plot combine, on Aug. 3, 2010.

Fungicide treatments: Fungicides were applied according to the protocol established for 2010 by the FHB Management Area – Uniform fungicide trials. Because spring barley in ND flowers in the boot prior to head emergence, growth stages of application were adjusted slightly from wheat, to reflect barley growth and that FHB infections occur in barley after flowering, with head emergence.

Nine fungicide treatments were applied (Table 1). Two registered products, Prosaro (prothioconazole + tebuconazole) from Bayer, and Caramba (metconazole) from BASF, were applied at three growth stages, head half emerged (Feekes 10.3), head fully emerged (Feekes 10.5) and kernel watery ripe (Feekes 10.54). Two unregistered products also were tested and applied at Feekes 10.5. LEM 17 is a penthiopyrad chemistry from DuPont, and A9234D is a mixture of difenoconazole + tebuconazole, from Syngenta. Headline (pyraclostrobin) from BASF, a Quinone outside inhibitor (QoI) fungicide, also was applied at Feekes 9 to see if mid-season application of this product would result in DON levels greater than the untreated check. All fungicide applications applied to grain heads were applied with a CO2 backpack-type sprayer equipped with XR8001 flat fan nozzles angled 30 degrees from the horizontal in 18 gpa, at 40 psi. The Headline application was applied with XR8002 flat fan nozzles oriented straight down.

Inoculum application: On the evening of the Feekes 10.5 fungicide applications (which were applied in early morning hours), inoculum of *Fusarium graminearum* was applied with a CO_2 backpack-type sprayer, equipped with XR8001 flat fan nozzles angled 30 degrees from the horizontal, in 30 gpa. The spore concentration was at 100,000 spores/ml, delivering 250 ml solution/plot.

Disease ratings and DON determinations: FHB ratings and leaf disease ratings were taken at soft dough kernel stage, the third week of July (Table 1). Disease severity was fairly low in the plots, as no rainfall had occurred from the time of spore inoculation until shortly after disease ratings occurred. Once grain was mature, the plots were harvested and grain was cleaned once with a Clipper mill prior to weighing to determine yield and test weight and grinding grain for DON determination. DON levels were determined by the NDSU Veterinary Toxicology Lab using gas chromatography and electron capture techniques. Disease and yield and quality parameters were analyzed using ANOVA at P = 0.05.

RESULTS AND DISCUSSSION

Net blotch ratings, FHB field severity and DON levels were low, but significant differences among treatments did occur (Table 1). Net blotch was significantly reduced by all fungicide treatments. FHB incidence was significantly reduced by all treatments but Headline applied at Feekes 9, a too early of an application to expect any FHB reduction. FHB field severity was reduced by the Prosaro treatments applied at Feekes 10.5 and 10.54 and the Caramba treatment at Feekes 10.54, while applications of either product were not as efficacious when applied at Feekes 10.3. The two unregistered products, LEM 17 and A9232D, were not as efficacious as Prosaro or Caramba at the Feekes 10.5 application timing, in reducing FHB field severity or DON. DON levels were very low in the study, most likely because FHB did not develop well, even with inoculations, due to dry conditions and high winds that occurred at this site for four weeks following inoculation.

Although disease levels recorded were relatively low, all fungicide treatments significantly improved barley yield, with the greatest yield increase (12 bu > than untreated) with the Prosaro treatment applied at full head emergence (Feekes 10.5). This bushel increase would have more than paid for the cost of the fungicide and application costs, if malting barley received $3.00/bu. Test weights were not significantly impacted by any fungicide treatment.

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Table 1. Fungicide treatments, disease ratings, and yield and quality parameters of the 2010 uniform fungicide trial on 'Tradition' spring barley, Fargo, ND.

Treatment[a]	Feekes application stage	Rate/ Acre	Net Blotch % severity Flag – 1	FHB[b] Incidence %	FHB[b] head severity %	FHB[b] Field severity %	DON ppm	Yield Bu/acre	Test wt. Lbs/bu
Untreated			11.1 a	42.2 a	2.3 a	1.0 a	0.35 ab	95.5 d	46.3 a
LEM 17	10.5	24 fl oz	2.4 c	30.1 bc	2.1 a	0.6 abc	0.45 a	101.6 c	46.6 a
A9232D	10.5	7 fl oz	2.7 bc	26.7 cd	1.9 a	0.5 bc	0.33 ab	104.5 abc	46.8 a
Prosaro	10.3	6.5 fl oz	2.1 c	26.7 cd	2.3 a	0.6 abc	0.20 b	106.3 ab	46.4 a
Prosaro	10.5	6.5 fl oz	3.2 bc	14.3 c	1.8 a	0.3 c	0.20 b	107.8 a	46.3 a
Prosaro	10.54	6.5 fl oz	2.1 c	22.2 cde	1.9 a	0.4 c	0.20 b	103.3 bc	46.7 a
Caramba	10.3	13.5 fl oz	5.1 b	20.0 cde	2.6 a	0.5 bc	0.38 ab	106.2 ab	46.4 a
Caramba	10.5	13.5 fl oz	3.6 bc	24.6 cde	2.0 a	0.5 bc	0.20 b	104.9 abc	46.3 a
Caramba	10.54	13.5 fl oz	3.7 bc	19.0 de	2.1 a	0.4 c	0.20 b	106.6 ab	46.9 a
Headline	9	6 fl oz	2.3 c	37.8 ab	2.2 a	0.9 ab	0.43 a	101.2 c	46.5 a
LSD (P = .05)			2.7	11	0.8	0.4	0.21	8.4	1.3
Standard Deviation			1.6	6.4	0.5	0.3	0.1	5.7	0.9
CV			40.9	24.2	25.4	39.7	48.5	4.9	2.0

Means followed by same letter do not significantly differ (P = 0.05)

[a] Lem 17 = penthiopyrad (DuPont); A9232D = difenoconazole + tebuconazole (Syngenta); Prosaro = prothioconazole + tebuconazole (Bayer); Caramba = metconazole (BASF); Headline = pyraclostrobin (BASF); Induce nonionic surfactant was added to each fungicide treatment at rate of 0.125% v/v;

[b] FHB = Fusarium head blight; Incidence = % tillers with symptoms; head severity = % of kernels showing symptoms; Field severity = (incidence x head severity)/100; DON = deoxynivalenol

THE RECOVERY OF RELEASED CLONES OF *GIBBERELLA ZEAE* FROM WINTER WHEAT AND BARLEY IS INFLUENCED BY THE AMOUNT OF LOCAL CORN STALK RESIDUE

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ABSTRACT

The amount of corn residue remaining in winter wheat and barley fields may influence Fusarium head blight (FHB) incidence, severity, and deoxynivalenol levels. We hypothesized that the recovery of a released clone of *G. zeae* would increase as inoculum levels (i.e., corn residues) within a field increase. Clonal isolates of *G. zeae* containing unique alleles relative to background populations were released in two wheat fields (2009 and 2010) and two barley fields (2008 and 2009) in Virginia. Small plots of approximately one meter in diameter were placed within the fields with varying amounts of corn residue. Wheat and barley spikes were collected, observed for symptoms of FHB, disinfested, and plated onto *Fusarium*-selective medium. Approximately 1,400 isolates of *G. zeae* were recovered, and amplified fragment length polymorphisms (AFLPs) were used to determine the recovery of the released clones to FHB. The presence of larger amounts of *G. zeae*-infested corn residue contributed more to recovery of the clone in years when environmental conditions were low or moderately favorable to FHB than when conditions were highly favorable for infection. Recovery of the released clone was never observed to be 100%, thus confirming the presence of atmospheric (background) sources of inoculum in all years. Knowledge of the inoculum potential of existing corn residues will enable producers to make informed decisions regarding management of FHB.

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DATA MINING OF WEATHER AND CLIMATIC DATA TO IMPROVE RISK PREDICTION OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB) of wheat has been known to be a sporadic disease for at least 80 years. Intensity of the disease and concentration of DON in harvested grain vary tremendously from year to year and from location to location. The uncertainty in FHB and DON levels greatly complicates the development of efficient management tactics and the recommendations made in a given location-year for FHB control. Variation in environmental conditions is considered one of the primary reasons for the sporadic nature of the disease. In particular, several authors have shown that environmental conditions around the time of wheat flowering—about the time that infection occurs—are correlated with disease intensity and DON. Measurements of atmospheric moisture or wetness (such as average relative humidity [RH]), or combinations of moisture and temperature (e.g., hours of high RH with temperature between different thresholds) have been found to be significantly related to the risk of FHB. However, the risk of FHB can, in principle, be a function of environment at any time during the growing season. For instance, late season (post-flowering) conditions can affect spike colonization and DON production, and conditions during the months before flowering can affect perithecia maturation and ascospore production. Conditions during the winter can affect overwintering of the pathogen in infested debris, and conditions during the previous year can affect the magnitude of pathogen infestation of plant debris. The key issue is whether or not the relationship between environment at a particular time and disease/DON risk at harvest is strong enough to provide useful information for prediction purposes.

Three specific questions can be addressed. (1) Which environmental variables (or combinations of variables) are the best predictors of risk? (2) What are the time windows (both in terms of starting or ending times, and the duration of the windows) when there are high correlations between environment and risk? (3) What other (confounding) factors (such as wheat type, cropping system, location, etc.) affect the environment-risk relationships? Various data-mining techniques can be used to address these questions when data sets over multiple years or multiple locations are available. Window-pane analysis is one specialized data-mining procedure that has been successfully used for the environment-risk problem for different plant diseases, including FHB (Kriss et al. 2010. Phytopathology 100: 784-797). This approach to data analysis will be demonstrated for both published and unpublished results from the USA and Europe to show that FHB risk is associated with moisture-related variables for several time windows. The results can contribute to the development, refinement, or modification of prediction models for FHB.

DETERMINANTS OF ADOPTION OF SCAB MANAGEMENT TECHNIQUES

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OBJECTIVES

To understand how farm management practices and technology delivery systems affects the selection of scab management techniques. Specifically, to detect and estimate the effect of these factors on choice of the number of technologies wheat farmers in North Dakota and Minnesota adopt for scab management. Our analysis aims to provide insights into the role played by social and farm-specific factors in the scab management portfolio adoption.

INTRODUCTION

Fusarium head blight (FHB), commonly known as scab, is a disease affecting all classes of wheat and other small grains. This fungal disease rapidly destroys a crop within a few weeks of harvest. Physical damage from scab is multifold: reduced yields; discolored, shriveled kernels; contamination with mycotoxins, and reduction in seed quality, leading to economic loss. These losses suggest the need for management practices which reduce losses from scab. Common scab management practices include planting varieties which have resistance to scab; cultural practices, such as crop rotations within the same crop field or planting varieties with a range of flowering dates; and chemical application, such as fungicides (McMullen, Jones, and Gellenberg, 1997).

MATERIALS AND METHODS

Variables, Assumptions, Statistics: Following Lohr and Park (2002), we constructed a dependent variable, the number of adopted scab management practices, based on response to the question "Which of the following do you consistently practice in order to reduce the amount of damage by scab?" Respondents select from five techniques for managing scab. These were (1) grow resistant varieties, (2) apply a recommended fungicide at heading, (3) rotate so that I never grow wheat immediately following another small grain or corn crop, (4) grow varieties that differ in flowering dates, and (5) stagger planting dates so that not all fields flower on the same date. The respondent was free to select as many as they believed applied to their situation. We left the interpretation of "consistently" to the mind of the respondent. We avoided making assumptions about the best combination of management techniques. Hence, any combination of one, two, three, four, or five techniques were regarded similarly for purposes of analysis as any other combination of one, two, three, four, or five techniques. A farmer is assumed to be an adopter of only one combination of techniques.

We expect that economies of scale will hold in scab management in wheat production. Farmers with larger operations, in terms of acreage, will attempt to reduce long-run average costs. We also expect variations in management practices based on ownership structure. We assume farmers who own greater fractions of the land they cultivate may have different scab management preferences from those who rent their land. We constructed the variable *land* as the product of the response to the categorical variable describing average annual wheat acreage between 2006 and 2009, and the categorical variable describing the percentage of cultivated land owned by the producer. To more precisely control for the effect of scab management on owned wheat acreage, we also constructed the variable *owned wheat* as the product of the categorical variable describing the number of acres farmed, percentage of cultivated land owned, and the range of farmed acres planted as wheat.

The availability of information sources describing new scab management practices may also be a constraint for selecting best scab management practices. It may be that information sources that are the most familiar to the farmers have the most influence on adoption. We asked respondents to indicate their level of use of two categories of information sources. Firstly, professional groups which provide expertise on scab management practices. Secondly, university extension resources and professional resources which provide scab management expertise. We constructed the variables *extension* and *prof* as the sum of dichotomous responses to questions about use of these sources.

We also assume the size of the farm workforce, time spent by the decision maker on the farm, and experience with farming affect the number of techniques selected to manage scab. We inquired as to the number of employees on the farm, the level of schooling of the producer, and whether the respondent earned a degree in an agriculture-related. A combination of the last two farmer characteristics was combined into the variable *wise*, which was constructed by the product of the questions "What is your highest level of education" and "how many years have you been farming."

We assume the number of scab management techniques would depend on the number of benefits producers expect to obtain from using them. Observations of the variable *benefits* are the total number of expected benefits respondents selected from the following: increased yield, increased profitability, reduced discounts at the elevator. Alternatively, farmers could indicate their belief that benefits of scab management techniques do not justify their cost.

Lastly, we expect variation in geographic characteristics will affect the choice of scab management techniques, but not necessarily the number of techniques. North Dakota and Minnesota have variations in climate, crop production practices, and support infrastructure for various scab management techniques.

Our assumed relationship between the number of scab management techniques selected by producers is: probability of selecting a number of techniques = *f*(*land, owned wheat, extension, prof, workforce, wise, benefits, region*). The relationship between the number of techniques selected and the explanatory factors was statistically estimated using multivariate logistic regression. An iterative process was used to eliminate factors not statistically significant.

Data collection: The data used to estimate this relationship were obtained from a postal questionnaire sent to North Dakota and Minnesota wheat growers. The survey was sent to 5150 wheat producers in North Dakota and Minnesota USA. The sample of producers was drawn from the National Agricultural Statistics Service (NASS) list of North Dakota and Minnesota wheat producers which have at least 100 acres of wheat. NASS mailed the questionnaires. Completed questionnaires were mailed by respondents directly to the NASS North Dakota Field Office. All completed questionnaires, with no identifying information, were given to the project scientists at North Dakota State University. We received 1092 responses of which 1038 are usable.

RESULTS AND DISCUSSION

Our dependent variable is the intensity of scab management technique adoption, measured as the number of scab management techniques adopted. Survey respondents adopted an average of 2.6 techniques. The least number of techniques adopted by any grower was one and the most was five. The largest percentage of farmers (35.3%) used three techniques; 28.3 percent adopted two. About 7.7 percent adopted five techniques.

The most commonly adopted technique was use of variety resistance, with 81.5 percent of respondents; the least commonly used was staggering planting dates, with 21.6 percent percent of respondents. There were 28 observed combinations of techniques. The top four combinations were use of variety resistance, fungicides, plus crop rotation (26.4% of sample). Use of variety resistance plus crop rotation was reported by 13.4 percent of the sample. Among five management strategies, four combinations accounted for 56.0 percent of all responses.

The relatively low adoption rate for the remaining 24 combinations suggests general agreement on effective combinations of techniques.

In this sample, the largest farms were larger than 5000 acres, the smallest were less than 1000 acres, and the average size farm of respondents was between 2001 and 3000 acres. The fraction of land planted as wheat during between 2006 and 2009 ranged from between 25 and 50 percent to more than 75 percent, with the mean response being between 25 and 50 percent. Average annual wheat acreage during this period was between 500 and 1000 acres in our sample.

Respondents also indicated use of scab management information sources: 69 percent used university extension resources at least once every three years; 64 percent used some sort of professional resources; and 49 percent used both. In ranking of the importance of information sources, of the 563 complete responses to this question, extension resources were ranked highest by 72 percent; the professional resources category was ranked highest by 20 percent. Respondents indicated crop consultants were the most important single professional source of information on scab management. Publications prepared by the extension service and extension meetings featuring scab management techniques were the second and third most important media sources used among respondents.

Respondents indicated they use the internet regularly. 78 percent indicated their internet connection speed was best described as "high speed internet access." They also indicated they use their internet connections to do 2.4 tasks, on average, of those provided, with e-mail being the most common task. About 75 percent indicated they do one or more of the following: read blogs, Twitter, Facebook, YouTube, or listserv messages to their cell phone. The media resources category was ranked their most important source of information about scab management techniques by 7 percent. Television programs or advertisements were the source ranked the least important sources of information in the sample.

About 94 percent of the respondents described themselves as farming full time or having full-time employees. Experience in farming averaged between 31 and 40 years). About 36 percent completed a four-year college degree or more, and 50 percent of these obtained a degree in an agriculture related field.

Farmers described benefits they expected to obtain from adopting scab management techniques. The most common desired benefit from adopting a scab management program was increased yield, but 90 percent expected more than one benefit and 11 percent indicated they did not believe the benefits justified the cost of adopting a scab management technique.

Respondents identified the county in which they principally plant wheat. Responses were divided into three regions, the Red River Valley (what counties), central ND, and MN, with 33 percent of farmers grow wheat in the RRV, 31 percent in central ND, and 36 percent in MN. The variable *region* is the observed value of these regions.

The statistical analysis of the relationship between number of techniques used and the determinants of the number of techniques showed that, relative to no techniques being used, land ownership attributes, planting wheat on owned land, use of the university extension service for information about scab management techniques, and expectation of benefits from technique use are significant determinants of the number of scab management techniques adopted. Five models were estimated, one for every possible number of techniques adopted.

The statistical results suggest four overall results. First, the intercept, *land*, *owned wheat*, *extension*, and *benefits* are significant predictors of the number of scab management techniques used. Second, these variables are primarily significant when two, three, or four techniques are selected. This indicates that additional information has no value when one technique is being used or all techniques are being used. It also indicates that the effect of land ownership and use practices are of decreasing importance as two, three, or four techniques are used, respectively.

Estimated Coefficients of Logistic Model:

	One technique	Two techniques	Three techniques	Four techniques	five techniques
Intercept	0.4921	*2.5000***	*3.3531***	*2.5203***	**0.8191***
	1.3727	*0.4020*	*0.3736*	*0.3621*	**0.4245**
Land	-0.1577	*-0.1895***	*-0.2379***	*-0.1555***	-0.045
	0.3883	*0.0759*	*0.0690*	*0.0626*	0.0717
Owned wheat	0.0157	*0.0499***	*0.0437***	*0.0396***	0.0143
	0.1398	*0.0267*	*0.0246*	*0.0227*	0.0262
Extension	-0.4266	*-0.4116***	*-0.3519***	*-0.1779***	-0.1128
	0.3845	*0.0856*	*0.0768*	*0.0735*	0.0866
Benefits	**-1.8179***	*-0.1299***	*-0.1165***	-0.0203	-0.0131
	1.038	*0.0571*	*0.0493*	0.0447	0.0531

** indicates statistical significance with 95% confidence
* indicates statistical significance with 90% confidence
Numbers in small type are standard errors.

We also note the negative signs of the *land*, *extension*, and *benefits* variables. This indicates that as the value of these variables increases, the odds ratio for preferring two, three, or four techniques relative to zero techniques declines. This suggests that the effect of land ownership, use of extension information, and increasing number of expected benefits is to dissipate the value of any one technique for scab management. In other words, expecting one more benefit or using more information from the university extension service when using two, three, or four techniques already, tends to make the producer prefer to concentrate their benefits into a smaller number of techniques, allowing them to take maximum advantage of the benefits from any one technique. An extension of this result is that the effect of information provided by the university extension service tends not so much to increase the number of techniques used to manage scab. Instead, it encourages producers to select a narrow set of techniques.

We also note the positive sign on the *owned wheat* variable. This suggests that as the fraction of owned acreage is planted in wheat increases, producers view additional techniques as a source of loss prevention in assets for which they bear the risk of that loss, instead of being able to share that loss through a rental contract.

Finally, we note that farm workforce size, producer education and experience, and regional differences in production were not statistically significant in this model.

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INOCULATION TIMING, MIST DURATION AND ISOLATE EFFECTS ON FHB AND DON IN TWO DURUM CULTIVARS

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ABSTRACT

We continue to do greenhouse investigations on inoculation growth stage, moisture duration, and *Fusarium graminearum* isolate genotype effects on Fusarium head blight severity (FHB) and deoxynivalenol (DON) levels in spring grains. The most recent work has concentrated on these effects in durum wheat. Our previous research on hard red spring wheat and that of others on winter wheat have shown that FHB severity and DON increases as duration of post-flowering misting increases. However, our previous greenhouse studies showed that inoculations at growth stages before full head emergence did not result in substantial disease or DON, regardless of mist period. Also, our recent greenhouse studies with hard red spring wheat indicated that inoculations with a 3ADON trichothecene genotype isolate resulted in higher DON than inoculation with a 15ADON isolate, but differences were only significant in the susceptible spring wheat cultivar (abstract presented at NC Division Meeting of the American Phytopathological Society, Rapid City, SD, June, 2010). The study reported here evaluated: three inoculation timings, early flowering (Feekes 10.51), kernels watery ripe (Feekes 10.54) and early soft dough (Feekes 11.2); three mist periods of two, five or 10 days; and two *F. graminearum* isolates for inoculation, a 3ADON and a 15ADON trichothecene genotype - all on two durum cultivars (Monroe = susceptible; Divide = moderately resistant). The data analyses were over a total of three trials per cultivar, all with 18 treatments. Results indicated that:

- the 10 day mist periods gave the highest FHB index and DON levels, regardless of inoculation timing, isolate genotype, or cultivar;
- inoculations at Feekes 10.51 or Feekes 10.54 gave higher FHB and DON values than inoculations at Feekes 11.1, over cultivars, isolate type, or cultivar;
- the 3ADON isolate produced significantly higher DON than the 15ADON isolate only in the susceptible Monroe cultivar, not in Divide;
- the Monroe cultivar had approximate 2.5 times greater FHB index and approximately 2 to 3 times higher DON than the Divide cultivar, when averaged across inoculation stages and mist durations;
- DON levels significantly correlated with the FHB index (r = 0.6370; P=0.0001), across all treatments.

POPULATIONS OF *BACILLUS* STRAINS APPLIED TO WHEAT HEADS FOR BIOLOGICAL CONTROL OF FHB: RESULTS OF BROOKINGS, SD 2010 FIELD PLOTS

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ABSTRACT

After spray application of biological control agents (BCAs) onto grain heads for FHB control, assaying numbers of BCAs on the inoculated grain heads is important for understanding how the BCAs colonize and grow on plant surfaces, and how they might help control FHB. We have focused our research on *Bacillus* strains 1BA and 1D3 for use as BCAs to control FHB, as well as a mutant strain 1BAC. Our hypothesis was that the population counts of *Bacillus* strains (1BA, 1D3 and 1BAC) fluctuate over time on the sprayed wheat heads (Feekes stage 10.51). After application of BCAs on the wheat heads, sampling was done every three days for 24 days. In the 2010 biocontrol trials conducted at Brookings, SD the treatments with strains 1BA and 1D3 used the most probable number (MPN) method employing high temperature and high salt selection in the MPN growth media, while treatments with the antibiotic-resistant mutant 1BAC used rifampicin in growth media to track mutant numbers. The control plots that did not receive spray application of BCAs had very low bacterial numbers, indicating that a small number of native bacteria can tolerate the high salt and temperature conditions and/or the rifampicin antibiotic. The plots inoculated with BCAs had detectable numbers of BCAs, with highest counts being about 1.5 X 10^4 CFU/g fresh weight plant mass. The population counts of BCAs on wheat heads fluctuated between the sampling days and treatments. In most of the treatments at Brookings, the vegetative cell count of BCAs fluctuated between the sampling days of different treatments, and over time in the same treatment. In the heat pasteurized MPN assay of most treatments, the endospore counts did not increase appreciably till sampling day 21. The treatment 1BA with plant oil + Chelated Mn + Induce NIS showed higher population counts in comparison to other treatments.

It was clear that the BCA *Bacillus* strains that were sprayed onto heads were able to colonize and sustain detectable populations, and were not washed entirely off plant surfaces despite the excessive rainfall amounts in summer of 2010.

PROCEDURE TO ISOLATE AND IDENTIFY *FUSARIUM GRAMINEARUM* IN CEREAL SEED AND PLANT TISSUES WITH THE AID OF A SELECTIVE MEDIUM

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ABSTRACT

Identification of *Fusarium* species using compound microscope is time consuming and laborious. The goal of this research was to find a method to accelerate and simplify the procedure for isolating and identifying *Fusarium graminearum*. First, we developed a selective agar medium on which only this fungus produce a red pigmentation. We confirmed the sensitivity and specificity of the developed *Fusarium graminearum* agar (FGA) by observing the reaction of 115 strains representing 14 *Fusarium* species. FGA medium was very specific since all and only isolates of *F. graminearum* (35/35) produced the characteristic red colour. Significantly, none of the 15 isolates of *F. pseudograminearum* produced a red colour, permitting the separation, without molecular techniques, of this morphologically identical species from *F. graminearum*. In a second step, for detecting and enumerating *F. graminearum* in cereal seed, we tested a procedure utilizing first peptone-pentachloronitrobenzene agar (PPA), a semi-selective medium to isolate *Fusarium* spp., then the FGA. To validate the method, eight laboratories were each sent two wheat and one barley sample, prepared media, and detailed instructions. The protocol consisted of first placing 200 surface disinfested seeds per sample onto PPA, then, after 7 days at 25°C in the dark, transferring mycelium from each typical *Fusarium* colony to FGA. After another 7 days, colonies that formed a red zone from the point of inoculation on FGA were counted as *F. graminearum*. Mean numbers of *F. graminearum* obtained from the laboratories were 33 ±5, 5 ±3, and 4 ±2 for samples 1 to 3 respectively, confirming the reliability of the procedure. These results were similar to the ones found with the standard method of plating seeds onto PDA followed by microscopic observation. Moreover, in one lab, all *Fusarium* colonies from PPA were also transferred to PDA, then microscopically examined. All 47 red colonies on FGA were confirmed as *F. graminearum* on PDA. In a third trial, the method was tested to isolate *F. graminearum* from cereal crowns. Segments of the crown from 1743 barley plants were placed onto PPA. After 7 days, the 1520 colonies of *Fusarium* spp. that developed were transferred to FGA. To validate results, the 195 colonies that produced a red zone on FGA were transferred to PDA where *F. graminearum* was identified by microscopic examination. *F. graminearum* was confirmed for 184 of the 195 red colonies obtained from crowns for a precision of 94%. These results proved that PPA/FGA method reliable for detection and enumeration of *F. graminearum* in cereal seeds or crowns without the need for microscopic examination or extensive training. FGA alone could be used to identify *F. graminearum* isolates among other Fusaria, and it could be a very useful alternative to PCR for distinguishing *F. pseudograminearum* from *F. graminearum*. Our method is a reliable and labour-saving tool for research on Fusarium head blight (FHB) and seed health testing.

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INFLUENCE OF ROW SPACING, SEEDING RATE, FUNGICIDE AND VARIETY ON YIELD AND FHB DEVELOPMENT IN SPRING WHEAT, DURUM AND BARLEY

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ABSTRACT

Research was conducted at three locations in 2010 to determine the effect of row spacing, seeding rate, variety, fungicide and their interactions on FHB development and grain yield of bread wheat, durum and barley. Experiments consisted of a factorial combination of three row spacings (seven inches, 14 inches, and paired rows with 7 inches between rows and 14 inches between pairs), two seeding rates (recommended and 1.5 times recommended), two varieties within each crop (most resistant and non-resistant check), and fungicide (Prosario™ applied at the recommended stage, and no fungicide applied). In durum, FHB field severity was significantly reduced by fungicide and variety (Divide less than Alkabo), but was not affected by row spacing or plant population. Yield increased by 15% when fungicide was applied. The seven inch row spacing had superior yield to the other two spacings, and Divide was higher yielding than Alkabo. No other factors or interactions were significant for yield in durum. In bread wheat, FHB field severity was not reduced by fungicides but DON was and both FHB field severity and DON were less in Glenn (0.2 ppm DON) compared to Sampson (2.1 ppm DON). Yields were significantly affected by fungicide, seeding rate, plant spacing and variety. In barley, fungicide and the higher seeding rate reduced DON levels. Furthermore, ND20448 had less DON than Tradition, regardless of spatial arrangement and seeding rate. Yield was reduced when row spacings were widened. Tradition was consistently higher yielding than ND20448 across all other treatments. These data suggest that seeding rate (except maybe in barley) and row spacing have little impact on FHB and DON development, but are either neutral (in the case of seeding rate), or have a negative impact on yield. Altering these practices, therefore, does not appear to offer any improvement over the existing practices, with regards to FHB control and yield. Fungicide applied at flowering and the use of resistant varieties offered the best disease control. In the studies reported here, there was no variety by fungicide interactions. Fungicide improved yields across all small grain crops. The variety with the least FHB, however, was not always the highest yielding, complicating variety selection for growers wishing to produce the highest yield with the least FHB damage.

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2010 TRIAL FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium Head Blight (FHB, or scab) is still a potential problem for wheat and barley producers in South Dakota. The objective of this study was to continue evaluating the efficacy of selected biological control agents (BCAs), alone or in combination with fungicide that can suppress different measures of FHB under South Dakota field conditions. Briggs hard red spring wheat was planted at Brookings, SD. Trial treatments included an untreated check; the fungicide premix Prosaro; *Bacillus* strain 1BA and its mutant 1BAC cultured in different broth formulations; *Bacillus* strain 1D3 cultured in different broth formulations; a combination of *Bacillus* strain 1BA and *Bacillus* strain 1D3; and combinations of Prosaro with one or more of the *Bacillus* BCAs. Chelated manganese was added to the spray mix for some treatments. All treatments were applied at anthesis, and included Induce NIS. Plots were treated with pathogen by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field, and applying overhead mist irrigation each day for 10 days following anthesis. Following the treatments, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for Fusarium damaged kernels (FDK) and deoxynivalenol (DON).

Grain yield was less than some years, probably due in large part to the excessive rainfall. Statistically significant reduction in FDK was observed for the application of 1BA, 1D3, chelated manganese, and Prosaro. Test weight was significantly greater for this treatment, too; as it was for treating with 1BA, 1D3, Prosaro, and Induce NIS; and for treating with 1D3, Prosaro, and Induce NIS. This contrasted with results from 2009 field plots where FHB incidence, FHB index, yield, and FDK were all significant for at least some of the BCA treatments. Many of these significant treatment differences in 2009 were in treatments that omitted Induce NIS. We hypothesize that inclusion of Induce NIS may not be beneficial as part of some of these BCA treatments, and want to test this hypothesis in future trials. Results from some of our BCA treatments at Langdon, ND in summer 2010 further suggest that Induce NIS may not help in promoting efficacy of some of these BCA formulations.

GRAIN HARVESTING STRATEGIES TO MITIGATE LOSSES DUE TO FUSARIUM HEAD BLIGHT: A COST/BENEFIT ASSESSMENT

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ABSTRACT

Management of Fusarium head blight (FHB) through the integration of cultivar resistance, fungicide application, and agronomic practices has been more effective than any individual strategy at reducing FHB and deoxynivalenol (DON) accumulation. However, when conditions for disease development are highly favorable, Fusarium damaged kernels (FDK) and DON contamination cannot be avoided, even when integrated management practices are implemented. Grain harvesting strategies have also been recommended as a way of reducing losses due to FHB and DON. Research has shown that reducing FDK generally leads to reductions in DON, thus adjusting combine harvester settings could help to reduce FDK and DON in harvested wheat grain through the removal of diseased, lightweight kernels. In 2009 and 2010, the influence of varying combine harvester configurations on FDK and DON was evaluated in inoculated wheat plots of a susceptible SRWW cultivar (Hopewell). Plots were harvested using four different combine harvester configurations (C1 as default setting and C2, C3 and C4 as experimental settings) established through modification of the default fan speed (airflow speed) and shutter opening (volume of air flowing through the system). Wheat grain yield, test weight, FDK and DON were determined for each configuration, at different levels of FHB index. Grain yield increase and quality loss reduction were also evaluated. The results showed that averaged across high disease levels (23 to 35% index), C2, C3 and C4 reduced FDK by 2.44, 7.00 and 5.89% respectively, relative to the default setting (C1); C3 and C4 reduced DON by 3.75 and 2.80 ppm, respectively; and C3 and C4 resulted in mean test weight increases of 2.50 and 2.63 lb/bu, respectively. However, the configuration (C3) that was most consistent across disease levels at reducing FDK and DON and increasing test weight led to a 6.52 to 14.46 bu/ac reduction in grain yield at mean index levels greater than 30%. This was largely because the higher fan speed resulted in excessive removal of healthy kernels along with diseased kernels. Average SRWW grain prices for the last five growing seasons and grain discount schedules were used to determine whether the gain in grain quality through increased test weight and reduced FDK and DON was sufficient to offset losses associated with the removal of healthy grain (overall yield reduction). For instance, at 30% index the baseline yield without combine modification was 32.39 bu/ac. At an average grain price of $4.38/bu, this grain yield would have resulted in a gross income over $70,000.00 for a 500 acre field (~$141.87/acre), if there were no discounts. However, at 30% index, mean test weight (TW) was 47.77 lb/bu, FDK was 11.67%, and DON was 15.20 ppm. Grain from a field with this level of DON will likely be rejected at grain elevators. However, assuming discounts of $0.08/bu for every pound reduction in test weight below 57 lb/bu; $0.05/bu for every percent increase in FDK above 4%, and $0.10/bu for every 0.5 ppm DON increase above 2 ppm, the estimated income would be approximately $6,700.00 for a 500 acre field (~$13.50/acre). For treatment 3 (combine configuration C3), mean grain yield, TW, FDK, and DON were 25.87 bu/ac, 50.92 lb/bu, 8.67%, and 8.23 ppm, respectively. Using the same discount schedule and grain price, the estimated gross income would be approximately $27,000.00 for a 500 acre field (~$55.00/acre). Both of these estimates are well below the amount expected for a FHB-free field of the same size (~$158,000.00), with average grain yield for the state of Ohio (72 bu/acre). However, for the scenario presented, the TW increase and FDK and DON reductions were sufficient to offset the yield reduction resulting from the use of the C3 combine configuration. A more comprehen-

sive analysis will be conducted to evaluate the effects of other scenarios (baseline yield and disease levels, grain prices, and discount schedules) on grain yield, quality, and the economic benefit of using modified combine harvester configuration to harvest grain from scabby wheat fields.

EFFECT OF LATE INFECTION AND POST-INOCULATION MOISTURE ON FHB DEVELOPMENT IN WHEAT AND BARLEY

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ABSTRACT

Field experiments were conducted to examine the effect of late infection of *Fusarium graminearum* and post-inoculation moisture on FHB in wheat and barley. The 2010 experiment was a randomized split-split-plot design with four replications and followed a preliminary study in 2009. The main plot treatments were duration of mist-irrigation (14, 21, 28, or 35 d after inoculation). Sub-plots were host genetic background (3 cultivars of each wheat and barley). The sub-sub-plots were timing of inoculation (0, 7, or 14 days after anthesis (daa)) of *F. graminearum*. The three wheat cultivars included were Tom (moderately resistant), 2375 (moderately resistant-moderately susceptible), and Wheaton (susceptible). The three barley cultivars were Quest (moderately resistant), Robust (moderately resistant-moderately susceptible), and Stander (susceptible). Individual plots consisted of three rows, 1.8 m in length, at 30 cm spacing. All plots were inoculated twice, with the second inoculation applied 3 d after the initial inoculation. The first inoculation of the 0 daa treatment was applied at anthesis for wheat and at head emergence for barley. Inoculum consisted of macroconidia (1 x 10^5 spores ml^{-1}) and 2 ml L^{-1} of Tween 20 (polysorbate) from a mixture of ca. 50 *F. graminearum* isolates. The inoculum was applied at a rate of 30 ml per meter of plot row. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10 ml sec^{-1} at a working pressure of 275 kPa. Visual assessment of FHB was determined on whole heads (10 per plot) that were arbitrarily sampled 0, 5, 10, 15, 20, 25, and 30 d after inoculation (DAI). The sampled heads were stored at -20°C for later processing. In 2009, the window of infection extended at least 7 days after anthesis for both wheat and barley. In 2010, an additional inoculation treatment (14 daa) was examined and our data indicated that initial infections are still effective in establishing disease up to 14 daa. Our results also suggest that the rate of disease development increases as the plants near physiological maturity. The rate of disease development was also greater in the susceptible cultivars, Wheaton (wheat) and Stander (barley). Our results support other research indicating that wheat and barley may be susceptible to infection by *F. graminearum* for a prolonged period after anthesis. As FHB appears to develop more rapidly in plant tissues nearing natural senescence, late infections may contribute proportionally more to disease symptoms than would be expected in comparison to infections that occur closer to anthesis. The analysis of the toxin time course data (not yet available) from the sampled heads will provide additional information to test these findings.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-069. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

COLONIZATION OF WHEAT HEADS BY ANTAGONIST *CRYPTOCOCCUS FLAVESCENS* OH 182.9 WHEN APPLIED ALONE OR IN COMBINATION WITH DIFFERENT CONCENTRATIONS OF PROSARO® AND THE EFFECT ON FUSARIUM HEAD BLIGHT DEVELOPMENT IN FIELD-GROWN WHEAT

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OBJECTIVES

1) Conduct a second year of studies to quantify the colonization of infection court tissues of field-grown wheat by yeast antagonist *Cryptococcus flavescens* OH 182.9 in the presence or absence of full or 1/10th strength rates of Prosaro® and 2) determine Fusarium head blight (FHB) disease development for the same treatments applied in the colonization work.

INTRODUCTION

The significant and consistent reduction of FHB and deoxynivalenol (DON) contamination of wheat and barley remains elusive though research results indicate that utilizing an integrated pest management approach achieves the greatest level of disease/toxin control. The use of yeast biological control agent *Cyptococcus flavescens* OH 182.9 (NRRL Y-30216) as part of an integrated management strategy against FHB is understudied yet has the potential to contribute to the reduction of FHB and DON. We have isolated a prothioconazole-tolerant (PTCT) variant of OH 182.9 (OH 182.9 3C) that frequently exhibits enhanced biocontrol activity over its wild type progenitor strain. This variant also is tolerant of tebuconazole. As part of an integrated control protocol, strain OH 182.9 3C could be applied to heads after flowering when fungicides are not approved for use but when new infections by *F. graminearum* can occur (Cowger and Arrellano, 2010). Alternatively, a tank mixed Prosaro® (Bayer Crop-Science product with a.i. of prothioconazole and tebuconzaole) and OH 182.9 combination treatment applied at flowering could provide immediate and lasting protection against FHB and DON due to OH 182.9 survival on wheat head infection courts after protection from the fungicide component has diminished. By understanding the colonization dynamics of strain OH 182.9 under differing integrated application protocols, the direction of fermentation and formulation research could be focused on enhancing colonization of infection courts and thereby improve biocontrol effectiveness.

MATERIALS AND METHODS

PTCT variant 3C of FHB antagonist *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) was generated by growing cells of OH 182.9 in liquid culture medium containing prothioconazole (PTC) and selecting for naturally occurring variants with enhanced tolerance to the fungicide (Schisler et al., 2009) . Field trials with OH 182.9 wild type and PTCT variant 3C were conducted in Peoria, IL, Wooster, OH and Urbana, IL in 2010. Inoculation techniques utilized in the Urbana, IL trial introduced small quantities of OH 182.9 between treatments. Multiple rain events permitted populations of OH182.9 3C to reach levels on non-inoculated plots that were nearly as high as those on inoculated plots, compromising the interpretation of treatment effects. At Peoria, IL and Wooster, OH, soft red winter wheat cultivar

Freedom (moderately resistant to FHB) was grown using standard agronomic practices (Schisler et al., 2006). Corn kernels colonized by native *G. zeae* isolates were scattered through plots (~25-40 kernels/m^2) three weeks prior to wheat flowering. Biomass of WT OH 182.9 and PTCT variant 3C (~3 x 10^8 cfu/ml and 40 gal/acre at Peoria and ~3 x 10^8 cfu/ml and 20 gal/acre at Wooster) was produced in a B Braun Biostat B fermentors (B. Braun Biotech Inc., Allentown, PA) charged with 1 L of SDCL medium (Schisler et al., 2009). Treatments are shown in Table 1 and included OH 182.9 WT and PTCT variant 3C treatments, Prosaro® at 6.5 oz/acre or 0.65 oz/acre, and combinations of Prosaro® and OH 182.9 3C applied at flowering (Feekes 10.5) or with OH 182.9 3C applied 7 days after the Prosaro® application at flowering. Sixteen, 88, 184 and 256 hours after treatment application to wheat heads at flowering in Peoria, three replicate samples of glume and lemma tissues were taken from selected treatments (Figs 1, 2; lemma data not shown) and plated on one-fifth strength tryptic soy broth agar (TSA/5) and TSA/5 with 50 ppm streptomycin and 2 ppm prothioconazole to enumerate "total" microbial populations and populations of yeast OH 182.9 3C, respectively. Natural rainfall was supplemented with overhead irrigation in one instance (Figs 1, 2). Heads were scored for disease severity and incidence and grain evaluated for 100 kernel weight and DON (DON data were not available at the time of publication). Analysis of variance and Fisher's Protected LSD test (FPLSD, $P \leq 0.05$) was used to compare all treatment means.

RESULTS AND DISCUSSION

Due to similar trends in colonization of lemma and glume tissues by strain OH 182.9 3C, only glume colonization data are presented. Initially low colonization by OH 182.9 3C was at least partially due to the higher concentrations of PTC present in the selective medium used for the 16 h plating versus the 2 ppm PTC concentration used subsequently. Rain events occurred regularly throughout the time of the experiment and populations of total microflora and that of 3C inoculated at flowering or 7 days after flowering increased in total numbers and as a percentage of the total microflora recovered (Figs 1, 2). By 184 h and continuing through the 256 hour evaluation, OH 182.9 3C made up >50% of the recoverable microflora, when used alone or in combination with full or 1/10th rate Prosaro®, demonstrating for a second year the propensity of OH 182.9 3C to aggressively compete in colonizing glume and lemma tissues when free moisture is regularly available. Results from Dunlap and Schisler (2009) and initial analysis of a similar study this year (data not shown) indicate that the hydrophobicity of the surfaces of glume and lemma tissues increases leading up to flowering and then drops after flowering. While a strong relationship between physiochemical changes on wheat head tissues and the level of colonization achieved by OH 182.9 3C is not immediately apparent, genomic characterization of OH 182.9 should enable more accurate population characterization in situ and the determination of the effectiveness of modifying tissue environments via formulation to support the efficacy of OH 182.9 3C throughout flowering and kernel development.

In Peoria, IL all but two treatments reduced disease severity on cultivar Pioneer Brand 2545 (Table 1, $P \leq 0.05$, FPLSD) though OH 182.9 3C applied at 7 days after flowering did not. Though in most cases significantly different from the control, treatments rarely differed one from the other. Similarly, all treatments but OH 182.9 3C applied at 7 days reduced disease incidence. Full strength Prosaro® tank mixed with OH 182.9 3C and applied at flowering had the lowest recorded level of FHB incidence compared to the control (8.3% vs 20%, respectively; Table 1). Disease reduction associated with the various treatments supported the observation that the population of OH 182.9 C3 on infection court tissues was not inhibited by the presence of PTC. No treatment differences occurred on wheat cultivar Freedom in Peoria. In Wooster, OH on Pioneer Brand 2545, all treatments reduced FHB severity and incidence (data not shown). Full strength Prosaro® reduce severity to a greater extent than OH 182.9 3C (FHB severity values of 4%, 19%, 23% for full strength Prosaro®, OH 182.9 3C treatments and control respectively, $P \leq 0.05$, FPLSD). Similarly, full strength Prosaro® and OH 182.9 3C treatments significantly reduced incidence compared to the control (22%, 49% and 61%, respectively). As was the case in the 2009 studies, PTCT variant 3C of OH 182.9 often exhibited efficacy in reducing FHB and mixed results in enhancing the performance of Prosaro® used at full or reduced rates.

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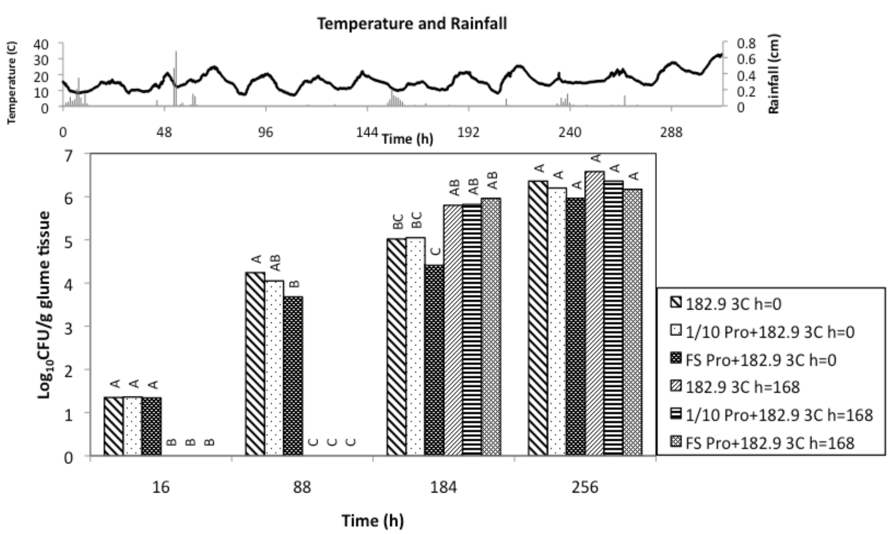
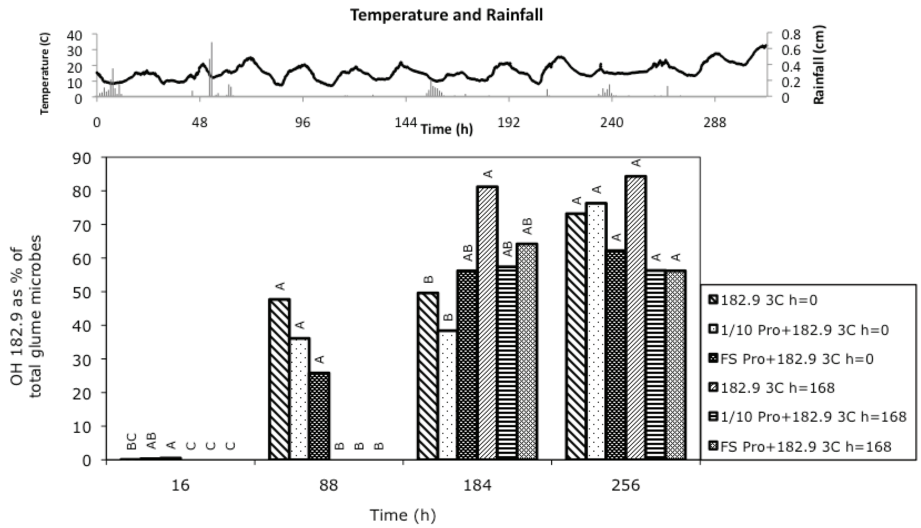


[a] Between 220 hours and 230 hours a total of 0.5 cm of irrigation was applied aerially during six separate irrigation events, each of 3 minutes duration. Within plating times, bars with no letters in common are significantly different ($p<0.05$, FPLSD).

Fig. 1. Log_{10} population of *Cryptococcus flavescens* OH 182.9 3C on glume tissue when applied alone or in combination with Prosaro® at or seven days (168 hours) after wheat flowering[a].



[a] Between 220 hours and 230 hours a total of 0.5 cm of irrigation was applied aerially during six separate irrigation events, each of 3 minutes duration. Within plating times, bars with no letters in common are significantly different (p<0.05, FPLSD).

Fig. 2. Population of *Cryptococcus flavescens* OH 182.9 3C on glume tissue expressed as a percentage of the total recoverable microbial population when strain 3C was applied alone or in combination with Prosaro® at or seven days (168 hours) after wheat flowering[a].

Table 1. 2010 field trial results at Peoria, IL: Influence of Prosaro®, yeast antagonist OH 182.9, prothioconazole-tolerant variant 3C of OH 182.9, and combinations thereof on FHB disease parameters on winter wheat cultivar Pioneer Brand 2545[a,b,c]

	Wheat Cultivar Pioneer Brand 2545		
Treatment	**DS (%)**	**DI (%)**	**100 KWT (g)**
Untreated control	3.8[A]	20.0[A]	4.0[A]
OH 182.9 variant 3C T=0h	2.3 [BCD]	11.9[BCDEF]	4.2[A]
1/10 Pro + 3C T=0h	3.2[AB]	14.7[BC]	4.3[A]
FS Pro T=0h; 3C T=7d	1.8[D]	8.9[EF]	4.3[A]
1/10 Pro T=0h; 3C T=7d	2.6[BCD]	14.4[BC]	4.1[A]
1/10 Pro T=0h	1.9[CD]	9.4[DEF]	4.1[A]
OH 182.9 variant 3C T=7d	2.8[AB]	16.4[AB]	4.0[A]
FS Pro + 3C T=0h	1.9[D]	8.3[F]	4.2[A]
FS Pro T=0h	2.0[CD]	9.2[EF]	4.1[A]
1/10 Pro T=0; 182.9CY T=7	2.7[BCD]	13.6[BCDE]	4.0[A]
OH 182.9 wild type T=0h	2.6[BCD]	12.8[BCDEF]	4.0[A]
P value	0.05	0.05	0.05

[a]Within a column, means not followed by the same letter are significantly different (P≤0.05, Fisher's Protected LSD). Mean separation was performed on arcsine transformed values while non-transformed values are presented.

[b]DS= Disease severity, DI= Disease incidence, 100 KWT= One hundred kernel weight, DON=Deoxynivalenol

[c]Prosaro® (Pro)= Commercial fungicide formulation applied at a rate equivalent to 6.5 oz/acre (full strength=FS) or at 1/10th this rate; OH 182.9 variant 3C (3C)= prothioconazole tolerant variant of OH 182.9; OH 182.9 WT= Wild type strain of OH 182.9; T= time of application of treatment (T= 0h represents at flowering, T= 7d represents seven days after flowering); 182.9CY= OH 182.9 variant that is cycloheximide tolerant.

PROSARO® FUNGICIDE PERFORMANCE IN WINTER WHEAT IN THE PRAIRIE POTHOLE REGION OF SOUTH DAKOTA AND NORTH DAKOTA

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ABSTRACT

Prosaro™ is a broad spectrum head and leaf disease foliar fungicide that was introduced in 2009 by Bayer CropScience. Prosaro is registered for use in spring wheat, durum wheat, winter wheat, and barley. Prosaro is a mixture of prothioconazole and tebuconazole; these two active ingredients provide control of Fusarium head blight was well as several important cereal leaf diseases. Prosaro is formulated as a soluble concentrate for ease of handling. It is applied at 6.5 to 8.2 fl oz/ac with a non-ionic surfactant to wheat or barley up to 30 days prior to harvest.

Winter Cereals: Sustainability in Action is a unique, joint research and education initiative of Ducks Unlimited and Bayer CropScience. The initiative promotes improving agricultural productivity while maintaining habitat for wildlife by increasing winter wheat acres in the Prairie Pothole Region. Winter wheat can improve a producer's operation by increasing yield and profitability while spreading out workload for labor and equipment. The fall-planted crop also provides adequate nesting cover for waterfowl because there is little field disturbance during the nesting period.

Replicated trials were coordinated by Ducks Unlimited agronomists in 2009 and 2010 at several locations in the Prairie Pothole Region of North Dakota and South Dakota. Additionally, in 2009 and 2010, large demonstration plots were established with private growers in commercial fields comparing Prosaro to either an untreated check or tebuconazole. The objective of these trials was to evaluate the effect of Prosaro on grain yield and grain quality in winter wheat. Prosaro was applied at 6.5 fl oz/ac with a non-ionic surfactant when the winter wheat initiated flowering (Feekes 10.51). Disease ratings were recorded where applicable. The trials were harvested and grain yield and grain quality was determined.

Prosaro applied at Feekes 10.51 increased the grain yield of winter wheat by an average of more than 12 bu/ac compared to the untreated check in 2009. Test weight was also increased by an average of more than 0.8 lb/bu by Prosaro. In a similar set of trials conducted in 2010, the grain yield was increased more than 10 bu/ac by Prosaro applied at Feekes 10.51 compared to the untreated check. Test weight was also increased by an average of 1.0 lb/bu by Prosaro. In the case of grower applied Prosaro, yield was increased more than 8 bu/ac compared to the untreated check.

EVALUATION OF INTEGRATED METHODS FOR MANAGING FHB AND DON IN WINTER WHEAT IN NEW YORK IN 2010

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and foliar fungicides (Caramba, Folicur and Prosaro) on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) under two environments in New York.

INTRODUCTION

In response to the USWBSI goal to validate integrated management strategies for FHB and DON, the Disease Management RAC of USWBSI initiated a multi-state, multi-year, coordinated field study. In New York during 2010, we conducted two separate experiments each with unique environmental conditions during flowering and early grain development.

MATERIALS AND METHODS

All experiments were performed at the Musgrave Research Farm in Aurora, NY following cultural practices recommended for soft winter wheat in the region. The four cultivars included in the experiment were 'Pioneer 25R47' (red, susceptible to FHB), 'Truman' (red, moderately resistant to FHB), 'Jensen' (white, moderately resistant to FHB), and 'Richland' (white, susceptible to FHB). The two experimental wheat environments were characterized by the planting of winter wheat 1) no-till into soybean residue on 10/12/09 and 2) no-till into a fallow field on 10/20/09. Each experimental design was a split plot with four wheat cultivars as whole plots and four spray treatments as subplots, and four replicate blocks. Main plots were planted with a 10 ft wide commercial grain drill. Sprayed areas in each subplot were 8 ft wide by 20 ft long. Spray treatments applied at Feekes GS10.5.1 were 1) non-sprayed; 2) Caramba 13.5 fl oz/A & Induce 0.125%; 3) Folicur 4.0 fl oz/A & Induce 0.125%; and 4) Prosaro 6.5 fl oz/A & Induce 0.125%. The experiment was inoculated with conidial suspension of Fusarium graminearum (100,000 conidia/ml) on the same day as treatments were applied after the fungicides had dried. Fungicide and Fusarium application was made with paired Twinjet nozzles mounted at an angle (30^o from horizontal) forward and backward and calibrated to deliver at 35 gallons per A. FHB and foliar diseases were assessed at soft dough stages. Grain was harvested from a 4 ft wide x 20 ft long area in each subplot using a Hege plot combine. Grain moistures, plot yields, and test weights were recorded and the latter two were adjusted for moisture. Means were calculated and subjected to Analysis of Variance. Fisher's protected LSD was calculated at P=0.05. Analysis of DON content in grain was conducted in an USWBSI-supported mycotoxin laboratory.

RESULTS AND DISCUSSION

Although planting dates varied by a week, any developmental head start in the earlier planted environment was inconsequential by spring. Flowering occurred simultaneously in both environments during a dry and hot period unfavorable to FHB development. The two weeks following flowering were considered medium risk of FHB due to increased rainfall and more moderate temperatures. The overall average of FHB incidence observed for both environments was 20%. Inoculation with the conidial suspension proved fruitful as only 2% FHB incidence was observed in adjacent non-inoculated experiments. There is little evidence to suggest inocula from within-plot crop residues impacted FHB development. Only two cultivars, Jensen and Richland, had significantly higher FHB indexes in the soybean residue plot (environment 1). There were no significant differences observed in the DON levels between the two environments for all cultivars. Due to the similar levels of FHB development in both environments, FHB is unlikely an explanation to the greater yields observed in environment 1. One potential explanation of yield differences is that more foliar disease, especially Stagonospora leaf blotch, was observed in the previously fallow plot (environment 2). In both environments, foliar diseases were reduced significantly by application of any of the fungicides.

All fungicide treatments impacted at least some aspect of FHB development. All fungicide treatments resulted in FHB indices lower than nontreated. In two plots, Jensen and Richland in environment 2, there was no significant difference in FHB index between any of the treatments. When significant reductions of FHB index due to fungicide application were observed, there was no difference between fungicides except for Pioneer 25R47 in environment 1 where Folicur did not differ from nontreated. Pooling cultivar data to determine treatment averages minimized the observed decrease in FHB index due to fungicide application.

Contamination of grain by DON was decreased significantly by all fungicide applications in all cultivars and in both environments, but not always below the 2.0 ppm threshold for sale at flour mills. In both environments, nontreated grain had DON levels greater than the threshold with the exception of the Truman plots. For the Jensen plots in both environments, no fungicide treatment reduced DON levels below 2.0 ppm. In Pioneer 25R47 and Richland plots in both environments, Prosaro and Caramba reduced DON below the threshold. Reduction of DON below 2.0 ppm was observed only once with the Folicur treatment (in environment 2 on Pioneer 25R47).

Fungicide application increased yield for all cultivars in both environments. Prosaro was the only fungicide that resulted in significantly higher yields than the nontreated in all cultivars in both environments. The other two fungicides had significantly higher yields in all plots except for the Folicur treatments on Truman in environment 1 and on Pioneer 25R47 in environment 2, and the Caramba treatment on Richland in environment 2. Of the three fungicide treatments, none consistently increased yields significantly in comparison with the others. However, in situations where FHB is severe, treatment with Caramba or Prosaro would more likely reduce DON below 2.0 ppm than would treatment with Folicur.

The four cultivars demonstrated differences in both yield capability and disease response. Observations did not necessarily conform with expectations based on defined FHB response. The white wheat varieties, Jensen (previously categorized as moderately resistant) and Richland (susceptible), had similarly high FHB indexes and DON levels. Richland had significantly higher yields than Jensen in environment 2. The red wheat varieties, Pioneer 25R47 (susceptible) and Truman (moderately resistant), had similarly low FHB indexes and DON levels. Pioneer 25R47 had significantly higher yields than Truman in both environments. Only Truman demonstrated cultivar FHB resistance with DON levels below 2.0 ppm in the nontreated plots. Under the moderate disease levels of this experiment, fungicide application resulted in marketable grain even in the highest yielding, albeit more susceptible, cultivar Pioneer 25R47.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported in part by the U.S. Department of Agriculture under agreement No. 59-0206-9-056. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. Main effect of treatment on grain yield, Fusarium head blight index, and deoxynivalenol contamination at Aurora, NY.

	Adjusted grain yield (bu/A)		
Treatment:	Environment 1	Environment 2	Average
No treatment	70.2	53.0	61.6
Caramba	88.3	67.2	77.8
Folicur	83.9	63.8	73.9
Prosaro	90.6	73.0	81.8
LSD (*P*=0.05)	8.8	6.5	

	Fusarium head blight index (%)		
Treatment:	Environment 1	Environment 2	Average
No treatment	18.9	11.7	15.3
Caramba	9.5	6.4	8.0
Folicur	11.5	7.7	9.6
Prosaro	4.4	5.5	5.0
LSD (*P*=0.05)	7.4	NS	

	Contamination of grain by DON (ppm)		
Treatment:	Environment 1	Environment 2	Average
No treatment	5.5	4.4	5.0
Caramba	1.8	1.7	1.8
Folicur	2.9	2.2	2.6
Prosaro	1.0	1.3	1.2
LSD (*P*=0.05)	1.8	6.5	

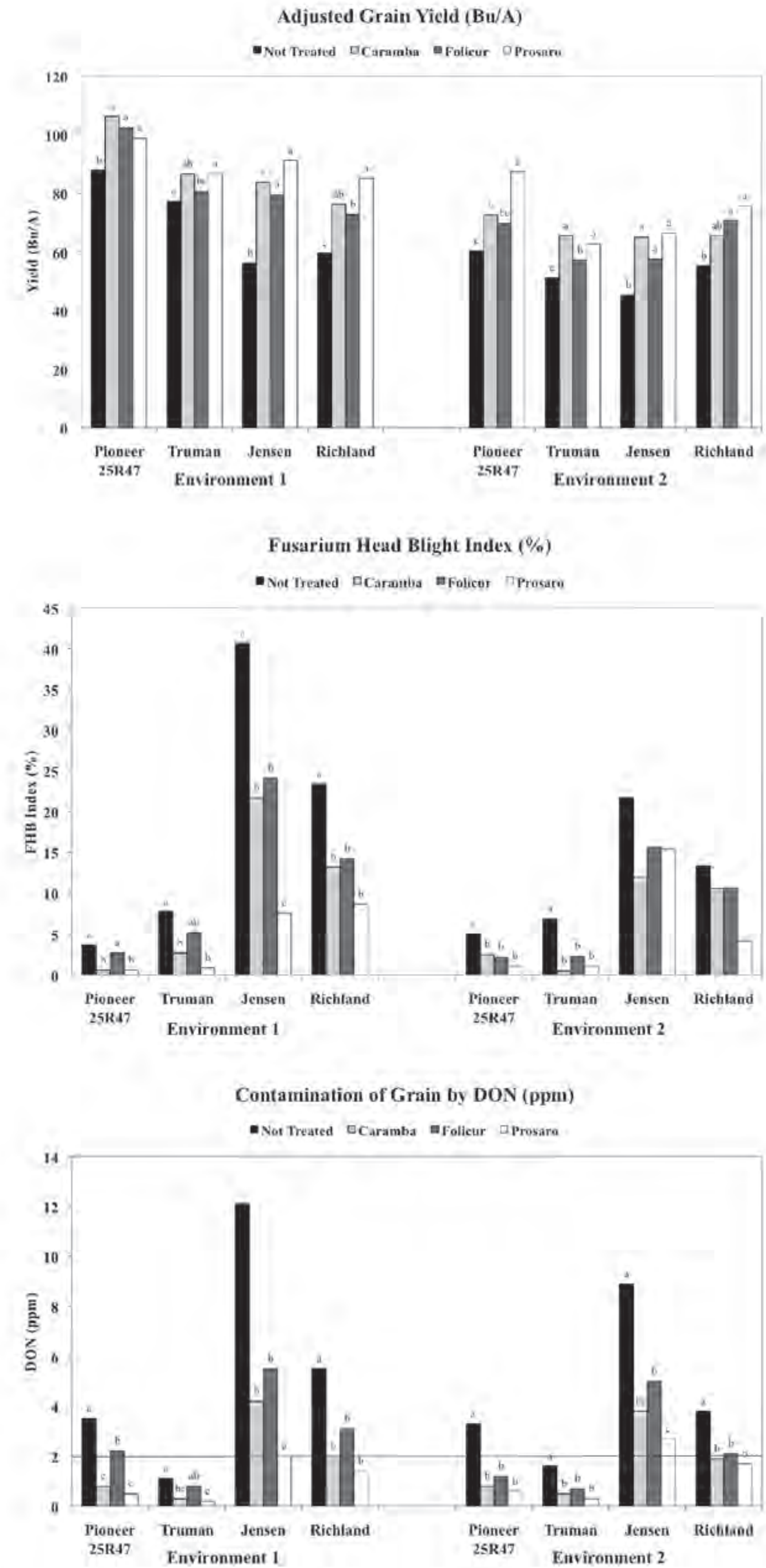

Figure 1. Effect of flowering stage application of Prosaro fungicide on yield, FHB index and DON contamination of four winter wheat cultivars in Aurora, NY. Letters denote treatment means that differ significantly at P=0.05.

CONTROL OF FHB AND DON BY PROSARO FUNGICIDE IN MODERATELY RESISTANT AND SUSCEPTIBLE WINTER WHEAT CULTIVARS

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a devastating disease of wheat and other small grain cereals. Losses to FHB are due to yield reduction, presence of *Fusarium*-damaged kernels, and production by *F. graminearum* of the mycotoxin deoxynivalenol (DON) which accumulates in grain. One strategy for management of FHB is the integration of cultivar resistance with fungicide application at early flowering. To evaluate the effectiveness of this strategy, five experiments were conducted in 2007-2009 in Kansas and Nebraska, USA. The fungicide prothioconazole + tebuconazole (Prosaro 421 SC) was applied or not applied to winter wheat cultivars differing in levels of resistance to FHB. FHB index, yield, the percentage of *Fusarium*-damaged kernels (FDK), and DON concentration were measured. Based on FHB index, moderately resistant cultivars (Harry, Heyne, Roane, and Truman) were grouped into one treatment (resistant treatment) and susceptible cultivars (2137, Jagalene, Overley, and Tomahawk) were grouped into a second treatment (susceptible treatment). The efficacy of Prosaro (fungicide efficacy) in reducing FHB index, FDK, and DON and increasing yield was calculated for each treatment. The effect of treatment on fungicide efficacy was highly significant for FHB index ($P < 0.0001$) and DON ($P = 0.0057$). It was significant at the 10% level for FDK ($P = 0.0903$); however, it was not significant for yield ($P = 0.4175$). Fungicide efficacy for FHB index, DON, and FDK was higher in the moderately resistant cultivars (46, 35, and 32%, respectively) than in the susceptible cultivars (22, 14, and 11%, respectively). Fungicide efficacy for yield was 25% in the moderately resistant cultivars and 20% in the susceptible cultivars. These results indicate that integrating cultivar resistance with fungicide application is an effective strategy for managing FHB in winter wheat. Hence, producers are more likely to realize greater benefits from fungicide application to control FHB if they choose moderately resistant cultivars over susceptible ones.

ACKNOWLEDGMENT AND DISCLAMER

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INOCULATED FIELD TRIALS FOR EVALUATING FHB/DON INTEGRATED MANAGEMENT STRATEGIES

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ABSTRACT

In recent years there have been discussions amongst researchers involved in the USWBSI integrated management coordinated program regarding the value of using artificial inoculation in field trials. In many small grain regions, moderate to severe FHB epidemics (> 10% FHB index) are intermittent events and are strongly dependent on environmental conditions critical for inoculum production and dispersal and infection. In years in which nominal levels of disease develops, it is difficult to evaluate the main and interaction effects of variety and fungicide treatments in integrated management trials. From 2007 to 2010, 27 out of 42 total trials had less than 10% index, even in the untreated, susceptible check. For the same period, 13 out of 31 trials with available DON data had less than 2 ppm mean DON in the check. In addition, it is common for researchers to forgo DON testing altogether when observed disease levels are low in the field. These sorts of "negative" results make it difficult to recommend best-management practices to growers based on research-based conclusions. It was hypothesized that inoculating these trials will consistently provide more meaningful FHB and DON data for integrated management studies. However, some researchers are concerned that such a system is not representative of what happens in growers' fields and may result in elevated levels of disease and DON that could potentially alter the individual and combined effects of different management strategies relative to what would be expected under natural infection. A subset of the naturally infected FHB integrated management trials (trials with FHB index above 1% in the untreated, susceptible check [n=22]) and all available artificially inoculated trials (n=6) for the period between 2007 to 2010 were combined and analyzed using linear mixed models to determine the effects of fungicide treatement (check and Folicur or Prosaro® at anthesis) x variety resistance (susceptible, moderately susceptible, and moderately resistant) interaction on percent control of FHB and DON and whether these effects vary with study type (inoculated vs. non-inoculated). Only one inoculated trial had index below 10% in the untreated, susceptible check. All inoculated trials with DON data (5) had mean DON greater than 2 ppm in the untreated, susceptible check. In non-inoculated winter wheat trials, index and DON ranged from to 0 to 80% and 0 to 52 ppm, respectively. In inoculated winter wheat trials, index and DON ranged from 0 to 48% and 0 to 30 ppm, respectively. For spring wheat, one non-inoculated trial resulted in index and DON ranges of 0 to 33% and 0 to 8 ppm, respectively. For the inoculated spring wheat trial, index and DON ranged from 0 to 15.8% and 0.2 to 6.6 ppm. All variety resistance x fungicide treatment combinations

significantly reduced FHB index relative to the untreated susceptible check. Percent FHB control ranges from 18 to 50% for untreated moderately susceptible variety; 35 to 68% for untreated moderately resistant variety; 39 to 62% for treated susceptible variety; 46 to 68% for treated moderately susceptible variety; and 56 to 81% for treated moderately resistant variety. Inoculated trials tended to have higher overall mean percent control (46 to 89%) than non-inoculated trials (34 to 65%), however the effect of inoculation on percent FHB control was only statistically significant for the comparison between untreated+resistance and untreated+susceptible (26 to 64% for non-inoculated compared to 67 to 95% for inoculated) and marginally significant for the comparison between treated+resistance and untreated+susceptible (50 to 75% for non-inoculated compared to 62 to 97% for inoculated). These analyses will be repeated for incidence, index, DON, and FDK as more data become available. The effect of baseline disease level on percent control of each of these responses will be evaluated.

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IMPLICATIONS OF FHB MULTI-SPECIES COMPLEX ON DISEASE DEVELOPMENT AND MYCOTOXIN

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ABSTRACT

Field development of FHB was surveyed in many sites over a period of four years in four European countries. This set of survey data included incidence of visual FHB symptoms, fungal presence and the amount of its DNA at GS69 and at the harvest, weather conditions and mycotoxin at harvest. Controlled inoculations were also carried out investigate the effects of temperature, wetness duration, fungal competition and cultivars on FHB development and mycotoxin accumulation.

Main findings are:

1. Overall, *F. poae* was the most frequent species detected; more pathogen species was detected in the UK and Ireland than in Italy and Hungary

2. In cooler regions the frequency of *F. graminearum* increased at the expense of *F. culmorum*

3. Presence of FHB pathogens at a given field appeared to be positively associated with each other. However, at a finer scale, presence of FHB pathogens appears not to be related to the presence of each other

4. The mycotoxin level was in general very low in field samples and its relationship with disease incidence and fungal biomass was weak

5. The presence of *F. graminearum* appeared to be most related to toxin production

6. FHB pathogen differed significantly in their pathogenicity and toxin-producing capability; inoculation studies showed that *F. graminearum* was most pathogenic and *F. poae* the least

7. Under controlled inoculation conditions, there were significant positive correlations among disease incidence, fungal biomass and mycotoxins

8. *F. graminearum* was most competitive species and the other three toxigenic species were similar in their competitiveness,

9. Mycotoxin production per unit of fungal biomass increased in mixed inoculations although fungal DNA of individual pathogens in such inoculations appeared to have decreased due to competition.

10. There were evidences for supporting the existence of ecotypes within each FHB pathogen,

11. Cultivars also differed significantly in their response to FHB and mycotoxin production.

2010 UNIFORM BIOLOGICAL CONTROL TRIALS - PRELIMINARY RESULTS

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OBJECTIVE

To evaluate, using standardized methodology, two biological materials applied alone and in combination with a fungicide for effectiveness in managing Fusarium head blight (FHB) in wheat across a range of environmental conditions.

INTRODUCTION

Great strides have been made in the breeding of new scab resistant wheat cultivars and the creation of new chemical fungicides to address Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation in grain. Nevertheless, effective disease resistance is not available in all wheat market class and new fungicides are not completely effective in stemming DON accumulation under disease favorable conditions. Therefore, the use of biological agents is continuing to be explored as a strategy to augment host resistance and fungicides by individual laboratories and as part of the USWBSI-funded coordinated efforts to evaluate new management tools across different wheat production environments. In the Uniform Biocontrol Trials of 2009, two biological control materials were evaluated, one being a novel organism mixture involving two yeast strains. This "double yeast" treatment consisted of *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) and *C. aureus* OH 71.4 (NRRL Y-30213) that were produced together in a fermentor. Mixtures of these individually-effective, compatible strains had potential to reduce FHB symptoms on wheat in greenhouse trials (Schisler et al., 2007). The other biological material was Taegro (Novozymes Biologicals, Salem, VA), a product containing *Bacillus amyloliquefaciens* FZB24. Taegro is commercially available but not yet registered for use in wheat. The biological materials applied alone or in combination with the fungicide Prosaro 421 SC (a formulation of prothioconazole and tebucanozole; Bayer CropScience), were comparable in consistency to the standard fungicide and in a few instances provided higher levels of control than the fungicide (Yuen et al., 2009). In addition, Prosaro followed by the double yeast was the only treatment to significantly reduce DON when averaged across all locations. The 2010 Uniform Biocontrol Trials focused on the same set of treatments as those evaluated in 2009 with the objective of determining whether or not the biological materials could be consistently effective over years when applied as stand-alone treatment or in combination with a commercial fungicide.

MATERIALS AND METHODS

Six trials involving a range of wheat market classes were conducted across four states (Table 1). The biological materials tested were the double yeast, supplied by D. Schisler in frozen cell concentrate form, and Taegro, supplied as a dry formulation by Novozymes Biologicals, Salem, VA). The same set of treatments (Table 2) was tested in every trial. All treatment liquids were amended with 0.125% Induce. One application was made per treatment at early flowering (Feekes 10.51) or 5 days later (late-bloom) in 20 gal/acre using a CO_2-pressurized sprayer. The size and number of replicate plots varied among trials, as did the use of mist irrigation systems to stimulate infection and the application of *Fusarium graminearum*-infested corn kernels as a source of pathogen inoculum. In all trials, FHB incidence, severity, and index were determined from at least 40 heads per plot around 3 weeks after anthesis. Plot yields, test weight, and the incidence of Fusarium-damaged kernels (FDK) were determined after harvest. Kernel samples from each plot were analyzed for DON content by the North Dakota State University Veterinary Diagnostic Laboratory in Fargo. Data from each trial were analyzed separately by analysis of variance or, in cases in which data was incomplete, by ProcMixed (SAS), with arithmetic means or LSmeans being separated by the LSD test at the 95% confidence level. Data from all experiments also were pooled together and analysis by ProcMixed.

RESULTS AND DISCUSSION

Wet weather was experienced at most sites at the onset of anthesis and, thus, moderate to high incidence levels were recorded. Drier conditions, however, occurred during the flowering periods in Nebraska and Michigan resulting in severity levels being relatively low. Taegro alone, applied either at early anthesis or 5 days later, and the double yeast treatment alone reduced scab incidence compared to the control in 4 out of 5 experiments in which there was a significant treatment effect on incidence (Table 3). The biological control agents alone were effective in reducing severity or index in only one out of the 4 or 5 experiments with significant treatment effects. Nevertheless, Taegro and the double yeast treatments alone significantly reduced severity and index when averaged across all experiments. Prosaro and Prosaro/biocontrol agent combinations were similarly effective in reducing incidence, severity and index in all experiments having significant treatment effects. Significant treatment effects for %FDK were found in only one experiment, with the Prosaro/biocontrol agent combinations, but not Prosaro alone, providing significant reductions in %FDK compared to the control. The biological materials alone largely were ineffective in reducing DON in harvested seed. However, the Prosaro/biological agent combinations exhibited numerically lower or statistically lower levels of DON in individual experiments and in the preliminary (data from one experiment not yet available) pooled analysis. Double yeast alone and treatments involving Prosaro increased yields over the control in one experiment (Michigan), but there were no significant treatment effects for yield or seed weight when averaged across trials (data not shown).

In this year's trials, as in 2009, the biological treatments were more effective in reducing scab incidence than severity. This may reflect population levels of the applied organisms being in sufficiently high numbers to address pathogen inoculum arriving on wheat heads for a short period. The population levels on the wheat heads subsequently may have declined, and thus, were not effective in inhibition by inoculum arriving days later. Another explanation may be that organism population on the surface of treated wheat head had little effect on the spread of the pathogen from initial infected florets through the rachis. Unlike the 2009 results, Prosaro alone was consistently effective in reducing field disease parameters in 2010, and therefore, no there was no advantage to combining Prosaro with a biological agent. But, as was found previously, Prosaro-biocontrol agent combinations appear to be the best treatments in these trials in regard to DON reduction. These data lend support to the hypothesis that biocontrol agents, particularly when applied at bloom stage after a fungicide, can inhibit late infections by *F. graminearum* when fungicide activity is reduced.

ACKNOWLEDGEMENT AND DISCLAIMER

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Table 1. 2010 Uniform Biocontrol Trial locations, wheat cultivars, and researchers

State (location)	Crop market class and cultivar	PI and institution
MO (Columbia)	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
MO (Columbia)	Soft red winter wheat 'Elkhart'	L. Sweets, University of Missouri
NE (Mead)	Hard red winter wheat 'Karl 92'	G. Yuen, University of Nebraska
NE (Lincoln)	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
ND (Langdon)	Hard red spring wheat 'Howard'	S. Halley, North Dakota State University
MI (Clarksville)	Soft white winter wheat 'Pearl'	W. Kirk, Michigan State University

Table 2. Treatments tested in 2009 uniform trials.

Treatment code	Treatment
Control	Nontreated
Pro	Prosaro 6.5 fl oz /acre at 10.51
Tae	Taegro 3.5 oz/acre at 10.51
Tae late	Taegro at late bloom
Pro + Tae	Tank mix of Prosaro and Taegro at 10.51
Pro early/Tae late	Prosaro at 10.51 followed by Taegro at late bloom
DYs	Double yeast at 10.51
Pro early/DYs late	Prosaro at 10.51 followed by double yeast at late bloom

Table 3. 2010 results from uniform biocontrol trials denoted by state and location (or cultivar)

Treatment	NE Mead	NE Lincoln	ND Langdon	MO 'Elkhart'	MO 'Roane'	MI Clarksville	LS means
INCIDENCE (%)							
Control	84	94	96	64	84	48	78
Pro	70*	80	85*	50*	70*	10*	61*
Tae	80	87	98	50*	73*	37*	71*
Tae late	67*	89	100	53*	75	29*	69*
Pro + Tae	66*	74	85*	48*	70*	7.5*	58*
Pro early/Tae late	54*#	80	85*	46*	80	4.7*	58*
DYs	73	88	99	48*	78	32*	70*
Pro early/DYs late	65*	79	85*	50*	66*	11*	59*
P	.0014	Ns	.0002	0.0128	.0099	.0005	<.0001
$LSD_{0.05}$	12.9		8.8	8.8	9.0	6.7	4.4
SEVERITY (%)							
Control	21	30	23	80	44	25	37
Pro	15*	16*	14*	63	41	4*	25*
Tae	23	26	28	64	39	19*	33*
Tae late	22	30	25	63	41	22	33*
Pro + Tae	14*	15*	15*	66	43	2*	26*
Pro early/Tae late	13*	17*	14*	63	40	1*	25*
DYs	21	24	22	63	38	15*	30*
Pro early/DYs late	16*	19*	13*	66	39	2*	26*
P	<.0001	.0048	<.0001	Ns	Ns	.001	<.0001
$LSD_{0.05}$	3.8	8.4	3.9			4.2	2.8

Table 3 (continued)

Treatment	NE Mead	NE Lincoln	ND Langdon	MO 'Elkhart'	MO 'Roane'	MI Clarksville	LS means
INDEX (%)							
Control	*19*	*28*	*21*	*51*	*37*	*12*	**28**
Pro	10*	13*	11*	32*	29	0.4*	16*
Tae	17	23	27	32*	28	7*	23*
Tae late	16*	26	25	33*	31	7*	23*
Pro + Tae	10*	12*	12*	31*	30	0.1*	16*
Pro early/Tae late	8*	14*	10*	29*	32	0.1*	16*
DYs	15	22	23	30*	30	5*	21*
Pro early/DYs late	11*	15*	9*	33*	26	0.2*	16*
P	<.0001	.0059	<.0001	0.007	Ns	.001	<.0001
$LSD_{0.05}$	3.8	10.2	4.3	10.5		2.6	2.6
FDK (%)							
Control	*15*	*61*	*Nd*	*27*	*19*	*Nd*	**31**
Pro	12	39	Nd	35	19	Nd	26
Tae	20	50	Nd	29	16	Nd	29
Tae late	13	40	Nd	25	15	Nd	23
Pro + Tae	9*	51	Nd	35	14	Nd	27
Pro early/Tae late	7*	20	Nd	31	15	Nd	19
DYs	15	53	Nd	29	14	Nd	28
Pro early/DYs late	7.5*	33	Nd	33	14	Nd	22
P	.0032	Ns		Ns	Ns		Ns
$LSD_{0.05}$	7.3						
DON (ppm)							
Control	*Tbd*	*1.4*	*2.0*	*16.9*	*5.9*	*Nd*	**6.5**
Pro	Tbd	0.58	1.4*	14.4*	4.5*	Nd	5.2*
Tae	Tbd	0.7	2.2	16.0	4.8*	Nd	5.9
Tae late	Tbd	0.9	2.1	15.9	5.5	Nd	6.1
Pro + Tae	Tbd	0.8	1.3*	12.8*	4.0*	Nd	4.7*
Pro early/Tae late	Tbd	0.7	1.1*	11.7*#	4.0*	Nd	4.4*#
DYs	Tbd	1.1	2.1	16.7	5.2	Nd	6.3
Pro early/DYs late	Tbd	0.9	1.1*	11.0*#	3.6*	Nd	4.1*#
P		Ns	.0003	.0001	.004		<.0001
$LSD_{0.05}$			0.63	2.5	1.1		0.6

* = Value is significantly lower than the control at the 95% confidence level
\# = Value is significantly lower than Prosaro at the 95% confidence level
Ns = not significant, i.e., *P*>0.1
Nd = No data
Tbd = to be determined

EVALUATION OF MATING PHEROMONE PEPTIDES FOR INHIBITION OF WHEAT SPIKELET INFECTION BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

Anti-fungal peptides are an emerging area of antibiotic therapy with potential application for control of Fusarium head blight. Improved blight control strategies can be based on the deployment of small peptides that interrupt critical steps in the *F. graminearum* life cycle. Recent in vitro experiments performed in our laboratories have established that mating pheromone peptides derived from *F. graminearum* and other ascomycetous fungi can inhibit germination of pathogen ascospores. Similarly, peptides have been selected from combinatorial peptide libraries that bind with *F. graminearum* ascospores and inhibit germination as well as further germling development. Recently, we began to integrate laboratory and greenhouse studies to expand evaluations of the potential for these peptides to protect wheat from infection by *F. graminearum*. If shown to be effective, these inhibitory peptides could be applied as a protective spray to wheat during flowering or alternatively, deployed in transgenic wheat. Our current experiments initially established the protective efficacy of mating pheromone and combinatorial peptides when applied at various concentrations to wheat spikelets. Each tested peptide was chemically synthesized and applied to individual spikelets in combination with a water droplet containing pathogen ascospores. Only 1% of spikelets were infected by ascospores in the presence of 20 μM Pgz, the mating pheromone peptide derived from *F. graminearum*. Pathogen mycelial growth on spikelets was also severely reduced at this peptide concentration. Significant reductions in percentage spikelet infection were also obtained with a representative combinatorial peptide at this concentration. The protective effect of either type of peptide declined with decreasing concentration. We have begun to evaluate the protective efficacy of additional mating pheromone peptides and their derivatives when compared to Pgz as a standard treatment. These assessments are being made over a range of concentrations to identify the best peptides for larger-scale greenhouse trials in which peptides are displayed on a carrier protein scaffold and applied as a protective spray to flowering wheat heads.

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