SESSION 2:

FHB MANAGEMENT

Co-Chairpersons: Christina Cowger and
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AGGRESSIVENESS AND DON PRODUCTION OF *FUSARIUM GRAMINEARUM* 3ADON AND 15ADON POPULATIONS AS AFFECTED BY WHEAT CULTIVAR RESISTANCE AND FUNGICIDE TREATMENT, UNDER ND FIELD CONDITIONS, 2009

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INTRODUCTION

Fusarium head blight (FHB) is primarily caused by *Fusarium graminearum* in North America. The disease affects yield, by reducing harvestable kernel numbers and weight, and affects quality, by contaminating grains with mycotoxins produced by the fungal pathogens. FHB management is mainly through use of wheat cultivars with moderate FHB resistance and through use of fungicides, when warranted. Population studies indicate that *F. graminearum* isolates can be identified having one of three chemotypes (15ADON, 3ADON and NIV) and isolates of 15ADON chemotype were predominant in the population of North America (Ward et al. 2002; Gale et al. 2007). However, several recent studies have shown that the frequency of 3ADON isolates have increased dramatically in recent years (Burlakoti et al. 2008; Gale et al. 2007; Ward et al. 2008). The newly emerging 3ADON population appears to be more aggressive than the 15-ADON population based on growth rate, virulence and DON production in culture (Ward et al. 2008). Characterization of the *F. graminearum* isolates collected from 1980 to 2000 (old collection) and those collected in 2008 (new collection) in North Dakota showed that 3ADON isolates accounted for only 3% of the old collection, while 45% of the isolates in the new collection was of 3ADON chemotype (Puri and Zhong et al., unpublished results). Greenhouse inoculation studies in North Dakota also indicated that most of the 3ADON isolates were more aggressive and produced higher DON than the 15ADON isolates on susceptible and resistant cultivars (Puri and Zhong et al., unpublished results). However, little information is available on aggressiveness and DON production of *F. graminearum* 3ADON and 15ADON populations under field conditions. The objective of this study was to compare the 3-ADON and 15-ADON populations for FHB development and DON production in spring wheat under North Dakota field conditions, as affected by cultivar susceptibility to FHB and as affected by fungicide treatment.

MATERIALS AND METHODS

Two wheat cultivars, Alsen (with the fhb1 gene from Sumai 3 and moderately resistant to FHB) and Briggs (susceptible to FHB), were planted at the NDSU Agricultural Experiment Station at Fargo on May 8, 2009. A split plot experimental design was used with three replications, where cultivars were main plot, inoculum type was sub plot, and fungicide application was sub subplot. The experiment was conducted in a field where soybean was planted in the previous year. The plot size was 10 x10 feet. Three plots of each cultivar, with or without fungicide treatment, were inoculated (100K spores/ml) at anthesis (Feekes 10.51) on July 3 with either spore suspensions from ten 3ADON isolates (A), spore suspensions from ten 15ADON isolates (B), or a balanced mixture of spore suspensions from A and B isolates. The *F. graminearum* isolates used in the inoculation were a random subset of isolates from the large collection of Dr. Robert Stack from 1980 to 2000 and isolates from a new 2008 collection from farmers' fields in different counties of North Dakota. The chemotypes of these isolates were determined by PCR using the primers and the conditions described by Ward et al (2002).

For plots treated with fungicide, “Prosaro” (6.5 fl oz/acre) was applied 12 hrs prior to inoculations. The mixture of ten isolates of each chemotype was used to mimic a population of each type and minimize genetic background differences between the two populations. Three plots of each cultivar were left un-inoculated and unsprayed as checks. The disease incidence and severity data was recorded on July 23 (three weeks after inoculation) when the cultivars were at early dough stage (Feekes 11.1). To test for DON accumulation, 50 spikes with a disease severity of >66 %, based on the rating scale of Stack and McMullen (1995), from each treatment were tagged, harvested at maturity and kept separately in zip lock bags. These grain samples were sent to the Veterinary Diagnostic Laboratory, NDSU, for DON analysis.

RESULTS AND DISCUSSION

FHB incidence and severity in spring wheat inoculated with 3ADON and 15ADON isolates

Due to the prolonged cool and dry weather throughout 2009 wheat growing season in North Dakota, conditions were not conducive for FHB development. This was reflected in the relative low disease incidence in all non-inoculated plots. The checks (without inoculation and without fungicide treatment) had traces of FHB, with very low incidence (2.0% and 2.3% in Briggs and Alsen, respectively) and low head severity (7.0% and 4.6% in Briggs and Alsen, respectively), suggesting that natural infection was very low and would not have a large impact on the results of the inoculated plots. As expected, disease incidence and severity were higher in the inoculated plots compared to the checks in both susceptible and resistant cultivars. In the plots without fungicide treatment, Briggs had higher disease incidence and severity than Alsen, but no significant differences in incidence or severity were observed between 3ADON and 15ADON isolate inoculations in either of the two cultivars (Table 1). Fungicide treatment significantly reduced FHB incidence and severity in both cultivars, generally by 50% or greater, compared to the untreated, but no significant differences were observed between the 3ADON vs 15ADON isolate inoculations, except for a higher severity in Briggs with the 3ADON isolate inoculations. Further field experiments are needed to test the interactions among fungicides, chemotypes and wheat genotypes.

DON production in grains harvested from spring wheat inoculated with 3ADON and 15ADON isolates

DON analysis from non-fungicide treated grain heads showing 66%=> FHB severity indicated that the 3ADON isolates produced approximately double (149.5 ppm) the amount of DON produced by the 15ADON isolates (77.7 ppm) in the FHB susceptible wheat cultivar Briggs. The DON level accumulated in the FHB moderately resistant cultivar Alsen was approximately one-third that found in susceptible Briggs, but the 3ADON isolates produced 32% higher DON (43.2 ppm) than the 15ADON isolates (32.7 ppm) (Table 1). It is notable that in non-fungicide treated Briggs, both 3ADON (2.4 ppm) and 15ADON (2.6 ppm) in addition to DON were detected in grains inoculated with the 15-ADON isolates, while only 3ADON was detected in grains inoculated with the 3ADON isolates. In the treatments with fungicide application, DON level was reduced compared to the untreated inoculated plots. DON accumulation was reduced with fungicide treatment by about 46% in Briggs for the inoculations with 3ADON isolates and by 41.7% for the 15ADON isolate inoculations. However, in fungicide treated plots of Briggs, the DON also was higher (80.8 ppm) in grains inoculated with the 3ADON isolates than those inoculated with the 15-ADON isolates (45.3 ppm). In Alsen, fungicide treatment reduced DON by 63.6% for the inoculations with 3ADON isolates and by 63.9% for those with 15ADON isolates (Table 1).

In summary, these preliminary studies have indicated that the 3ADON isolate inoculations accumulate higher DON than the 15ADON isolate inoculations in either of the cultivars tested although disease severity differences were not significant between the two inoculations. These preliminary studies have also indicated the value in using variety resistance and fungicide treatment in reducing FHB disease and DON levels, regardless of the chemotype of the inoculum source.

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Table1. Effect of *Fusarium graminearum* 3ADON and 15ADON populations on FHB development and DON production under field conditions in North Dakota.

Treatment	FHB		Trichothecene (ppm)		
	Incidence (%)	Severity (%)	DON	3ADON	15ADON
Briggs inoculated with A	22.00	43.87	149.50	8.20	<0.5
Briggs inoculated with B	22.00	43.29	77.70	2.40	2.60
Briggs inoculated with A+B	26.00	49.59	101.00	5.10	2.00
Briggs sprayed with F and inoculated with A	5.00	27.87	80.80	4.30	0.5
Briggs sprayed with F and inoculated with B	5.30	18.82	45.30	0.60	3.10
Briggs sprayed with F and inoculated with A+B	11.00	26.24	73.00	5.10	1.20
Briggs without treatments	2.00	7.00	<0.50	<0.50	<0.50
Alsen inoculated with A	9.66	31.46	43.20	2.00	<0.50
Alsen inoculated with B	9.66	29.78	32.70	<0.50	1.30
Alsen inoculated with A+B	18.33	34.28	34.40	1.00	0.50
Alsen sprayed with F and inoculated with A	4.33	15.33	15.70	<0.50	<0.50
Alsen sprayed with F and inoculated with B	4.00	14.25	11.80	<0.50	0.50
Alsen sprayed with F and inoculated with A+B	4.33	10.47	3.80	<0.50	<0.50
Alsen without treatments	2.33	4.66	<0.50	<0.50	<0.50

Briggs = FHB susceptible variety; Alsen = FHB moderately resistant variety with Sumai3 heritage
A=a mixture of spore suspensions (100K spores/ml) from ten 3ADON isolates, B= a mixture of spore suspensions from ten 15ADON isolates, F=fungicide "Prosaro" (a mixture of prothioconazole and tebuconazole, applied at 6.5 fl oz/acre at anthesis, Feekes 10.51)

EFFECTS OF WITHIN-FIELD CORN DEBRIS IN MICROPLOTS ON FHB AND DON IN TEN U.S. WHEAT ENVIRONMENTS IN 2009

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ABSTRACT

Knowledge of the relative contribution of within-field inoculum sources of *Gibberella zeae* to infection of local wheat and barley is important for developing and/or excluding strategies for managing FHB. 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 ten variable wheat environments in 2009, 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 two commercial-scale wheat fields in Illinois, Missouri, Nebraska, New York, and Virginia, each following an FHB nonsusceptible crop. Over these environments we encountered six severe epidemics (in Illinois, Missouri, and Virginia), two moderate epidemics (in New York), and two mild epidemics (in Nebraska). Locally overwintered, natural corn stalks were collected in spring from two different sources in each state or locale by placing a 33 inch diameter plastic 'Hoola Hoop' onto four 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 (four) microplots containing corn debris and without 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 'Hula Hoop', fastened with plastic zip-ties, and secured to the soil with metal ground staples. Wheat heads above each microplot were rated at soft dough stage for FHB incidence, severity, and index. At grain maturity, at least 100 heads from each microplot were harvested, dried and shipped to Cornell where grain was threshed from a subsample of heads and sent to Virginia Tech for DON analysis. Only in one field in Virginia did wheat heads from microplots containing locally overwintered corn debris show a slight but statistically significant increase in FHB incidence and index over those from microplots with no corn debris. The astounding result is that DON level did not differ significantly between corn debris and no debris microplots in any of the ten wheat environments. By inference of our results, it appears that elimination of corn debris from single wheat fields in a major corn producing region may have rather limited benefits in terms of reducing FHB and especially of reducing DON contamination of grain. The experiments will be repeated in ten additional environments in 2010.

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HOST RESISTANCE TO *FUSARIUM* METABOLITES: RELEVANCE OF MASKED MYCOTOXINS FOR RESISTANCE BREEDING AND TOXICOLOGY

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ABSTRACT

The working hypothesis of the special research program FUSARIUM at BOKU is that secondary metabolites of the fungal pathogen can suppress defense responses in host plants, thereby causing a lack of gene-for-gene interactions and the ability to cause disease on a broad range of host plants. According to our hypothesis, quantitative and polygenically inherited differences in host resistance are due to differences in the ability to antagonize such secondary metabolites. This can be achieved by multiple mechanisms. The products of multigene families are responsible for drug efflux across the plasma membrane (e.g. PDR genes), detoxification by conjugation to sugars (UDP-glycosyltransferases, UGTs) or glutathione (GSTs), and sequestration of conjugates in the vacuole (Multidrug Resistance-Related Proteins, MRPs). The *Fusarium graminearum* genome encodes multiple predicted secondary metabolite biosynthetic genes/clusters, such as terpenoide synthases, polyketide synthases and nonribosomal peptide synthases. For most of these the corresponding metabolites and their mode of action *in planta* are currently unknown, and the virulence function is expected to be masked by redundancy. The trichothecene deoxynivalenol (DON), a known virulence factor, is also acutely toxic for humans and animals and therefore received most of the attention. It was shown previously that the ability to detoxify DON into DON-3-O-glucoside (D3G) co-localized with wheat DON resistance and a major QTL for *Fusarium* spreading resistance. Since this QTL is heavily used by breeders, it is toxicologically relevant to which extent D3G accumulates and whether it is a “masked mycotoxin”. D3G is heat stable and also unaffected by the acidic pH in the stomach, and it is not hydrolyzed by the product of the human cytosolic β-glucosidase, or the commonly used almond β-glucosidase. Yet, certain intestinal bacteria can hydrolyze D3G, and reform the parental toxin DON. UGTs are encoded in diploid grasses by a family of 140+ genes. Recently we succeeded to identify a D3G forming UGT of barley. Furthermore, evidence for formation of unstable glutathione conjugates of DON has been obtained. For breeding purposes it seems important to consider also other virulence mechanisms of *Fusarium*. For instance, we could show that the metabolite zearalenone (ZON), which is known for its estrogenic activity in animals, is an inhibitor of ATPase activity of heat shock protein 90 (Hsp90). Formation of zearalenone-4-O-glucoside (Z4G) leads to a loss of ATPase inhibitor activity. Hsp90 is an important player in plant resistance. ZON-inactivation could therefore also be relevant for resistance breeding, at least in maize.

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POPULATIONS OF *BACILLUS* STRAINS APPLIED TO WHEAT HEADS FOR BIOLOGICAL CONTROL OF FHB: RESULTS OF BROOKINGS, SD 2009 FIELD PLOTS

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ABSTRACT

Following spray application of biological control agents (BCAs) onto grain heads for control of FHB, evaluating numbers of BCAs on the inoculated grain heads is important to understand how the BCAs colonize and grow on plant surfaces, and how they might act against FHB. We have focused our research on *Bacillus* strains 1BA and 1D3 for use as BCAs to control FHB. In the 2009 biocontrol plot trials conducted at Brookings, SD using most probable number (MPN) methodology employing high temperature and high salt selection in the MPN growth media, control plots that did not receive spray application of BCAs had very low bacterial numbers, being at most in the hundreds of cells per gram fresh plant mass, indicating that a small number of native bacteria can tolerate the high salt and temperature conditions used in recovering and counting the BCA bacterial strains. The plots inoculated with BCAs also had very low bacterial numbers, not statistically different from the control plots. This is the first year since 2006 when we started tracking BCA populations on grain heads using the MPN method that there has not been a noticeable difference between control and treatment plots.

Although BCA numbers were apparently low, results of the BCA trails in Brookings indicated several significant differences between control and BCA treatments. A new growth medium formulation was used in summer of 2009 for growing the BCAs, which might help account for both the change in MPN counts of BCAs and the desirable effects the BCAs apparently had on reducing measures of FHB in the Brookings plots. Whether the modified growth medium enhanced production of antifungal lipopeptides by the BCAs, resulting in the treatment differences in the Brookings plot trial, is not yet clear. Laboratory studies with *Bacillus* strains 1BA and 1D3 grown in the new broth formulation showed apparent differences between the two strains in the amount of lipopeptide produced, as indicated by an oil droplet collapse assay. *Bacillus* strain 1D3 appears to have greater biosurfactant activity than strain 1BA.

Wheat heads inoculated with the BCA strains were processed for extraction of bacterial DNA, and using primers specific for the surfactin and iturin genes, PCR was carried out on the extract to see if there was evidence of surfactin genes on the grain heads. The yield of DNA from grain heads was very low, making the PCR difficult. Future work will use a greater number of grain heads for DNA extraction, to provide enough DNA for PCR to be successful.

PROGRESS ON MODELING DEOXYNIVALENOL IN BARLEY

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INTRODUCTION

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe), continues to be a serious problem for barley producers in the U.S. Northern Great Plains and elsewhere. Economic losses associated with FHB occur because of the blighting of florets (reduction in grain number), disruption of grain fill (shriveled kernels leading to lower test weight), and most importantly through the contamination of grain with trichothecene mycotoxins, primarily deoxynivalenol (DON). Tolerances for DON in malting barley are generally lower than those for food or feed-grade barley because of the association between DON, *G. zeae*-infested kernels, and gushing in beer (Garbe, 2009). 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 through the application of fungicides after spike emergence to reduce the risk of infection. Inoculum management has been documented to reduce both the number of *G. zeae* propagules reaching the spikes as well as the final DON concentration in the grain (Dill-Macky and Jones, 2000; Stein et al, 2009); however, this approach is not sufficiently effective in all situations since the spores of *G. zeae* can become airborne and travel moderate distances (Markell and Francl, 2003; Schmale et al., 2006). The application of fungicide is also not completely effective at preventing FHB and DON accumulation, but may provide some reduction in both (Yoshida et al., 2008). The timing of application is critical and therefore a need exists for a risk-advisory system that predicts the risk of an economic level of DON occurring in a malt barley crop.

OBJECTIVES

To identify the weather variables that were predictive of economic DON levels and to develop accurate risk model(s) that predict FHB and/or DON based on these variables.

MATERIALS AND METHODS

Experiments were conducted during the 2005-8 growing seasons using a set of regionally adapted, malting barley varieties at multiple locations in North Dakota and South Dakota (2005-8), and Minnesota (2005, 2007-8) or Montana (2006). At least three varieties, namely ‘Conlon’ (2-row), ‘Robust’ and ‘Tradition’ (both 6-row), were common at all locations. Plots were sown with a small-plot planter, a minimum of 1.5m x 4.6m in size, replicated four times in a randomized complete block design (RCBD), and maintained using standard agronomic practices for the region. The plots were not inoculated, nor were they misted or manipulated in any way to increase the probability of disease.

Crop growth was monitored regularly throughout the growing season and the date at which each variety was at 50% Feekes 10.5 (heads fully emerged) noted, hereafter this is referred to as the ‘heading day'. The incidence and severity of FHB was recorded on a minimum of 25 heads per plot at the soft-dough stage (18-21 days after heading). Plots were harvested at the end of the growing season using a small plot combine and DON concentration was determined from a random sample of 100 g grain from each plot. The mean DON concentration for all replicate plots of a singe variety at each location was used in the analyses. A binary response variable, eDON (economic DON), was created based on whether the mean concentration for each variety at every location*year met or exceeded 0.5 ppm. For example, if the mean DON concentration for a variety*location*year was 0.7 ppm it would be assigned a value of 1.

In addition to disease and mycotoxin data, nearby weather stations were used to record hourly environmental conditions for at least 10 days proceeding, and including, the heading day for each variety at all locations. At a minimum this included temperature, relative humidity (RH), and precipitation (incidence and rate). A total of 117 weather predictors were calculated from this data based on previous studies and trends observed in the data over 7- and 10-day intervals, which included the heading day itself (Andersen, 1948; De Wolf et al., 2003). For example, if a variety headed on June 30, predictors were obtained by summarizing the weather observations from June 24-30 (7-day) and June 21-30 (10-day).

Predictors were analyzed individually to evaluate their relation with eDON using univariate logistic regression and all predictors that were significant at a p-value of 0.25 were selected for further evaluation (Hosmer and Limeshow, 1989). The remaining predictors were divided into two subsets depending on the weather data interval used (i.e. 7- or 10-day intervals). Each subset was analyzed individually due to potential correlations between the predictors as the durations overlapped. A classification tree approach was used as a selection tool to identify the variables from the two subsets that were most associated with eDON (Harrell Jr., 2001). Box plots and stepwise regression methods were also used as a guide to obtain the most significant predictors from the two subsets. Interaction terms were calculated between the most significant predictors and evaluated using univariate logistic and stepwise regression procedures as noted above. The predictors that were most associated with eDON were reduced to five in total, three from 10-day subset and two from 7-day subset.

Logistic regression models were developed using the selected predictors from the two subsets by evaluating them in two and three variable combinations. Predictive power statistics such as generalized R^2 index of Nagelkerke (R^2_N), C-statistic, Somer's D_{xy} rank correlation, sensitivity, specificity, and prediction accuracy were calculated for each (Allison, 1999; Harrell Jr., 2001). In all cases, larger values correspond to stronger associations between the predicted and observed values. In order to achieve the greatest prediction accuracy, the probability of a positive eDON value (p*) was selected at which the sum of sensitivity and specificity was highest (De Wolf et al., 2003). Akaike's Information Criterion (AIC) was used to compare the regression models.

RESULTS AND DISCUSSION

A total of 43 location*years over four years were used in modeling process. The 6-row cvs. Robust and Tradition tended to have numerically higher DON concentrations than the 2-row cv. Conlon (Figure 1); however, variety was found to be not significant and was excluded from further analyses. Ninety-four predictors were selected in the univariate analyses with 46 and 48 being from the 7- and 10-day intervals, respectively. Overall, predictors that included some measurement of humidity were more strongly associated with eDON than those without.

The classification tree and stepwise procedures selected five predictors, of which three were from the 10-day subset and two were from the 7-day subset (Figure 1). For the 10-day subset, the remaining predictors were the number of hours the air temperature was 26-34^{o}C (T2634_10), maximum duration of hours where relative humidity was continuously greater than 90% (DR_RH90_10) in the 10-day period, and the summation of a weighted-hour matrix (TR6_10). Specifically, TR6_10 was assigned a value of '0' for each hour if the temperature was <15°C or >28°C, a value of '2' was assigned if the temperature was 20-24°C, and a '1' assigned otherwise. For the 7-day interval subset, two predictors were selected: maximum duration of hours with relative humidity greater than 90% continuously (DR_RH90_7) in the 7-day period and number of hours the air temperature was 20-28°C with relative humidity greater than 90% (T2028RH90_7). All the two and three term interactions in both 10-day and 7-day subsets were deleted in the stepwise regression procedures since they were not significant.

Twenty logistic regression models from the five identified predictors in one, two, and three variable combinations were developed (intercorrelated variables were not used together in a regression model) and the prediction accuracies of all models ranged from 73-94% (data not shown). Two models were identified with p*≥0.3, R^2_N ≥0.60, Somer's Dxy ≥ 0.80 and C-statistic ≥0.90 (Table 1). Both models had smaller AIC values compared to the remaining models with same number of predictors. Model A had four false positives and six false negatives, whereas model B had three false positives and 11 false negatives. The fit statistics were higher for model A than model B, with the exception of sensitivity. However, lower p* for model B might increase the probability of misjudging a non-economic DON event as an eDON event, which is evident from the false negative rates of model A and model B (4% for model A and 11% for model B). Both models were under consideration and further analysis on validation of these two models is ongoing.

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DISCLAIMER

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Table 1. Predicting power statistics for the selected logistic regression models.

#	Predictor1	Predictor2	AIC	C-Stat	R^2_N	Dxy	p*	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)
A	T2634_10	DR_RH90_10	61.1	0.92	0.67	0.85	0.4	83	96	94
B	T2028RH90_10	DR_RH90_10	69.1	0.90	0.61	0.80	0.3	88	89	89

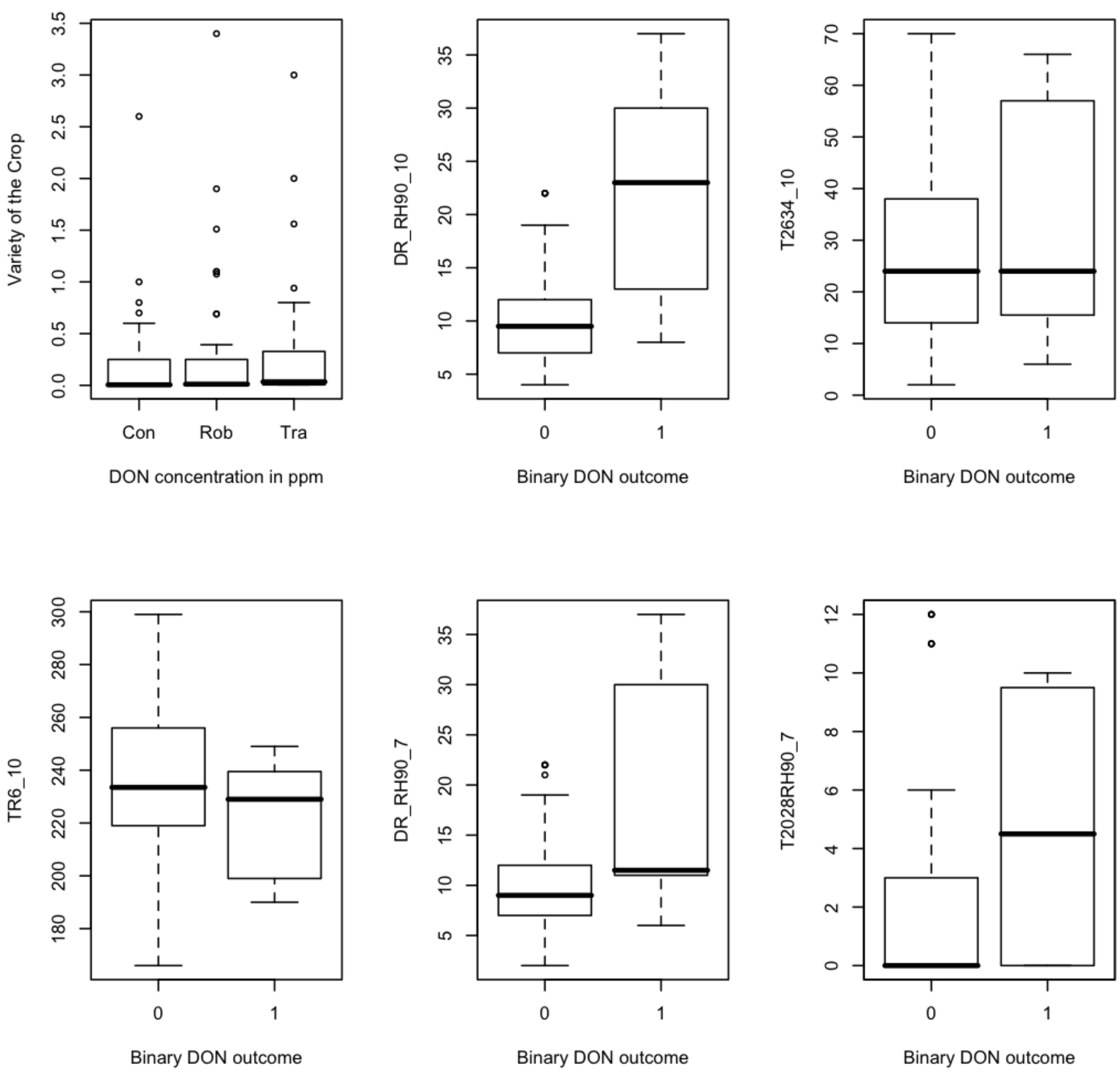

Figure 1. Box plots for variety of the crop based on the actual DON concentrations and the five most predictive variables divided based on non-economic (0) and economic (1) DON events.

USING FORECASTED WEATHER DATA AND NEURAL NETWORKS FOR DON PREDICTION IN BARLEY

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INTRODUCTION

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae* (Schwein) Petch (anamorph: *Fusarium graminearum* Schwabe), continues to be a serious problem for barley producers in the U.S. Northern Great Plains and elsewhere. Economic losses associated with FHB occur because of the blighting of florets (reduction in grain number), disruption of grain fill (shriveled kernels leading to lower test weight), and most importantly through the contamination of grain with trichothecene mycotoxins, primarily deoxynivalenol (DON). Tolerances for DON in malting barley are generally lower than those for food or feed-grade barley and discounts can be implemented if the level detected exceeds 0.5 parts per million (ppm).

Predictive models are being developed for estimating the risk of economic DON levels in barley (i.e. 0.5 ppm) using the 10-day interval leading up to, and including, the date of full head emergence. Generating risk advisories from such weather data does not allow for the pro-active management of FHB. That is, growers learn of highly conducive conditions <u>after</u> infection has probably occurred. The timing of fungicide application to limit losses from *G. zeae* is critical (Yoshida et al., 2008) and growers would be best served by having accurate risk advisories based on forecasted weather data so that management decisions could be made before infection occurs.

OBJECTIVE

To develop Artificial Neural Network (NN) model(s) that predict the risk of economic DON accumulation in barley (>0.5 ppm) based on 5-day forecasted weather leading up to the date of heading.

MATERIALS AND METHODS

Quality Controlled Local Climatological Data (QCLCD) and extended range forecast model output statistics (MOS) weather data were collected from 36 locations in the U.S. Northern Great Plains for the months of June and July, over an eight-year period (2001-08). Selected locations represent three geographical regions; namely, Red River Valley: 11 stations from Minnesota (MN), three stations from North Dakota (ND), and one station from South Dakota (SD); western ND (nine stations); and eastern South Dakota (11 stations from SD and one station from MN). The daily risk of economic DON accumulation in barley was calculated with the QCLCD data over each 10-day interval using the best available barley-DON logistic regression model (see other report by Bondalapati in these proceedings). This represented the 'Gold Standard' and was binary, where '0' represented no risk and '1' represented risk. The proportion of risk to non-risk days in the complete data set was 1:7, respectively.

For each date, 120 h (5 days) of forecasted temperature and relatively humidity were calculated from the MOS daily minimum and maximum values using an algorithm described previously (Baker and Kirk, 2007). Five additional data sets were created from this data using a combination of the QCLCD (true) and MOS (forecasted) hourly weather values by replacing 1-5 days of QCLCD data with an equal number of MOS data. That is, if June 10th was the day of full head emergence, weather data from June 1st-10th was used to predict the 'true' DON risk (response variable). In addition, QCLCD data from June 1st-9th plus MOS data from June 10th was used for a one-day forecast, QCLCD data from June 1st-8th plus MOS data from June 9th-10th was used for a two-day forecast, etc. In other words, increasingly larger proportions of the QCLCD (true) data set were replaced by MOS (forecasted) data until each 10-day interval consisted of 5 days each of QCLCD and MOS data.

To observe the impact of using forecasted data directly in the aforementioned barley-DON model, daily risk predictions were calculated for each combination of QCLCD and MOS data. That is, the risk was re-calculated for each 10-day interval after replacement one day of true (QCLCD) with forecasted (MOS) weather data, two days QCLCD with MOS, etc. These were then compared to those computed based on the QCLCD (true) weather and the accuracies for each combination were calculated.

Feed-forward back propagation NN with one hidden layer was then used to model the relationship between weather predictors and the outcome (Ripley, 1994). A random selection of 90% of observations was considered for training and the remaining were set aside for testing. Since NN models do not perform very well on unbalanced data sets (Ha et al., 2005), the training set was balanced by sub-sampling of the non-risk class so that the each set had an equal number of observations in both risk and non-risk classes. The package "*nnet*" from the statistical software R was used to perform the analysis (Venables and Ripley, 2002). Four variables were considered as input variables in NN models and were obtained by summarizing weather variables from the barley-DON logistic regression model over QCLCD and MOS periods individually. For example, if the mean temperature of 10-day period was used in the barley FHB model, two variables were constructed with one being the mean temperature of QCLCD weather interval and other being the mean temperature of MOS weather interval. The optimal number of hidden nodes in the hidden layer was considered where the misclassification error rate was minimum. A larger number of hidden nodes in a NN model is extremely flexible and can approximate any smooth function; however, too many hidden nodes can result in low prediction on validation set (Venables and Ripley, 2002) and lead to poor accuracy, no matter how powerful a model is (Ha et al., 2005).

As the main objective of this research is to develop the NN model to predict the risk using 5-days forecasted data, additional potential weather variables were calculated for the fifth data set in order to increase the prediction accuracy for the NN model developed in the previous step. To obtain the most predictive variables from the set of 102 variables, traditional regression methodologies, such as univariate logistic regression, stepwise variable selection, and regression trees were used. Variable selection prior to model development was recommended in order to reduce the noise from unnecessary predictors (Faraway, 2002). NN models were then developed on the selected predictors and the performance of the each was examined in the region as well as for sub-regions.

RESULTS AND DISCUSSION

The performance of logistic regression model decreased with the inclusion of forecasted days (Table 1). The total prediction accuracy was 98% in case of one-day forecast and only dropped to 93% when extended to five-day forecast. However, the sensitivity dropped rapidly from 91% to 52%. Here the high prediction accuracies with low sensitivities were due to high proportion of non-risk days. From Table 1, it is evident that the efficiency of the logistic regression model was substantially reduced in predicting the true risk with the inclusion of forecasted weather data.

The optimal number of hidden nodes for NN models varied with the number of forecasted days (Table 2) due to complexity in the data. The number of hidden nodes was only 1 in case of the one-day forecast, whereas it was 5 in case of five day forecast. The performance of NN models were substantially better than the logistic regression models with the total prediction accuracies ranging from 94%-86% and sensitivities from 93% to 73%.

In the case of the five-day forecast, six variables were selected as the most predictive and were divided into two subsets. The first subset had four variables and second subset had five variables with three common to both subsets. Five and seven hidden nodes were selected as the optimal number based on the misclassification error rate and four NN models were developed with the optimal number of hidden nodes for each subset of predictors. The prediction accuracies of the four NN models varied from 81% to 84% (data not shown). Since four models had approximately equal prediction accuracies, the model with less number of parameters (four inputs and five hidden nodes) was selected for further evaluation and the prediction accuracies by each geographical region calculated (Table 2). The selected NN model had high accuracy in predicting risk days (sensitivity) in the Red River Valley region and high accuracy in predicting non-risk days (specificity) in western ND.

ACKNOWLEDGEMENTS

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Table 1. Prediction accuracies when comparing the logistic regression model response on historical data with logistic regression model response and NN model response after including the forecast data.

Number Forecast Days	Logistic Regression Model			Single hidden layer NN			
	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)	Architecture	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)
1	91	99	98	4-1-2*	93	92	92
2	82	99	97	4-3-2	86	95	94
3	73	99	96	4-5-2	81	93	92
4	63	99	94	4-5-2	80	89	88
5	52	99	93	4-5-2	73	88	86

* - 4 input variables, 1 hidden node and 2 output variables.

Table 2. Geographical prediction accuracy of NN model using five-day forecast data. The architecture of NN model is 4-5-2*.

	Sensitivity (%)	Specificity (%)	Prediction Accuracy (%)
Total Region	72	86	84
Red River valley region	74	84	82
Eastern South Dakota	71	84	82
Completely Dry region	68	91	89

* - 4 input variables, 5 hidden node and 2 output variables.

APPLICATION TIMINGS OF CARAMBA AND PROSARO FOLIAR FUNGICIDES FOR MANAGEMENT OF FHB AND DON

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ABSTRACT

As part of the USWBSI – funded uniform fungicide trial project for control of Fusarium head blight (FHB) and mycotoxins in wheat, a multi-state project was initiated to evaluate the efficacy of Caramba (metconazole; BASF Corporation) and Prosaro (tebuconazole + prothioconazole; Bayer CropScience) when applied at different Feekes growth stages (FGS). In field trials established at multiple locations in Arkansas, Illinois, Maryland, Michigan, Minnesota, North Dakota, and South Dakota, these two products were applied at FGS 10.5, 10.5.1, and five days following the 10.5.1 application. Caramba was evaluated at 13.5 fl oz/A, and Prosaro at 6.5 fl oz/A. A combination of Caramba + Proline (prothioconazole; Bayer CropScience) at 7 + 3 fl oz/A applied at FGS 10.5.1 also was evaluated. The trials were conducted on wheat cultivars in different market classes which included soft red winter, soft white winter, hard red winter, and hard red spring. The combination treatment of Caramba + Proline generally provided equal control of FHB and mycotoxins compared to Caramba or Prosaro treatments at most locations. In general, most applications of Caramba or Prosaro, regardless of application timing, significantly ($P \leq 0.05$) reduced FHB symptoms and mycotoxin levels in grain when compared to the untreated control; however, some application timings provided better fungicide performance than others, depending on location. These results indicate that the window of fungicide application for control of FHB and associated mycotoxins may be slightly wider than previously believed.

EFFECT OF PYRACLOSTROBIN APPLICATIONS TO WHEAT AT DIFFERENT GROWTH STAGES ON DON CONCENTRATIONS IN GRAIN

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ABSTRACT

As part of the USWBSI – funded uniform fungicide trial project for control of Fusarium head blight and mycotoxins in wheat, a multi-state project was initiated to evaluate the effect of pyraclostrobin (Headline fungicide applied at 6 fl oz/A; BASF Corporation) applications to wheat on deoxynivalenol (DON) concentrations in grain. Field research trials were established at multiple locations in Arkansas, Illinois, Maryland, Michigan, Minnesota, North Dakota, and South Dakota. These trials were conducted on wheat cultivars in different market classes which included soft red winter, soft white winter, hard red winter, and hard red spring. In addition to measuring DON concentration in grain, nivalenol (NIV) also was measured at the Arkansas location. At all locations, pyraclostrobin was applied at Feekes growth stage (FGS) 10.5. At some locations, pyraclostrobin also was applied at FGS 9 and 10.0. At the time this abstract was written, DON data were available for experiments at Fayetteville, Arkansas; Brownstown, Carbondale, Monmouth, and Urbana, IL; Clarksville, Michigan; Fargo, North Dakota; and Brookings, South Dakota. Concentrations of DON in the grain from the untreated controls ranged from 1.3 to 16.7 ppm. DON concentration was increased significantly ($P \leq 0.05$) in grain compared to the untreated control when pyraclostrobin was applied at FGS 10.5 at three out of eight locations, and when applied at FGS 10.0 at one out of six locations. Pyraclostrobin applications never significantly decreased DON concentrations compared to the untreated control. At the Arkansas location, pyraclostrobin applied at FGS 10.5 significantly increased NIV concentration compared to the untreated control (3.1 vs. 2.2 ppm). These results indicate that an application of a quinone outside inhibitor (QoI) or strobilurin fungicide such as pyraclostrobin to wheat at FGS 10.0 or 10.5 may increase mycotoxin concentrations in harvested grain.

INFECTION TIMING AND MOISTURE EFFECTS ON DON AND FDK IN WHEAT

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ABSTRACT

The USWBSI winter wheat forecasting tool provides forecasts of FHB severity that are timely for fungicide decisions based on weather in the seven days prior to anthesis (DeWolf *et al*, 2005). FHB severity and deoxynivalenol (DON) levels are also influenced by post-anthesis moisture (Cowger *et al*, 2009). Building on that foundation, our work has aimed to elucidate the influence of variable infection timing and post-anthesis moisture on disease development and DON concentrations. The questions addressed include:

1) When is wheat vulnerable; e.g., does it only need to be protected at anthesis? What factors change that window of vulnerability?

2) To what extent are visual FHB symptoms and *Fusarium*-damaged kernels (FDK) predictive of DON problems, and what factors influence that relationship? When should we be on the lookout for crops with higher DON levels than symptoms and FDK would predict?

3) What happens to DON levels during mid- and late grain-fill? How do environmental conditions at harvest affect DON? Can this information be helpful in high-DON situations, and should we modify our harvest-timing recommendations?

Field experiments with soft red winter wheat cultivars in North Carolina have been conducted to help clarify the relationship between DON, infection timing, and post-harvest moisture.

A) Infection timing

The effect of post-anthesis infection was examined by spray-inoculating individual heads of several wheat cultivars at anthesis, watery-ripe, or late-milk stages (0, 10, or 20 days after anthesis, or daa) with 10^5 *Fusarium graminearum* macroconidia/ml. Plots were subjected to 0, 10, 20, or 20 daa of misting. The threshed grain was assayed for FDK, DON, and (in one year) percent infected kernels. The experiment was conducted for three years.

The results indicated that the window of maximum FHB susceptibility of winter wheat in North Carolina is generally less than or close to 10 days after mid-anthesis. Wheat needs to be protected during this entire time period, not just during anthesis itself. In two of three years, FDK and DON were correlated for inoculations at anthesis and watery-ripe stage, but not at late-milk stage, and were more strongly associated for 0 or 10 daa of misting than for 30 daa of misting. In other words, late infection and protracted moisture reduced the association between FDK and DON. Samples with plump kernels (≤4% FDK) and unacceptable levels of DON (≥2 µg/g) were infrequent in two years (18-19%) but

were more frequent in a third year (41%). The "low-FDK, high DON" scenario was associated with late infections and was maximized by marginal disease conditions.

B) Changes in DON and kernel damage during grain development

A two-year experiment was conducted to assess the changes in FDK, infected kernels, and DON over the course of grain development. Plots of seven cultivars were spray-inoculated with 10^5 *Fusarium graminearum* macroconidia/ml at mid-anthesis and subjected to 0, 10, 20, or 30 daa of misting as described in (Cowger *et al*, 2009). On six dates about 10 days apart, from milk stage to about 20 days after harvest-ripeness, 30 spikes were blindly harvested from each plot. The spikes were threshed and the grain was weighed and assayed for FDK, DON, and percent *Fusarium*-infected kernels.

In both years, sample weights increased to a maximum in the 3rd sample, which was taken at hard dough stage approximately 7-10 days before harvest-ripeness. Mean DON per unit of sample weight, averaged across mist treatments, declined from the first to the second sample, and then remained roughly level. Mean DON per 30-spike sample, again averaged across mist treatments, fell by 42-47% between milk and harvest-ripeness. At the same time that DON levels were dropping, percent FDK and percent infected kernels increased until after harvest ripeness in 2006, and until harvest ripeness in 2007. Misting increased early DON levels, percent FDK, and percent infected seed, and decreased sample weights, to a greater extent in 2006 than in 2007.

These results are consistent with reports that DON biosynthesis occurs immediately following infection, with DON acting as a virulence factor that allows fungal hyphae to move into the wheat rachis and spread to other florets (Jansen *et al*, 2005). Early in grain fill, DON production apparently stops, and DON concentrations are reduced by host detoxification (Lemmens *et al*, 2005) micro-organism degradation, and/or by the leaching action of water. At the same time, according to our data, *Fusarium* continues to spread within spikes. Of interest for DON management are the changes in DON concentration in the period immediately before, during, and after harvest-ripeness. This is particularly relevant since growers confronted with severe FHB have traditionally been advised to harvest wheat early. This recommendation may make sense with respect to FDK. However, in 2006, DON continued to decline in the higher-DON treatments until the 5th sample, several days after normal harvest timing. In 2007, DON levels declined or stayed constant over the last three samples.

In summary, our work indicates that post-anthesis moisture increases disease symptoms, kernel damage, and initial DON levels by enhancing fungal spread within the head. Although this spread apparently continues to occur throughout grain-fill, DON concentrations decline over time. Late infection and extended post-anthesis moisture reduce the correlation between kernel damage and DON, and may account for occasional observations of high DON in apparently sound grain.

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

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ABSTRACT

The TrigoCor strain of *Bacillus subtilis* is one of a handful of biological control agents (BCAs) that show 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 the greenhouse and in two upstate New York locations during the 2008 and 2009 field seasons. Using dilution plating, we quantified *Bacillus* populations on wheat heads at 0h, 24h, 72h, 7d, and 14d after *Bacillus* application. The population levels of *Bacillus* remained constant throughout the sampling period in the greenhouse (10^8 CFUs/head) and at both field locations in 2009 (10^6-10^7 CFUs per head), and increased over the first week from 10^4 CFUs/head to a constant level 10^6 CFUs/head in both field locations in 2008. In 2008 and 2009 we also recovered *Bacillus* from wheat heads in significant quantities (10^5 CFUs/head) at harvest, suggesting that this BCA may also be present in sufficient numbers to protect plants against late-season *Fusarium* infections. In addition to these hand-sprayed field trials, we also quantified population levels on field plots commercially sprayed with *Bacillus*. We consistently recovered *Bacillus* populations of 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. The insufficient FHB control of *Bacillus* in the field compared with the greenhouse, despite its consistent persistence in both environments, suggests that some factor other than inadequate survival is responsible for the inconsistent performance of this BCA in the field. The absolute quantity of *Bacillus* on wheat heads in the field was lower than in the greenhouse, particularly in the 2008 field season and in the commercially sprayed plots, indicating that a reduction in the total amount of *Bacillus* on heads in the field may be responsible for its limited biocontrol efficacy in this environment. In addition to bacterial population dynamics, we are assessing the production and persistence of antifungal metabolites relative to biological control in field environments. Using LC and MS technologies we are currently evaluating the presence of *Bacillus* lipopeptides on wheat florets collected from the field at time points parallel to our population dynamics studies.

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EVALUATING THE USE AND POTENTIAL IMPACT OF FUSARIUM HEAD BLIGHT PREDICTION MODELS IN THE U.S., 2009

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ABSTRACT

A multi-state effort to predict the epidemics of Fusarium head blight continued during the 2009-growing season. These web-based prediction models provide daily estimates of disease risk for 24 states that can be used by growers to evaluate the need for disease management action. The prediction models combine estimates of temperature and relative humidity to predict the risk of an FHB epidemic with greater than 10% field severity. The prediction system incorporates weather observations generated by the Real Time Meso-scale Analysis (RTMA), and weather stations provided by the National Weather Service (NWS). This information is used to generate maps of disease risk throughout the region. Networks of weather stations independent from the NWS are also used by the system to display local estimates of disease risk. The independent nature of these weather networks allows for comparison of the map-based and local estimates of disease risk. In 2009, the prediction tools received more than 8,850 visits between April and August, the period when wheat is actively growing in the 24 states. A user survey conducted in the same year (n=593) indicated that 70% of these users were either farm advisors or farmers. Other users of the system included university extension personnel and members of the grain marketing and milling industries. The survey also indicated that 77% of the users applied the information provided by the prediction system for direct on-farm management decisions, or providing recommendations for disease management. In 2009, 92% of the users considered the information to be of high or moderate value for their farm operations or organization. The estimated net value of the disease prediction system to U.S. wheat growers exceeds $47 million.

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CHARACTERIZATION OF THE SURFACE PROPERTIES OF WHEAT SPIKELET COMPONENTS

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OBJECTIVES

To characterize the physicochemical properties of the surfaces of glume and lemma tissues of wheat spikelets immediately before and after anthesis.

INTRODUCTION

The surface of the aerial parts of terrestrial plants are covered by a waxy cuticle which mediates the interactions between the plant and the environment. The chemical and physical properties of the plant surface determines the nature of these interactions. These properties play an important role in plant-microorganism interactions including influencing the likelihood of a microbial propagule adhering to the plant and subsequently colonizing the surface. In the case of Fusarium Head Blight (FHB), these propagules would include ascospores and macroconidia of *Gibberella zeae*, the primary causal agent of FHB. In addition, the propagules of some beneficial microorganisms also need to interact and adhere to the plant surface, such as biological control agents.

Knowledge of these physicochemical properties of the spikelet surface is also important for developing formulations of sprayable pest control products. Understanding how a spray droplet interacts with the target surface can help in guiding formulation decisions. In order for a spray droplet to adhere to a surface, the droplet must first be able to wet the surface. In general terms, for a liquid to wet a solid, the surface tension of the liquid must be lower than the surface energy of the solid. Most of the targets for pest control spray application are low surface energy targets (plant surfaces). These types of surfaces are commonly referred to as being hydrophobic, since they repel water or the interaction with water is not energetically favorable. Items with a low surface energy are difficult to wet with aqueous solutions, since the surface tension of water (surface energy in the case of a solid) must be reduced below that of the solid for wetting to occur. In order to reduce the surface tension of aqueous solutions low enough to wet these surfaces, surfactants are added, which greatly lower the surface tension of water.

These surface properties are also needed as parameters for accurate spray droplet adhesion models such as the model of Forster (Forster et al. 2005) which incorporates spray droplet size, velocity, surface angle, dynamic surface tension and leaf contact angle measurements. These models can help provide guide decisions when developing new formulations or application technology for a specific crop system.

MATERIALS AND METHODS

Wheat samples - Field trials were conducted in Peoria, IL in 2009. Soft red winter wheat cultivar Freedom (moderately resistant to FHB) and Pioneer Brand 2545 (susceptible to FHB) were grown using standard agronomic conditions (Schisler et al. 2006). Whole wheat heads were cut from plots at split boot (early Feekes 10.1), out of boot (Feekes 10.1), flowering (Feekes 10.5) and at 4, 8, and 12 days after flowering. Heads were stored in zip lock bags on ice until analysis.

Contact angle measurements – To determine the contact angle measurement of glume or lemma tissues, samples were removed from a wheat spikelet and affixed to a microscope slide using doublesided tape. Contact angles were determined using a FTA4000 video drop shape analysis system (First Ten Angstroms, Portsmouth, VA). A droplet of 5 µl was deposited on to the sample and the image recorded every 100 milliseconds for 15 seconds. Under this time regime, the droplets reached equilibrium. The droplet images were analyzed using software provided by the instrument manufacturer. The images were fit to a non-spherical model. Ten replicate samples were tested for each sample and each liquid.

RESULTS AND DISCUSSION

Water contact angle measurements – The contact angle of a water droplet was determined for the wheat spikelet components (glume and lemma) for samples immediately before and after anthesis. The measurements were conducted on wheat cultivars Freedom and Pioneer Brand 2545. The results for glumes are reported in Figure 1A. The results show the glumes become slightly more hydrophobic leading up to and peaking at anthesis. Immediately after anthesis, a significant drop in hydrophobicity was observed. The most notable difference between the two wheat cultivars was during anthesis when moderately FHB resistant cultivar Freedom had a contact angle approximately 10 degrees higher than Pioneer Brand 2545.

The results for lemma tissue are reported in Figure 1B. The results show a similar pattern as found for glumes, with a drop in hydrophobicity after anthesis. The two cultivars were similar in their properties for the lemma at the different sample times.

Water/acetone contact angle measurements – The contact angle of a 1:1 (vol), water:acetone solution has been used to estimate the surface roughness of various leaves (Forester and Zabkiewicz 2001). It is also needed as a parameter in the spray droplet adhesion model(Forster et al. 2005). The results for the glume tissue for both cultivars are reported in Figure 2A. The results show a similar pattern as observed with the water only contact angle measurements. These results also suggests the glume of the two wheat cultivars may have a slightly different "roughness" or surface morphology. The acetone/water contact angle of the lemma tissue for both cultivars are reported in Figure 2B. The changes in the lemma tissue are less dramatic than those of the glumes although both show changes occurring at or immediately after anthesis.

Overall, these results suggest the surface chemistry and surface ultrastructure of the glume and lemma is changing during anthesis for these cultivars. These changes can also alter the ability of microorganisms to adhere to these surfaces (Lindow and Brandl 2003). It has previously be shown these properties can alter the binding of pathogen conidia to wheat surfaces (Stosch et al. 2007). These properties also play a role in pre-harvest sprouting, by regulating ear wetting via water repellence (King and Von Wettstein-Knowles 2000). Ultimately, it is our goal to determine the role of these properties in microbial colonization of these surfaces. This will include both colonization by the FHB pathogen and beneficial microbes (biocontrol organisms). This data will also be used to optimize pest control formulations for improved delivery and retention to the wheat head.

ACKNOWLEDGEMENT AND DISCLAIMER

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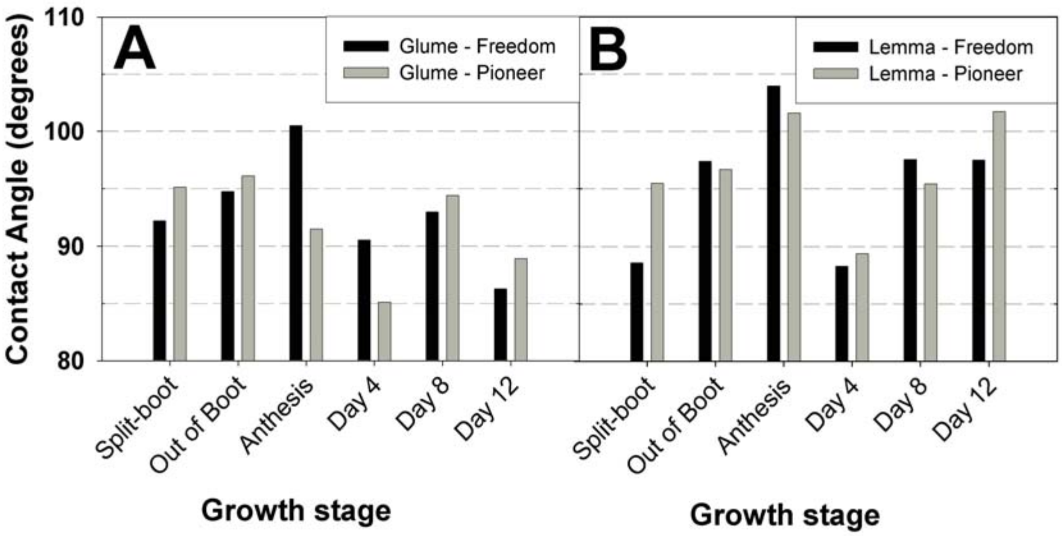

Figure 1. The contact angle of a water droplet on the: **A.)** glume and **B.)** the lemma of two wheat cultivars at different growth stages.

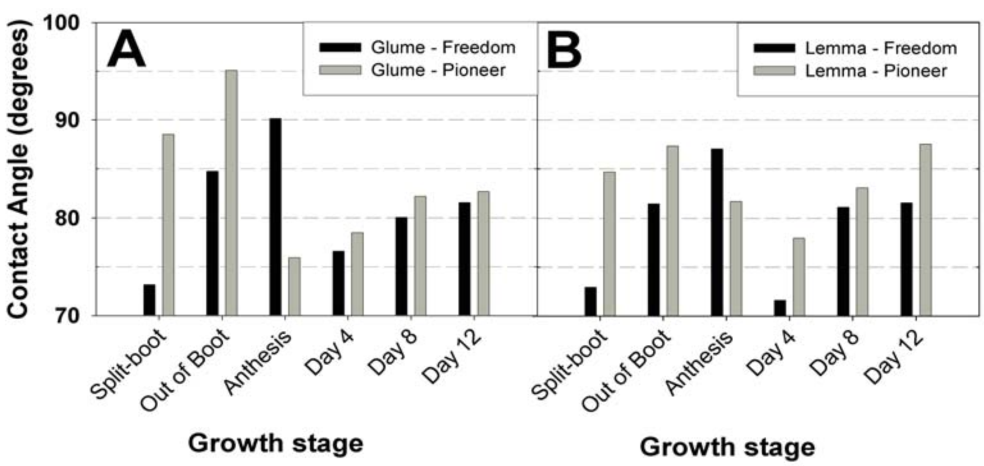

Figure 2. The contact angle of a 1:1 (vol), water:acetone droplet on the: **A.)** glume and **B.)** the lemma of Freedom and Pioneer Brand 2545 cultivars at different growth stages.

HEAD BLIGHTERS AND BLASTERS OF WHEAT: ARE WE READY?

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ABSTRACT

Wheat disease-causing agents can colonize different plant organs including the heads. These are of great importance because of the direct impact on the economic product of wheat – the kernels. In Brazil, there is a great concern about the increasing threat that two fungal diseases, known as wheat blast and head blight, now pose to wheat production. Wheat blast is incited by *Magnaporthe grisea* (*anamorph = Pyricularia grisea*). This disease was first described in wheat, in 1985, in northern Paraná, Brazil. Since then it has been reported in all wheat growing areas of the country. Up to this far, wheat blast occurrence is restricted to lower latitude wheat growing areas as those in Brazil, Paraguay and Bolivia. The disease may cause severe damage under conditions of high temperature (28°C) and high humidity (>93%). Fusarium head blight caused by *Gibberella zeae* is currently one of the most important diseases of wheat worldwide. Epidemics have been observed with a higher frequency in recent years in several regions, with damage on both yield and grain quality. In Brazil, the pathosystem has been studied for more than three decades, and recent reports indicate that a previous disease, previously sporadically outbreaking, achieved the status of major disease in wheat growing regions of southern Brazil causing significant economic impacts. Fusarium head blight is described as a disease of warm and humid climate, so that the rainfall and temperature are the main factors that influence the occurrence and severity of epidemics of this disease. Both fungi have other hosts besides wheat and can survive on crop residues. Depending on the region and time of the year, low temperatures or long dry periods may prevent growth and development of these fungi. Mechanistic simulations models have been developed for both diseases. The disease models take into account host development including details of the heading process (i.e., proportion of heads emerged, anther extrusion, grain filling and physiological maturity). Inoculum is considered not limiting. Hourly observed and 5-day forecast data for precipitation, relative humidity and temperature are used in mathematical equations to estimate infection risks. The models are implemented in a web platform which is intended to provide risk information to assist decision-making on crop management. An important wheat producing area in Brazil characterized by warm temperatures and moderately dry, is comprised by the North of Paraná, São Paulo and South of Mato Grosso do Sul states. This area, despite the likely occurrence of water stress during pre-flowering in some years, is considered a favorable environment for wheat production in terms of yield potential and quality. During the growing season of 2009, an abnormally high frequency of rainy days in July and August was observed. In the state of São Paulo, for example, July rainfall had record precipitation, four times the normal, since meteorological observations started in 1943. The resulting humid and warm climate observed in July coincided with the heading stage of wheat. Consequently, there were outbreaks of both Fusarium head blight and wheat blast. Crop yield declined 23% from pre-season estimates. The harvested product has been rejected by the milling industry due to low quality for bread and pasta making. Farmers suffered heavily from this climatic condition that resulted in crop failure due to head blighters of wheat. Continued global warming is likely to exacerbate Fusarium head blight problem. Moreover, it may contribute to the expansion of the geographical limits of new diseases like wheat blast. This hypothesis is supported by empirical evidence of the occurrence of wheat blast in

more southern regions of Brazil, which may relate to the warmer winters that have occurred in recent years. Therefore, efforts need to be made for better understanding of damaging head diseases of wheat in order to reduce impact on grain yield and quality, especially the mycotoxin issue related to Fusarium head blight. Genomic-based approaches promise to make a large and immediate impact through the identification of genes for disease resistance. However, the goal of lasting head blight disease control will depend on having an equally comprehensive understanding of the disease process from an epidemiological perspective.

RISK MAPPING FUSARIUM HEAD BLIGHT OF WHEAT IN BRAZIL

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ABSTRACT

Available epidemiological knowledge was previously used for developing a simulation model to predict Fusarium head blight infection risk in southern Brazilian conditions. The model has been successfully validated and incorporated into a web-based system to warn of FHB risk within-season using both site-specific observed and 5-day forecast weather. We have further used the model to assess disease risk under the influence of climate variability, especially under the effect of El Niño southern oscillation, and management practices (sowing dates) by using historical (50-year) records of weather data for a single location. We are now working on the development of tools to map disease risks over a broader geographic region. FHB risk maps are computer-generated images depicting the risk using special interpolation techniques within points indicated by the geographical location of automated weather stations. The final risk maps are made by color transparency layers which overlays a geographic map. Besides extending risk information for a large geographical region the use of intuitive images representing epidemic risks may facilitate dissemination and understanding of risks to guide decision-making on FHB management. In addition, maps may be useful for the fine tuning of wheat zoning and for the identification of post-harvest areas with lower probability risk of mycotoxin contamination.

AGRONOMIC FACTORS AFFECTING SPECIFIC MYCOTOXIN PRODUCTION IN FUSARIUM HEAD BLIGHT INFECTED WHEAT

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ABSTRACT

Several earlier studies have demonstrated a lack of correlation between the fungal biomass in grains from Fusarium head blight (FHB) infected ears of winter wheat and the level of mycotoxins such as deoxynivalenol (DON). However, the factors governing the specific mycotoxin synthesis are hardly known. In this study, results from experiments with four non-tillage crop rotations, three fungicide regimes and two cultivars run in two locations in Lower Saxony, Northern Germany, since 2007 are demonstrated. Pre-crops were wheat, sugar beets, maize and oil radish. A highly susceptible cultivar (cv. Ritmo) and a moderately resistant cultivar (cv. Centrum) were tested with three different fungicide regimes, one based on triazoles, a second on strobilurins and a third including chlorothalonil with no known physiological effects on the crop. Fungicides were applied twice on the foliage in growth stages (GS) 31 and GS 39, and no fungicides were applied after ear emergence or during anthesis. The FHB index was scored during milky ripening stage and compared with mycotoxin levels in grains after harvest. Furthermore, the fungal biomass of the predominant toxigenic species, *F. graminearum*, was measured with quantitative real-time PCR. The total DON content in wheat following maize was three times higher than following the other pre-crops. Similar levels of DON were recorded in wheat following either wheat or sugar beet pre-crops. Cultivar resistance reduced total DON content about 75%. Foliar application of strobilurins prior to ear emergence tended to increase the total mycotoxin level in grains. Specific mycotoxin production per µg *F. graminearum* DNA was not affected by fungicide treatments, while it was elevated in the susceptible cultivar and following maize and sugar beet pre-crops. However, specific toxin production was significantly affected by the location, indicating a predominant role for environmental factors in affecting the relative intensity of mycotoxin synthesis.

DEOXYNIVALENOL GENE EXPRESSION DURING WHEAT HEAD INFECTION BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is produced by several *Fusarium* species during infection of grain crops. *Tri5* encodes trichodiene synthase, necessary for DON production. Previous experiments, including Affymetrix GeneChip assays, indicated that *Tri5* is highly expressed during wheat infection. We dissected wheat heads from anthesis through kernel development with *Fusarium graminearum* and monitored *Tri5* gene expression using quantitative reverse transcript PCR (qRT-PCR). *Tri5* expression was compared with the expression of the housekeeping genes and relative abundances determined; the housekeeping genes also served as an indicator of fungal presence. The results present a detailed picture of fungal colonization and DON production during wheat head colonization.

EVALUATION OF BIOLOGICAL ALTERNATIVES FOR SINGLE TREATMENT FUNGICIDE ON HARD RED SPRING WHEAT FOR CONTROLLING FUSARIUM HEAD BLIGHT

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ABSTRACT

Several strategies using biological components were evaluated as alternatives to a single timing Prosaro fungicide treatment for reducing the effects of Fusarium head blight (FHB) in hard red spring wheat (HRSW) at Langdon, ND in 2008 and 2009. Biological alternatives tested were 1BA a *Bacillus* sp. provided by B. Bleakley from South Dakota State University, Brooking, SD, C3 *Lysobacter enzymongenes* from G. Yuen from University of Nebraska, Lincoln NE, TrigoCor 1448, *Bacillus subtilis* from G. Bergstrom, Cornell University, Ithaca, NY, a double yeast from D. Schisler of NCAUR, USDA-ARS Peoria, IL and Taegro, *Bacillus subtilis* var. amyloliquefacians from Novozyme Biologicals, Inc., Salem, VA, a product that was commercially available in 2008. Alternative strategies included a) biological treatment at Feekes growth stage (GS) 10.51, b) GS 10.54, c) a tank mix of Prosaro fungicide and a biological applied at GS 10.51 and d) Prosaro fungicide applied at GS 10.51 followed by a biological application at GS 10.54. A control treatment was included that received an application of water with the adjuvant Induce. All biological and fungicide treatments were applied with Induce at a v/v rate of 0.125%. The treatments were applied to Howard HRSW. Howard is a high yielding cultivar moderately susceptible to FHB from North Dakota State University. FHB incidence of the control ≥ GS 10.51 biologicals > Prosaro ≥ Prosaro/biological tank mixes ≥ Prosaro at GS 10.51 and GS 10.54 biologicals. FHB head severity of the GS 10.54 biological ≥ control ≥ GS 10.51 biologicals > Prosaro ≥ Prosaro/biological tank mixes ≥ Prosaro at GS 10.51and GS 10.54 biologicals. Deoxynivalenol (DON) concentrations from 2008 biologicals at GS 10.51> control > biologicals at GS 10.54 > Prosaro > Prosaro/biological tank mixes. Yield for the control ≤ biologicals at GS 10.51 and GS 10.54 < Prosaro/biological tank mixes and Prosaro fungicide.

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INTEGRATING RESISTANCE, BEST APPLICATION TIMING AND BEST FUNGICIDE DELIVERY TECHNIQUE FOR IMPROVED EFFICACY ON BARLEY, LANGDON, 2008

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ABSTRACT

Fusarium head blight (FHB) has reduced small grain yield and affected crop quality in the Northern Plains region of North America since 1993 and many other wheat and barley production regions worldwide. Genetic resistance to infection and spread of FHB will need to be combined with other management strategies to reduce losses in the short term. Control by fungicides has been inconsistent. This variability is due to environment, cultivar resistance, pathogen virulence, fungicide application method, coverage and fungicide timing. In the United States producers have been limited to one application of fungicide at the extended head growth stage in barley. Two cultivars were used in the trials including “Tradition” and a North Dakota Experimental labeled “ND20448”. No differences were determined for FHB incidence, severity, index, test weight and plump. Foliar leaf disease was greater on the “ND20448” as compared to “Tradition”, but foliar disease pressure was low in 2008. A significant interaction for yield was measured between cultivar and timing. The yield of “Tradition” barley was increased when fungicide was applied at Feekes GS 10.3 as compared to the GS 10.5. An interaction was measured for DON concentration between the cultivar and timing and also between the orifice orientation and timing. Deoxynivalenol levels decreased when the fungicide was applied to both cultivars at GS 10.5 as compared to 10.3. Deoxynivalenol levels also decreased further on cultivar ND20448 by the application timing at GS 10.5+5 days. A vertical oriented nozzle was less effective than a nozzle angled 30 degrees downward from horizontal and forward in depositing spray solution, 0.2 versus 0.34.

INTRODUCTION

Infection in small grains, by Fusarium head blight, also known as scab affects the food and feed quality of barley due to the toxin deoxynivalenol (DON). Damage from FHB affects the food and feed quality of barley because deoxynivalenol accumulates in the grain and cannot be cost-effectively removed from the end use product. The American Malting Barley Association recommends less than 0.5 ppm DON in barley used to brew beer. Grain with DON levels greater than 0.5 for barley are accepted for feed usage by some livestock industries but at reduced prices. Eastern North Dakota and northwest Minnesota growers have had barley yield and quality affected adversely by FHB since 1993 as environmental conditions have often been conducive for the development of the disease.

Applications of fungicide to headed barley are recommended as a management strategy to reduce damage in small grains caused by FHB. Fungicide application timing can be critical to obtaining favorable results. Other strategies such as planting resistant cultivars and using crop rotations are also recommended. Using resistant cultivars would be the preferred strategy by producers but developing cultivars with levels of resistance that would not require use of fungicides is slow to be achieved. The environment, temperature, rainfall and humidity usually determine the severity of the FHB epidemic.

Results from fungicide applications are often highly variable and range from effective to poor. This variability can be caused by environment, differences in cultivar resistance, pathogen virulence, fungicide coverage, and fungicide timing. Temperature and water activity have been shown to produce an extreme impact on deoxynivalenol growth. Efforts are ongoing to evaluate application technologies that will increase the amount of fungicide deposited on the grain spike. In North Dakota producers have been limited to one fungicide application at heading to provide disease control making deposition and timing a very critical component to increased efficacy. FHB has a multiple disease cycle making control difficult with one fungicide application.

NDSU studies have examined the effect of fungicide application at varying development stages to determine the effects on FHB incidence and field severity, incidence, yield, test weight, plump, DON accumulation, and head coverage. The studies were designed to compare a commonly grown malt barley with an experimental that has been shown to have about 1/3 less deoxynivalenol accumulation in the grain.

MATERIALS AND METHODS

A study was conducted in 2008 at the North Dakota State University Langdon Research Extension Center, Langdon North Dakota. The principle objective of the study was to integrate and test three 'recommended strategies' for reducing the impact of Fusarium head blight (FHB) in barley. The three strategies included comparing a standard malt type barley 'Tradition' with an experimental 'ND20448'. 'ND20448' is from the North Dakota State University barley breeding program. In previous studies 'ND20448' has had about one-third less deoxynivalenol accumulation in the seed than 'Tradition' and is being tested by the brewing industry for consideration as a malt type. The second strategy compared applying Prosaro fungicide application at growth stage (GS) Feekes 10.3, 10.5 and five days after the 10.5 GS application. GS 10.5 is the recommended GS for applying fungicide to barley. The third strategy compares a delivery method with a vertically oriented nozzle orifice that is typically used for the application of herbicides to field crops. This configuration comes as a standard with sprayer systems when they are purchased. Our comparative parameter was the recommended configuration for fungicide application for controlling FHB. This configuration has the nozzle orifices oriented 30° downward from horizontal and forward in the direction of travel of the tractor. The study was designed as a randomized complete block with a split split plot arrangement and four replications. Mean treatment comparison measured coverage reported as absorbance for coverage, disease incidence, severity and index, deoxynivalenol concentration (DON) in the grain, yield, test weight and plump. Prosaro 421 SC (203kg a.i./ha) fungicide, a 50/50 blend of prothioconazole and tebuconazole, is marketed by Bayer CropScience, Research Triangle Park, North Carolina 27709, USA. Prosaro was applied at 474.5 ml ha^{-1}in a tank mix that included the non-ionic surfactant Induce (Helena Chemical Co., Collerville, Tennessee 38017) at 0.125% v/v. The previous crop was field pea. The soil type was a Barnes/Svea complex (fine-loamy, mixed superactive Frigid, Calcic Hapludolls/mixed superactive Frigid, Pachic Hapludolls). Blocks of 'Tradition', a malt type barley, and 'ND20448', were planted on 7 May with an Almaco double-disk drill with a row spacing of 15 cm. After emergence and weed control were completed, each block was divided into plots 3.6 x 9.1 m. After delineation of the plots, a *Fusarium* inoculum was hand-broadcast on each plot to encourage development of disease. A food grade dye, FD&C blue #1, was mixed with the fungicide solutions at a rate of 108.7 gm ha^{-1}. The dye was included as an indirect type measurement to determine differences in coverage on the grain head. After agitation, the treatments were applied with a tractor using a side-mounted spray boom.

The tractor traveled 2.68 m s^{-1} (6 mph) delivering a solution 93.5 l ha^{-1} at 276 kPa psi using Spraying Systems XR8002 nozzles. The sprayer was equipped with a CO_2 type delivery system instead of a standard pump. After applying the treatments, a sample of 15 heads were collected from each plot, deposited in 250 ml Erlenmeyer flasks, sealed with stoppers and placed on ice. A solution of 80 ml 95% ethyl alcohol was added to each flask and shaken for three minutes with a Burrell wrist-type action shaker (Burrell Scientific Instruments and Laboratory Supplies, Model BT, Pittsburgh, Pennsylvania 15219). A sub sample of the solution was placed in a cuvette and placed in a Jenway photospectrometer (Jenway, Model 6300, Dunmow, Essex CM6 3LB England) to determine the absorbance of the solution. Each absorbance reading was indirectly used to determine differences in the amount of dye collected on the grain heads. After the fungicide was applied an impact type sprinkler irrigation system was installed (sprinkler heads were spaced on 9.1x 12.2 m centers) to modify the environment as needed and encourage the development of disease. North Dakota State University Extension recommended production practices for hard red spring wheat in Northeast North Dakota were followed. A visual disease evaluation was made from 20 samples per plot collected 20 days after the first fungicide application. The estimate of FHB incidence (number of spikes infected) head severity (average number of infected kernels per infected head), and FHB index (number of infected kernels per head divided by total kernels per individual spike), was determined for each plot. Five leaves were also sampled and scored to determine foliar disease severity. A rotary mower removed the front and back five feet from each plot prior to harvest to minimize any chance of interference by inaccurate application from the tractor sprayer when stopping or starting. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield and test weight. A sub sample was ground and analyzed for deoxynivalenol (DON) by the North Dakota State University barley quality lab. Data was analyzed with the general linear model (GLM) in SAS. Fisher's protected least significant differences (LSD) were used to compare means at the 95% probability level (Table 1).

RESULTS AND DISCUSSION

An untreated 'ND20448' and 'Tradition' were included in the trial for reference. However, data from the untreated plots were not included in the analysis because an untreated did not fit the timing and nozzle orientation factors. No differences were determined for FHB incidence, severity, index, test weight or plump. Foliar (leaf) disease severity was greater on the 'ND20448' compared to 'Tradition' (Table 2). The foliar disease pressure was quite low in 2008. Foliar disease was greater when the fungicide timing was Feekes 10.5 + 5 days. In other studies comparing the two cultivars, 'ND20448' has yielded less at times and had greater percentage plump kernels. Numerically, the 'ND20448' had both greater yield and plump. It has been theorized that the yield differences can possibly be attributed to differences in the cultivar's tolerance to root rot. This trial was conducted on previous crop field pea where the inoculum level in the soil may have been lower than trials conducted on previous crop small grains. A significant interaction for yield was measured between cultivar and timings. The yield of 'Tradition' barley was increased from 5649 kg ha^{-1} (105.1 bu a^{-1}) when the fungicide was applied at GS 10.3 to 6165 kg ha^{-1} (114.7 bu a^{-1}) at GS 10.5. Growth stage 10.5 would be the recommended fungicide application timing to maximize efficacy for control of FHB. Percentage plump of both cultivars were very good and numerically the 'ND20448' had greater plump. An interaction was measured for DON concentration between the cultivar and timing and also between the orifice orientation and timing. Deoxynivalenol levels were decreased when the fungicide applied to 'Tradition' was applied at GS 10.5 compared to 10.3. A similar decrease was measured on the 'ND20448'. An additional decrease was measured by the 5 day later fungicide application. While one study is not enough to change application timing recommendations, it certainly warrants further research. When the nozzle orifices were oriented forward the application timing 10.3 had significantly greater DON than the other growth stages. This would concur with North Dakota State University Extension recommendations on application techniques and timing. When fungicide was applied with vertically oriented nozzles, a reduction in DON concentration was measured from GS 10.3 to GS 10.5 on 'Tradition' and GS 10.5 to 5 days after GS 10.5 on 'ND20448'. While this reduction could be partially related to the environment at the late spray date, perhaps more air turbulence increasing coverage, our data does not statistically support this conclusion. Numerically the vertical oriented nozzles had increased coverage as the fungicide timing was delayed. Statistically the coverage was greater with the two earlier timings compared to the late timing with the 30° forward facing nozzles. The maximum coverage obtained coincided with the lowest DON concentration which occurred at the fungicide application GS 10.5 with the 30° forward facing nozzles.

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LITERATURE CITED

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Table 1. Source of variation, confidence intervals and coefficient of variation Langdon, 2008.

	Fusarium head blight			Leaf		Test			
	Incidence	Severity	Index	Severity	Yield	Weight	Plump	DON	Coverage
Cultivar (cult)	0.2362	0.0829	0.0894	0.0086	0.4497	0.8594	0.1421	0.9935	0.3946
Rep*cult	0.0949	0.0069	0.0042	0.8061	0.0934	0.0004	<0.0001	0.0015	0.0112
Orientation (orient)	0.3600	0.8041	0.9613	0.2610	0.5982	0.9536	0.6513	0.0497	<0.0001
Cult*orient	0.2346	0.5301	0.8864	0.6989	0.7494	0.9871	0.2149	0.5481	0.0914
Rep*orient	0.0258	0.3190	0.1700	0.2226	0.3785	0.4232	0.0858	0.7553	0.8739
Timing (tim)	0.2918	0.1757	0.1737	0.0141	0.8432	0.7518	0.8546	<0.0001	0.1665
Cult*tim	0.1250	0.3109	0.1995	0.3628	0.0224	0.9017	0.5501	0.0054	0.7822
Orient*tim	0.3924	0.4264	0.3841	0.2545	0.3520	0.2142	0.5159	0.0133	0.0004
Cult*orient*tim	0.7261	0.7962	0.8187	0.3731	0.3572	0.9799	0.2368	0.1493	0.2217
%C.V.	2.56	14.52	15.66	58.06	7.97	1.75	1.91	29.83	29.70

Table 2. Fusarium head blight incidence, severity and index, leaf severity, yield, test weight, plump, DON and head coverage by cultivar, nozzle orientation, and application timings (Feekes growth stage), Langdon 2008.

Cultivar, Nozzle Orientation or Appl. Growth Stage		Fusarium head blight Incidence	Fusarium head blight Severity	Fusarium head blight Index	Leaf Severity	Yield	Test Weight	Plump	DON	Coverage
		%	%		%	kg ha^{-1} (bu a^{-1})	kg m^{-3}	%	ppm	
ND20448 untreated		100.0	11.9	11.9	8.2	5617 (104.5)	607	95.5	1.37	0.02
Tradition untreated		100.0	11.9	11.9	10.8	5531 (102.9)	618	88.0	1.43	0.03
ND20448		99.8	12.0	12.0	9.1	6122 (113.9)	616	95.9	0.62	0.29
Tradition		98.1	9.4	9.14	5.0	5934 (110.4)	618	89.5	0.62	0.24
LSD $_{(0.05)}$		NS	NS	NS	2.1	NS	NS	NS	NS	NS
Vertical		99.6	10.6	10.6	7.9	6068 (112.9)	617	92.9	0.67	0.20
30° F down from Horizontal		98.3	10.8	10.5	6.1	5988 (111.4)	617	92.5	0.57	0.34
LSD $_{(0.05)}$		NS	NS	NS	NS	NS	NS	NS	0.1	0.03
10.3		99.4	11.3	11.2	6.3	5999 (111.6)	618	92.6	1.01	0.28
10.5		98.1	10.5	10.3	5.1	6004 (111.7)	618	92.9	0.43	0.29
10.5 + 5 days		99.4	10.3	10.2	9.6	6085 (113.2)	615	92.6	0.41	0.24
		NS	NS	NS	3.0	NS	NS	NS	NS	NS
Cultivar by fungicide timing averaged across both nozzle orientations										
ND20448	10.3					6343 (118.0)			1.12	
	10.5					5843 (108.7)			0.45	
	10.5+5					6181 (115.0)			0.29	
Tradition	10.3					5649 (105.1)			0.90	
	10.5					6165 (114.7)			0.42	
	10.5+5					5993 (111.5)			0.54	
Nozzle orientation by fungicide timing averaged across both cultivars										
Vertical	10.3								0.98	0.17
	10.5								0.48	0.18
	10.5+5								0.25	0.24
30° F	10.3								1.04	0.39
	10.5								0.39	0.39
	10.5+5								0.57	0.23

LSD Yield: To compare two-subplot means at the same levels of the whole plot (a_0b_0 vs a_0b_1)=511 (9.5) and to compare two whole plot means at the same or different levels of the subplot (a_0b_0 vs a_1b_0)=769 (14.3) ; LSD DON: To compare two-subplot means at the same levels of the whole plot (a_0b_0 vs a_0b_1)=0.14 and to compare two whole plot means at the same or different levels of the subplot (a_0b_0 vs a_1b_0)=0.43 ;LSD DON: To compare two-subplot means at the same levels of the subplot (b_0c_0 vs b_0c_1)=0.19 and to compare two subplot means at the same or different levels of the sub-subplot (b_0c_0 vs b_1c_0)=0.018 LSD Coverage: To compare two-subplot means at the same levels of the subplot (b_0c_0 vs b_0c_1)=0.082 and to compare two subplot means at the same or different levels of the sub-subplot (b_0c_0 vs b_1c_0)=0.075.

REACTION OF WINTER WHEAT CULTIVARS TO FHB AND DON

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, produces significant losses resulting from yield reduction, kernel damage, and presence of the mycotoxin deoxynivalenol (DON). One strategy for management of FHB and DON is to plant resistant/tolerant cultivars. In 2009, an experiment was conducted to study the reaction of winter wheat cultivars to FHB and DON. Twenty cultivars (Overley, Overland, Jagalene, Mace, Hawken, Goodstreak, Bond CL, Wahoo, Wesley, Camelot, Postrock, Millennium, 2137, Harry, Settler CL, Art, Infinity CL, Hatcher, Bill Brown, and Alliance) were planted in the fall of 2008 in a commercial field near Paxton, Nebraska. Plots were irrigated and not inoculated, but there was heavy natural inoculum of *F. graminearum*. Experimental design was a randomized complete block with three replications. FHB severity and incidence were determined on the 2nd of July 2009, on 10 heads in each of 10 arbitrarily selected locations in each plot and used to calculate FHB index. Plots were harvested with a small plot combine. The percentage of *Fusarium*-damaged kernels (FDK) was measured with an automated single-kernel near-infrared system at the USDA ARS Grain Marketing and Production Research Center in Manhattan, KS. Grain samples from all plots were ground and sent to the North Dakota Veterinary Diagnostic Laboratory at North Dakota State University, Fargo, ND for DON determination. Linear correlation analysis was used to determine relationships between FHB index, FDK, DON, and yield. Differences among cultivars were highly significant for FHB index ($P < 0.0001$) and yield ($P < 0.0001$). Overley had the highest FHB index (20%) followed by Jagalene (13%), 2137 (12%) Bond (12%), and Wesley (12%). Goodstreak had the lowest FHB index (2%) followed by Mace (4%), Infinity CL (4%), Art (4%), and Overland (4%). Bond CL had the highest yield (93 bu/acre) followed by Camelot (90 bu/acre), Settler CL (89 bu/acre), Infinity CL (89 bu/acre), Jagalene (89 bu/acre), Wesley (88 bu/acre), Harry (87 bu/acre), Art (87 bu/acre), Wahoo (85 bu/acre), and Overley (85 bu/acre). Mace had the lowest yield (62 bu/acre) followed by 2137 (67 bu/acre). FHB index in the rest of the cultivars ranged from 5% to 10%. FDK and DON were generally low and not significantly different among cultivars at $P = 0.05$. DON concentration ranged from 0.2 ppm (Overland) to 4.3 ppm (Postrock) and FDK ranged from 6% (Overley) to 24% (Harry). There was a significant positive correlation between index and DON ($r = 0.62$, $P = 0.0037$), and between incidence and DON ($r = 0.60$, $P = 0.0048$). All other correlations were not significant at $P = 0.05$. Linear regression analysis with DON as the dependent variable showed that the relationship between index and DON was the strongest ($R^2 = 0.38$, $P = 0.0037$), followed by the relationship between incidence and DON ($R^2 = 0.36$, $P = 0.0049$). The relationship between severity and DON was not significant ($R^2 = 0.16$, $P = 0.0843$).

This study demonstrated differences among winter wheat cultivars in their reaction to FHB and DON. It was interesting to note that Overley, Jagalene and Wesley were among the most susceptible cultivars for the second year in a row. Based on the data from this study, the best predictor of DON concentration was FHB index.

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RELATIONSHIP BETWEEN FUSARIUM HEAD BLIGHT SEVERITY AND DEOXYNIVALENOL CONCENTRATION IN THREE WINTER WHEAT CULTIVARS

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a destructive disease of wheat. *F. graminearum* produces the mycotoxin deoxynivalenol (DON) which accumulates in grain and has serious food safety implications. Additionally, it can cause significant losses resulting from yield reduction and kernel damage. *Fusarium*-damaged kernels, commonly referred to as FDK, are shriveled and/or discolored. The higher the percentage of FDK in grain, the lower the yield, test weight, and grain quality. The relationship between FHB severity (FHBsev) and DON can be used to estimate the level of DON to expect in grain, enabling producers to make informed decisions early regarding the marketing or end use of grain from fields affected by FHB. The objectives of this study were to i) investigate the nature of the relationship between FHBsev and DON in three winter wheat cultivars, Jagalene, Harry, and 2137, and ii) determine if there were differences among the three cultivars in in the levels of DON they accumulated.

The cultivars were planted following corn in October 2008 at the University of Nebraska Agricultural Research and Development Center near Mead, NE. In addition to natural inoculum, plots were inoculated with 1×10^5 spores/ml of *F. graminearum* at early anthesis in May/June 2009 and were not irrigated. Cultivars were arranged in a randomized complete block design with three replications. FHBsev was determined 21 days after inoculation on 20 heads tagged in each of 11 disease severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50%. After harvest, DON concentration in grain from each severity category was determined at the North Dakota Veterinary Diagnostic Laboratory.

There was a significant positive correlation between FHBsev in the 11 categories and DON for all three cultivars: Jagalene (r = 0.91, P = .0001), Harry (r = 0.77, P = 0.0053), and 2137 (r = 0.73, P = 0.0101). Linear regression analysis with DON as the dependent variable showed that the relationship between FHBsev and DON was strongest for Jagalene (R^2 = 0.83, P < 0.0001) followed by Harry (R^2 = 0.60, P = 0.0053), and 2137 (R^2 = 0.54, P = 0.0101).

This study demonstrated (i) a positive linear relationship between DON and FHBsev in three winter wheat cultivars and (ii) differences among the three cultivars in the levels of DON they accumulated. In 2007 and 2008, similar results were obtained.

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INFLUENCE OF CROP RESIDUES AND DISEASE RESISTANCE ON FHB IN VIRGINIA WHEAT

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ABSTRACT

Knowledge of the influence of crop residues and disease resistance on Fusarium head blight (FHB) in winter wheat is important for improving disease management strategies. Approximately 3.2 acres of the winter wheat cultivars Pioneer 26R12, Tribute, SS560, and Vigoro 9510 were planted in Blacksburg, Virginia in 2008. Pioneer 26R12 and SS560 are considered to be susceptible to FHB, and Tribute and Vigoro 9510 are considered to be moderately resistant to FHB. Varying amounts of corn stalk pieces (45g and 410g) infested with a clonal isolate of *G. zeae* were released in replicated 1 m diameter circular plots. Mature wheat spikes were collected at the released inoculum source, at a radius of 3.1 m (10 ft.) from the source, and from non-inoculated (control) locations separated 16.5 m (54 ft.) from the nearest released source. Spikes were observed for symptoms of FHB, disinfested, and plated onto a *Fusarium*-selective medium. Over 600 isolates of *G. zeae* were recovered from spikes approximately two weeks, four weeks, and six weeks after anthesis. For plots containing 45 g of inocula, disease incidence ranged from 55% to 79% and DON ranged from 18 to 45 ppm for all cultivars. For plots containing 410 g of inocula, disease incidence ranged from 88% to 100% and DON ranged from 19 to 109 ppm for all cultivars. Ongoing DNA-based methodologies are being used to determine the contribution of the released clone (relative to background sources) to FHB and DON in each of our experimental plots. Results from our first year of experimentation indicate that varying amounts of within-plot *G. zeae* inocula influence FHB and DON, despite varying levels of disease resistance that may be attributed to each of the cultivars used in this experiment.

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MULTI-STATE ASSESSMENT USING WINDOW PANE ANALYSIS CONFIRMING WEATHER VARIABLES RELATED TO FUSARIUM HEAD BLIGHT EPIDEMICS

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is a sporadic disease that is dependent, at least in part, on weather and climatic conditions. The objectives of this research were to determine whether the annual variability in FHB in Ohio can be related to variability from year-to-year in environmental conditions over short or long time scales, and to compare findings with results found for three other states, Indiana, Kansas, and North Dakota. Historical records of FHB intensity in each state were used to address this issue. In Ohio, overall FHB intensity in the state was rated on an ordinal scale from 0 to 9 for 44 years (1964-2008). In Indiana, disease was assessed for 36 years (1973-2008) in variety nurseries by Purdue University researchers. In Kansas, disease index was assessed for 28 years (1978, 1980, 1982-2007) by Kansas State University, Kansas Department of Agriculture, and USDA-ARS personnel, based on surveys of wheat in the state. In North Dakota, FHB intensity was rated for 23 years (1986-2008) on an ordinal scale from 0 to 9 (same numerical scale as in Ohio), based on results from field surveys by North Dakota State University extension agents and specialists. Weather data were gathered from local weather stations within each state, and summary variables (such as average RH, precipitation, temperature) were calculated for a wide range of time windows and starting times of the windows during the growing season. The windows ranged from 10 to 280 days in duration, beginning around physiological crop maturity and proceeding backwards to the fall of the previous year (for winter wheat). This methodology is a form of data mining that has been termed ‘Window-Pane’ analysis.

The relationship between each summary environmental variable and disease intensity was quantified with a Spearman rank correlation coefficient for each of the window lengths and starting times. This rank-based nonparametric correlation was used due to the ordinal nature of the FHB intensity data in Ohio and North Dakota, and because of the non-normal data in the other locations. Based on Spearman rank correlations, the FHB rating in all states was significantly ($P < 0.05$) associated with the mean average daily relative humidity for short time windows during and shortly after anthesis (Feeks 10.5.1), covering the periods for infection, spike colonization, and early DON production. In Ohio, significant associations were also found around late April, early March, and late December, covering the period of spore production (April) and pathogen winter survival. In all locations, total daily precipitation was significantly associated with FHB intensity around the time of heading and flowering for various time-window lengths. There were no significant relationships found between the mean average daily temperature and FHB intensity for any time window in any location. In general, correlations between FHB intensity and weather variables were stronger with shorter window lengths, and weather toward the end of the growing season was the strongest indicator of FHB epidemics.

EFFECT OF VARIETY, LOCATION, AND ENVIRONMENT ON DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT IN WISCONSIN

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OBJECTIVE

To investigate how variety, location, and environment affect the risk of Fusarium head blight in soft red winter wheat in Wisconsin.

INTRODUCTION

During epidemic years, Fusarium head blight (FHB, scab, *Fusarium graminearum* group 2, teleomorph: *Gibberella zeae*) has the potential to cause significant reductions in both yield and quality of wheat grain and seed. Because infection occurs in the anthers, the risk of infection is greatest from flowering (Feekes 10.51) through the soft dough stage (Feekes 11.2) (Dill-Macky, 1997). During this period, disease development is favored by prolonged periods of moisture, high relative humidity, and temperatures from 64 to 80^{o}F (Nyvall, 1999; Weise, 1989).

Symptoms of FHB are typically not observed until soft dough, and at this growth stage, it may be too late to implement management tactics. In Wisconsin, wheat acreage has increased and many growers lack the necessary information about how variety, location, and environment may interact to affect the risk of FHB development. Therefore, in order to improve recommendations for managing FHB in Wisconsin, we are investigating the role of each of these factors in FHB development.

MATERIALS AND METHODS

To examine the effect location, variety, and environment on the development of FHB, data were collected as part of the Wisconsin Winter Wheat Performance Tests (Results available at: http://coolbean.info). The performance tests were conducted at four locations around Wisconsin: Arlington, Chilton, Janesville, and Lancaster. At three of the locations (Arlington, Chilton, and Lancaster), 58 varieties of soft red winter wheat (*Triticum aestivum*) were planted in a randomized complete block design with four replications. At the Janesville location, the experimental design was a split-plot design with fungicide treatmentas the whole plot level and variety as the subplot level. Fungicide application (Quilt (R) Syngenta, azoxystrobin and propiconazole) was done at Feekes stage 9 for control of foliar diseases. Plots were 8' wide (7.5" row spacing) by 25' long, with seven center harvest rows and two non-harvest rows; all disease assessments were made in the non-harvested rows.

Fields were seeded at 1.5 million viable seeds per acre using a grain drill with cone units. The center seven rows were harvested using a self-propelled combine. Previous crop history, planting, flowering, and harvest date information are shown in Table 1.

Disease assessments were made for both foliar diseases and FHB. Assessments for foliar diseases were made at Feekes 4/5, Feekes 6/7, Feekes 8/9, and Feekes 10.51. During each assessment, 6 stems were destructively sampled from the non-harvested rows of each plot. Whole plant incidence and severity on the upper four leaves (flag, flag-1, flag-2, flag-3) were assessed for each stem. A weighted severity score was calculated for each plant as: weighted disease severity = (4 × severity on flag leaf) + (3 × severity on flag-1 leaf) + (2 × severity on flag-2 leaf) + (severity on flag-3 leaf) (Lipps and Madden, 1989). Incidence and severity of FHB were assessed on 100 heads per plot at Feekes 11.2. The Fusarium head blight index (FHBI) value was also calculated for each plot as: FHBI = (% incidence × % severity) ÷ 100 (Conley *et al*, 2009; Paul *et al*, 2005). Following harvest, the percentage of *Fusarium* damaged kernels (FDK) was assessed for a 200 kernel sample from each plot.

Weather data during the spring and summer period were obtained using HOBO U30 weather stations (Onset Computer Corporation, Bourne, MA). Each station was equipped with sensors for temperature, relative humidity (hygrometer), rainfall, and leaf wetness (sensors at 76.2 cm and 121.9 cm above the ground, oriented according to manufacturer recommendations).

Data were analyzed in SAS (v. 9.1.3, SAS Inc., Cary, NC) as a mixed model (PROC MIXED) for the following measures: FHB incidence and severity, FHBI, and percentage of FDK. Two analyses were conducted, the first specifically at Janesville to examine the effect of fungicide and variety, and the second a multi-location analysis (Littell et al., 2006). Fungicide-treated plots from Janesville were excluded from the multi-location analysis. Mean separations were based on a protected LSD to compare locations, varieties, and the interaction. The level of significance for all analyses was 0.05.

As a result of significant winterkill at the Arlington and Chilton locations, disease assessments were not made for a number of plots at these two locations (out of 256 total plots at each location: 172 plots were not assessed at Arlington and 162 plots were not assessed at Chilton). Due to the large number of missing values, the within-location effects on FHB incidence, FHB severity, and FHBI could not be analyzed for the Arlington and Chilton locations. However, grain was harvested from many of these unassessed plots, so the number of missing plots for the percentage of FDK analysis was smaller (96 of 256 plots were missing from Arlington, 51 of 256 plots were missing from Chilton, 3 plots were missing from Janesville, and 1 plot was missing from Lancaster) and statistical analysis of the within-location effects on percentage of FDK was carried out for all locations. All locations are also included in the among-locations analysis of the effects on FHB incidence, FHB severity, FHBI and percentage of FDK.

RESULTS AND DISCUSSION

Environmental conditions during the 7 days prior to flowering differed among locations (Table 2). The effect of fungicide and variety and the fungicide*variety interaction at Janesville on FHB incidence, FHB severity, FHBI, and percentage of FDK are shown in Table 3. There was a marginal effect of fungicide treatment on FHB incidence (P=0.065) (Table 3). This may indicate that the Feekes 9 fungicide application targeted toward control of foliar pathogens may provide some residual protection against FHB.

Table 4 shows the results of the among-locations analysis of the effect of location, variety, and the location*variety interaction on FHB incidence, FHB severity, FHBI, and percentage of FDK. The public variety Truman had consistently low incidence, severity, and FHBI values at both Janesville and Lancaster. The commercial variety Kaltenberg KW 70 had low severity at both Janesville and Lancaster. The varieties Growmark FS 637 and Kaltenberg KW 63 were both consistently high for incidence, severity and FHBI at Janesville and Lancaster. The variety Diener D 496W had a consistently high percentage of FDK at Janesville, Lancaster, and Chilton. The varieties PIP 717 and Pro Seed Genetics Pro 220 all had a consistently low percentage of FDK at Janesville, Lancaster, and Chilton.

The correlations between yield and FHB incidence, FHB severity, FHBI, and percentage of FDK for each location and across all locations are shown in Table 5.

These results demonstrate that variety selection is an important component for scab management in Wisconsin. Although there is variability among locations, specific varieties performed consistently well across locations.

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Table 1. Previous crop history, planting, flowering, and harvest dates at each winter wheat performance trial location.

Location	Previous crop	Planting date	Flowering date	Harvest date
Arlington	Soybeans	26 September 2008	7 June 2009	30 July 2009
Chilton	Peas	30 September 2008	8 June 2009	5 August 2009
Janesville	Soybeans	13 October 2008	3 June 2009	29 July 2009
Lancaster	Alfalfa	26 September 2008	6 June 2009	4 August 2009

Table 2. Environmental conditions at each field location during the 7 days (168 hours) prior to flowering.

Location	Relative humidity (%)[1]	Rain (hours)[2]	Rain (inches)[3]	Temperature (hours)[4]
Arlington	63	1.3	0.19	44
Chilton	70	13.0	1.54	22
Janesville	67	3.5	0.28	44
Lancaster	65	1.0	0.04	52

[1]Average relative humidity.
[2]Hours (out of 168) during which rainfall was recorded.
[3]Total rainfall recorded.
[4]Hours (out of 168) during which the air temperature was 64 to 80^{o}F.

Table 3. *P*-values for the effects of variety (Var), fungicide (Fung) and the fungicide*variety interaction (Fung*Var) on FHB incidence and severity, FHBI, and percentage of FDK at Janesville.

Location	Effect	FHB incidence	FHB severity	FHBI	% of FDK
Janesville	Fung	0.0605	0.5370	0.1392	0.0825
	Var	<0.0001	<0.0001	<0.0001	<0.0001
	Fung*Var	0.8794	0.3314	0.9972	0.9992

Table 4. *P*-values for the among-locations effects of location (Loc), variety (Var), and the location*variety interaction (Loc*Var) on FHB incidence and severity, FHBI, and percentage of FDK.

Effect	FHB incidence	FHB severity	FHBI	% of FDK
Loc	0.0001	0.0001	0.0001	0.0001
Var	0.0001	0.0001	0.0001	0.0001
Loc*Var	0.0001	0.0001	0.0001	0.0001

Table 5. Correlations between yield and FHB incidence and severity, FHBI, and percentage of FDK and *P*-values for those correlations.

	Correlations with yield			
Location	**Incidence**	**Severity**	**FHBI**	**FDK**
Arlington	-0.0214 (*P*=0.8522)	-0.1421 (*P*=0.2146)	-0.1097 (*P*=0.3392)	-0.2605 (*P*=0.0014)
Chilton	-0.0351 (*P*=0.751)	-0.0545 (*P*=0.6225)	-0.0773 (*P*=0.4845)	-0.0631 (*P*=0.4168)
Janesville	-0.0353 (*P*=0.5976)	-0.0277 (*P*=0.6785)	-0.0642 (*P*=0.3368)	-0.0707 (*P*=0.2902)
Lancaster	-0.2266 (*P*=0.0006)	-0.2050 (*P*=0.0019)	-0.2261 (*P*=0.0006)	-0.3257 (*P*<0.0001)
All locations	0.1206 (*P*=0.0027)	0.0073 (*P*=0.857)	-0.0040 (*P*=0.9217)	-0.0711 (*P*=0.0488)

EFFECT OF PRECEDING FORAGE CROPS ON DON CONTENT IN BARLEY

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ABSTRACT

In Northern Quebec, Canada (Saguenay-Lac-Saint-Jean area), barley (*Hordeum vulgare* L.) production is very important but Fusarium head blight (FHB) has become a major problem in this region. In 2002 and 2003, FHB affected 47% and 76 % respectively of total area seeded in barley. The economic losses associated with FHB in barley were estimated at 2 million Canadian dollars during those 2 years in the Saguenay-Lac-Saint-Jean region. In this region, dairy producers usually seed barley after 3-4 years of a forage crop. A study was conducted at the research farm of Agriculture and Agri-Food Canada in Normandin (Quebec, Canada), to evaluate if preceding forage crops could affect deoxynivalenol (DON) content in barley. Previous crops consisted in barley monoculture (seeded in 2006 and 2007), 10 different forage crops (seeded in 2006 and harvested in 2007 as recommended for each species) and a summer fallow. In fall 2007, glyphosate was applied at a rate of 2700 g a.e. ha $^{-1}$ on each treatment and in 2008, barley cv. Païdia was direct seeded. Some forage crops seemed to increase DON content. Higher DON content could have been induced by high lodging index. More data are needed to evaluate if some forage crops residues could be more conducive to FHB than others.

INTEGRATED MANAGEMENT OF SCAB IN WHEAT USING RESISTANT VARIETIES AND FUNGICIDE

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ABSTRACT

A study was conducted to assess the effectiveness of host resistance, Proline® or Prosaro® fungicide, and a combination of both control measures in reducing losses in grain yield and quality resulting from Fusarium head blight (FHB). Four soft red winter (SRW) wheat cultivars and eight winter durum wheat varieties were evaluated in a complete block design comprised of three replications and two treatments (varieties with and without fungicide). Experiments were conducted at two locations in Virginia in 2008 and 2009. Scabby corn seeds were applied to plots at the boot stage, and a spray inoculation using conidia of *Fusarium graminearum* was applied to each variety at 50% flowering in the mist-irrigated test at Blacksburg, VA. Plots in the mist-irrigated test at Mt. Holly, VA were inoculated using only scabby corn seed. Proline (2008) or Prosaro (2009) was applied at 5.5 oz/ac before flowering at both locations. Data were collected for test weight, grain yield, 100 grain weight, and FHB assessment parameters including incidence, severity, index, *Fusarium* damaged kernels (FDK), and DON concentration.

Variance analyses indicated that variety, fungicide treatment, locations and years had significant effects on most traits. Year and location interaction effects were common for all traits. All of the scab assessment parameters, except for severity, were significant and correlated ($r = 0.23$ to 0.79, $P < 0.001$) with each other. All of the scab parameters, except for FHB severity, had a significant ($r = -0.34$ to -0.89, $P < 0.001$) negative effect on test weight and grain yield. Results of this study indicate that a single fungicide application significantly reduced FHB incidence in 6 of 8 tested varieties. Significant reductions in FHB index, and FDK were also identified with a single fungicide application. As a result, the fungicide treatment significantly increased grain weight (3 varieties) and yield (5 varieties). Fungicide application resulted in a significantly higher yield in two SRW wheat varieties and five durum wheat varieties.

Fungicide application had the greatest effect on FHB infection in susceptible winter durum wheat varieties and less effect in moderately resistant SRW wheat varieties. Only the susceptible SRW cultivar Coker 9835 benefited from the fungicide treatment, which resulted in a significant reduction in scab infection and significant increase in test weight and grain yield. FHB resistance in the SRW wheat cultivars was more effective than in the winter durum varieties in reducing scab infection, and protecting grain yield and quality. Results of this study indicate that utilization of wheat varieties having moderate scab resistance provides a baseline of protection against FHB that is equal to or better than fungicide application to cultivars having little or no FHB resistance. Nevertheless, fungicide application is beneficial and critical under severe FHB epidemics especially when susceptible cultivars are grown.

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DEVELOPMENT OF THE *SCABSMART* WEB SITE - A QUICK GUIDE TO U.S. SCAB MANAGEMENT INFORMATION

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ABSTRACT

The U.S. Wheat and Barley Scab Initiative (USWBSI) supported the development of a website that provides scab management information for all small grain classes affected by this disease in the U.S. The website is called ScabSmart and can be found at www.scabsmart.org/. The purpose of the site is to allow ready access to collective information on management strategies that reduce head scab and the associated mycotoxins, including deoxynivalenol (DON). ScabSmart provides the latest information on variety resistance for eight grain classes, plus the ability to rapidly get basic information on other strategies, including fungicide management and disease forecasting, seed treatment, crop rotation, and residue management. This information resource is a result of a cooperative effort across regions and grain classes in the U.S. affected by this disease. The web site also serves as a portal for links to get further information from the USWBSI web site or from other, localized resources on management strategies for Fusarium head blight (scab), resources often available through many state's Extension and Experiment Station publications. The ScabSmart website tool will be updated periodically with new information as it becomes available. ScabSmart was launched on September 24, 2009, and its availability has been announced through press releases and contact with commodity organizations. Further use of such a tool may be aided by promotion of its availability through various grain industries.

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UNIFORM FUNGICIDE TRIAL RESULTS ON HRS WHEAT AND SPRING BARLEY, FARGO, ND 2009

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ABSTRACT

Nine fungicide treatments were compared to the untreated check for efficacy in reduction of Fusarium head blight (FHB) and deoxynivalenol (DON) in 'Tradition' spring barley and 'Steele ND" hard red spring wheat, at Fargo, ND. Both crops were planted on May 8 into previous wheat ground that had been chisel plowed twice prior to planting. Plots were 5' wide and 20' long, with 4 replicates per treatment, arranged in a randomized complete block design, and with winter wheat seeded between plots. At heading, an overhead misting system provided added water to the plots when the nighttime humidity dropped below 90%. Fungicides were applied between 6:00 am and 8:00 am with a backpack-type sprayer equipped with two XR8001 flat fan nozzles oriented toward the grain head at a 30^{o} angle from the horizontal. The fungicides were applied at 18.5 gpa with 40 psi. Conidia (100,000 spores/ml) of *Fusarium graminearum* were applied to grain heads with a backpack sprayer in 30 gpa of water, on the same evening of the early full head emergence applications in barley (Feekes 10.5) and the anthesis (Feekes 10.51) applications in wheat. Disease notes were taken at soft dough stage of development and crops were harvested at kernel maturity. Sub-samples of the harvested grain were ground and analyzed for deoxynivalenol (DON) by the NDSU Veterinary Toxicology Laboratory using gas chromatography and electron capture techniques.

The fungicide treatments included: Proline (3 fl oz/A) + Caramba (7.0 fl oz/A) at Feekes 10.5 in barley, Feekes 10.51 in wheat; Prosaro (6.5 fl oz/A) or Caramba (13.5 fl oz/A) applied once at three separate growth stages - Feekes 10.3, 10.5 and 10.54 in barley and Feekes 10.5, 10.51 and 10.54 in wheat; and two treatments of Headline (6 fl oz/A), one at Feekes 10.0 and one at Feekes 10.5.

Very cold July temperatures (average temperature of 67^{o}F and average lows of 55^{o}F) and rainfall amounts 78% below normal resulted in very low FHB levels in Fargo in 2009, even with added mist. The untreated FHB field severity was 1.5 % in barley and 5.0 % in wheat. Despite low disease, all fungicide treatments reduced FHB field severity (P = 0.05) for both crops. DON levels were 1.4 ppm for untreated barley, and 2.0 ppm for untreated spring wheat. All fungicide treatments, except for the two Headline treatments, significantly (P = 0.05) reduced DON in both crops. The fungicide treatments applied before flowering in wheat and before full head emergence in barley resulted in greater DON levels than the other two fungicide timings. In wheat, only the two fungicide treatments applied at anthesis (Feekes 10.51) resulted in significantly improved yield.

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DEOXYNIVALENOL ACCUMULATION DURING MATURATION OF BARLEY GRAIN

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ABSTRACT

Fusarium head blight (FHB) associated with the presence of the fungus *Fusarium graminearum* is probably one of the most feared diseases in barley (*Hordeum vulgare*) production in Eastern Canada. In addition to reducing grain yields, the fungus produces a toxin (deoxynivalenol or DON) which can affect the health of livestock. A study was conducted at the research farm of Agriculture and Agri-Food Canada in Normandin (Quebec, Canada), to evaluate deoxynivalenol (DON) content in barley during the maturation of the grain. In 2007 and 2008, 6 barley cultivars were planted at a seeding rate of 375 grains m^{-2}. The fertilization was applied according to the provincial recommendations. Each plot consisted of 8 rows of 3.5 m long. The experimental design was a randomized complete block design with 6 repetitions. From Zadoks growth stage 73 (milky stage) to Zadoks 90 (maturity), a section of 1m long of each plot was harvested for DON determination. The experiment was conducted under natural conditions of infestation. In 2007, DON content was low for all cultivars. At the first harvest, DON content was 0.24 ppm and at maturity DON content reached 0.54 ppm. In 2008, FHB was more important. At Zadoks 73, DON content was 0.8 ppm. At maturity, DON content reached 6.0 ppm. It seems that DON is present very early during the grain formation and high and rapid accumulation could be observed during grain maturation of barley.

INTEGRATED MANAGEMENT OF FHB AND DON: A 2009 UPDATE

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ABSTRACT

Coordinated integrated management trials were conducted in 2007, 2008 and 2009 in vastly different regions of the country to evaluate the overall efficacy and consistency of an integrated approach relative to an approach based solely on fungicide application or cultivar resistance to manage FHB and DON in all major grain classes. The experimental design was a split-plot, with fungicide treatment and cultivar as the whole- and -sub-plots or vice versa. In a few trials, a split-split-plot treatment arrangement was used, with cropping sequence (crop rotation) or surface residue management as the whole-plot. One plot of each cultivar was treated with Prosaro (6.5 fl oz/A + 0.125% Induce) or Proline 3+3 (a tank mix of Folicur and Proline, each at 3 fl. oz/A + 0.125% Induce) at anthesis, while the other was left untreated. Application was made using a sprayer equipped with paired Twinjet or flat fan XR8001 nozzles, mounted at an angle (30^o from the horizontal) forward and backward and calibrated to deliver at a rate of 10 to 20 gallons per acre. FHB, DON, FDK, yield, and test weight data were collected in all trials and analyzed to determine the effect of fungicide and cultivar resistance (and cropping sequence, were appropriate) on each of these variables.

In this summary, percent control relative to the untreated susceptible check (worst case scenario) will be used as the basis for evaluating efficacy against FHB and DON. Over the three years, disease intensity and DON contamination were low at most locations due to conditions unfavorable for FHB development; however, there were a few location-years with some level of disease and toxin, allowing for the comparison of treatments. Results thus far indicate that, at moderate to high levels of FHB and DON, the efficacy of both individual (fungicide or resistance) and integrated (fungicide + resistance or fungicide + resistance + residue management) approaches varies among trials, possibly reflecting, among other factors, differences in baseline levels of disease and DON, cultivar resistance (level and designation), overall weather conditions, and weather conditions at the time of anthesis of the individual cultivars. In spite of the relatively small dataset, a range of responses were observed over the three years. There were situations in which fungicide alone (applied to the susceptible check) was just as effective as resistance alone (resistant cultivar without fungicide) or resistance + fungicide. In other situations, resistance alone was more effective than fungicide alone (susceptible cultivar + fungicide) or just as effective as resistance + fungicide at reducing FHB index and DON. For trials with surface residue management or cropping sequence as a treatment factor, in most cases, there was a significant advantage in terms of disease and toxin reduction to planting wheat after a non-host crop as opposed to a cereal crop. The gain in FHB/DON reduction from using an additional control strategy was not always significant. However, in spite of these variations, a few general conclusions can be made. In general, moderately resistant cultivar + fungicide treatment combination resulted in higher percent control (relative to the untreated susceptible check) than that achieved by either approach used alone. Comparing trials with the same treatment combinations, but planted into different types of crop residue, non-host crop + moderately resistant cultivar + fungicide generally resulted in higher percent control than host crop + susceptible cultivar + without fungicide or other combinations of these variables. Under severe epidemic conditions, a three tier management approach of crop rotation with a non-host,

moderately resistant cultivars and fungicide application is required to achieve close to < 2 ppm DON and reduce index. Across all trials, based on FHB index, percent control ranged from 11.5 to 92.4% for fungicide or resistance alone and from 37 to 98% for fungicide + resistance. For trials with crop rotation or cropping sequence as a treatment factor, percent control (relative to the untreated susceptible planted into host residue) for non-host + resistance + fungicide combination ranged from 8.45 to 99% for index and from 33 to 96% for DON. (*note: trials with unusually high negative percent control [see Willyerd et al in this volume] were not considered in this summary, pending further information from the individual PIs*).

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FACTORS INFLUENCING THE ADOPTION FHB CONTROL PRACTICES IN ND AND MN: RESULTS OF A SURVEY

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ABSTRACT

In 2009 a survey was given to producers attending a wheat production workshop to determine the level of adoption of the various FHB control strategies and where they obtained the information they used to manage scab. Of the 161 respondents, 82% had adopted the use of tolerant varieties, 79% fungicides applied at heading, and 63% crop rotation to control FHB. The decision on whether to apply fungicide was largely based on the perceived likelihood of disease development by the growers themselves. Less than 20% used a disease forecasting model available through the internet, or guidance from crop consultants to help make that decision. Many growers reported the use of non-FHB tolerant varieties after having used them in the past. Farmers indicated that yield, and other desirable characteristics were the main reason from switching to non-FHB tolerant cultivars. Growers reported that the most important sources of information on scab control were extension meetings, crop consultants, articles in farm magazines and newspapers, and other extension publications. Less than 15% of the farmers considered the internet, other farmers, and personnel at the local elevator to be important sources of information on scab control.

2009 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) continues to be a potential problem for wheat and barley producers in South Dakota. The objective of this study was to continue evaluation of the efficacy of selected biological control agents (BCAs), alone or in combination with fungicide, that can suppress different measures of FHB under South Dakota conditions. Briggs hard red spring wheat was planted at Brookings, SD. Trial treatments included an untreated check; the fungicide premix Prosaro; *Bacillus* strain 1BA 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 both of the *Bacillus* BCAs. The treatments were applied at anthesis. 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).

Disease development was better than in some recent very dry years. The FHB incidence, FHB index, yield, and FDK were all significant for at least some of the BCA treatments. For FHB incidence, some BCA treatments resulted in significant differences in the absence of Prosaro. It appeared that there was desirable synergistic activity between Prosaro and one or more BCAs used in combination with the fungicide product. Data for DON will not be available until late 2009.

EFFECT OF VARYING COMBINE HARVESTER CONFIGURATIONS ON FUSARIUM DAMAGED KERNELS (FDK) AND DEOXYNIVALENOL ACCUMULATION IN WHEAT GRAIN HARVESTED FROM PLOTS WITH DIFFERENT LEVELS OF FUSARIUM HEAD BLIGHT

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OBJECTIVE

To evaluate the influence of varying combine harvester configurations on *Fusarium* damaged kernels (FDK) and DON in wheat grain harvested from plots with different levels of FHB.

INTRODUCTION

Fusarium Head Blight (FHB), caused predominantly by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae*) in North America, is a serious disease of wheat (*Triticum aestivum* L.) and other small grain in all wheat-growing regions. Infection of wheat spikes may cause significant grain yield and quality losses due to poor grain fill, high percentage of damaged (scabby) kernels, and low test weights. In addition, infected grain accumulates deoxynivalenol (DON), a mycotoxin produced by this pathogen (Bai and Shaner, 1994, and McMullen et al, 1997). DON, also known as vomitoxin, represents a health threat to humans and livestock, therefore mycotoxin-contaminated grain is either rejected or priced down in commerce. Research has shown that DON levels are positively correlated to *Fusarium* damaged kernel (FDK) and other visual estimates of FHB (Paul et al, 2005). As such, reducing FDK generally leads to reduction in DON. Integration of resistant cultivars, fungicide applications and agronomic practices are commonly recommended to reduce FDK and DON in harvested grain (McMullen, 2007). Management recommendations also include strategies to eliminate scabby, light-weight kernels during harvest by adjusting combine settings. However, specific information pertaining to how combine configurations should be adjusted to accomplish this is lacking. In response to this lack of specific information, research is currently being conducted at the Ohio Agricultural Research and Development Center (OARDC) to evaluate the influence of varying combine harvester configurations on FDK and DON.

MATERIALS AND METHODS

Wheat plots of moderately susceptible SRWW cultivar Hopewell were established in a conventionally tilled field, previously planted with soybeans. A total of 60 plots (5-ft x 20-ft) were planted for the experiment. The experimental design was a randomized complete block, with three replicate blocks and harvester configuration and inoculation treatments in a split-plot arrangement. Configuration was the whole plot, whereas inoculum density was the sub-plot. Subplots were spray inoculated with five different inoculum densities (0, 1.5, 3, 4.5 and 6 $x10^4$ spores per mL) prepared using a 1:1 mixture of ascospores and macroconidia of 10 isolates of *Fusarium graminearum*. All inoculations were done at anthesis (Feekes 10.5.1). At soft dough (Feekes 11.2), FHB incidence and index (Stack and McMullen, 1998) were visually estimated in five groups of 20 spikes randomly chosen within each subplot. Assessments were done by estimating percent spike area diseased on an individual plant basis. Plots were harvested using an ALMACO SPC20 plot combine harvester. Prior to harvesting the research plots, the combine was calibrated on non-inoculated, disease-free plots of Hopewell. Threshing, separation and cleaning devices, along with fan speed (airflow speed and volume) were regulated to minimize excessive removal of healthy kernels. Considering that the volume of air flowing through the combine is controlled by the shutter adjustment, configuration settings were regulated as follows: the initial setting, **C1** = Fan speed of 1375 rpm with Shutter opening adjustment of 2 ¾ inches, was used as the default (manufacturer-recommended setting). The three other configurations tested were: **C2** = Fan speed of 1475 rpm and Shutter opening of 2 ¾ inches; **C3** = Fan speed of 1475 rpm with Shutter opening increased to 3 ½ inches; and finally, **C4** = Fan speed of 1375 rpm and Shutter opening of 3 ½ inches. Throughout the harvest of this trial, Winnowing Blower and Threshing Cylinder speeds were held constant. Yield (bu/ac), test weight (lb/bu), and moisture (%) content were determined for each subplot. Grain harvested from each plot was visually rated for FDK (%). During the harvest of each plot, a sample of discarded material was collected at the back end of the combine using a sweep net-type collector attached to a pole. These samples were cleaned and examined for healthy and FDK. Samples of harvested and discarded grain from each subplot were ground and sent for DON analysis at the USWBSI-funded DON testing laboratory at the University of Minnesota

RESULTS AND DISCUSSION

Based on Linear Mixed model analysis (Littell et al, 2006), our results revealed significant differences ($P < 0.05$) among *F. graminerum* inoculation treatments for both FHB incidence (INC) and severity (IND). Mean INC and IND increased with inoculum density, reaching the highest levels in plots inoculated with 6 x10^4 spores/mL (Table 1). Based on Pearson's correlation coefficient (*r*), FDK values from harvested and discarded grain samples were positively correlated ($r = 0.62$ and 0.67, respectively, both with $P < 0.001$) with DON levels. Averaged across all disease levels (inoculum densities), configuration C3 yielded the lowest mean percent FDK and DON and the highest mean test weight (TW) for both harvested and discarded grain samples (Table 2). For harvested grain, the difference between C1, the default setting, and C3 was statistically significant for FDK and TW but not for DON. Comparing configurations at specific mean levels of FHB index, C3 consistently resulted in lower FDK and DON and higher TW than the other configurations (Figures 1, 2, and 3), however, the differences were not statistically significant at all levels of FHB. For instance, C3 resulted in significantly lower DON content than C1 at 29.38% index but not at the other levels of disease (Figure 2). C3 and C4 resulted in the highest percent reduction in FDK relative to C1 at all levels of disease (Table 3). For DON, C3 resulted in the highest percent reduction relative to C1 at the three highest index levels (Table 3). These results suggest that modifying combine configuration to discard scabby, lightweight kernels could minimize wheat grain quality losses due to FHB by reducing the FDK and DON levels of harvested grain. However, for the configurations tested, the effects varied with disease levels. C3 proved to be the most consistent configuration across all tested levels of disease.

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Table 1. Mean percent FHB incidence (INC) and index (IND) for five different inoculum concentrations of *F. graminearum* applied at anthesis (Feekes 10.5.1) to SRWW cultivar Hopewell

Inoculum Concentration	**FHB Intensity (%)***			
(x 10 ^{4} spores/ml)	**INC ^{x}**		**IND^y**	
0	19.50	a	6.72	a
1.5	43.92	b	23.47	b
3.0	53.75	c	29.38	c
4.5	57.92	cd	31.81	cd
6.0	62.92	d	34.82	d
Mean	47.60		25.24	

* Within each column, means followed by the same letter are not significantly different from each other at $P < 0.05$.
x INC = Fusarium head blight incidence (proportion of diseased spikes)
y IND = Fusarium head blight index (mean proportion of diseased spikelets per spike)

Table 2. Mean percent FDK, DON content (ppm), and test weight (TW, lb/bu) of wheat grain harvested with four different combine configurations from plots of SRWW cultivar Hopewell inoculated at anthesis (Feekes 10.5.1) with five different inoculum concentrations of *F. graminearum*

Combine Configuration ^{v}: Fan Speed (rpm) / Shutter opening (inches)	**Harvested Grain***						**Discarded grain*^{w}**			
	FDK1^y		**DON1**		**TW**		**FDK2**		**DON2**	
C1 = 1375/ open 2 ¾	14.73	a	10.53	a	48.48	a	88.33	a	42.73	a
C2 = 1475/ open 2 ¾	11.47	ab	10.78	a	48.75	a	84.00	ab	36.08	a
C3 = 1475/ open 3 ½	8.33	b	8.72	a	50.69	b	71.00	c	18.15	b
C4 = 1375/ open 3 ½	8.60	b	9.35	a	50.37	b	78.00	b	36.17	a
Mean			5		57		.33		3.28	

* Within each column, means followed by the same letter are not significantly different from each other at $P < 0.05$
v Combine configurations, with C1 as the default (manufacturer recommended).
w Discarded grain collected at the back of the combine
y FDK = Percentage of visibly scabby kernels

Table 3. Percent reduction of FDK and DON in harvested grain for different combine configurations relative to the default setting (C1) for plots with different mean levels of FHB

Inoculum Concentration (x 10 ^{4} spores/ml)	Mean FHB Index	Combine Configuration	Mean FDK (%)	Mean DON (ppm)	% Reduction* FDK	% Reduction* DON
0	6.72 %	C1	3.33	1.41	…	…
		C2	3.33	1.34	0	4.55
		C3	2.00	1.40	39.94	0.50
		C4	1.33	1.43	60.06	(1.85)
1.5	23.47 %	C1	17.00	5.63	…	…
		C2	8.00	8.00	52.94	(42.02)
		C3	7.33	7.83	56.86	(39.00)
		C4	6.00	7.13	64.71	(26.63)
3.0	29.38 %	C1	11.67	15.20	…	…
		C2	15.00	12.50	(28.53)	17.76
		C3	8.67	8.23	25.73	45.86
		C4	8.67	8.57	25.73	43.62
4.5	31.81 %	C1	18.33	14.13	…	…
		C2	16.00	15.63	12.71	(10.62)
		C3	11.67	12.07	36.33	14.60
		C4	10.33	12.73	43.64	9.91
6.0	34.82 %	C1	23.33	16.30	…	…
		C2	15.00	16.40	35.71	(0.61)
		C3	12.00	14.07	48.56	13.68
		C4	16.67	16.90	28.55	(3.68)

* Values in parenthesis are negative (Increments in FDK or DON relative to C1)

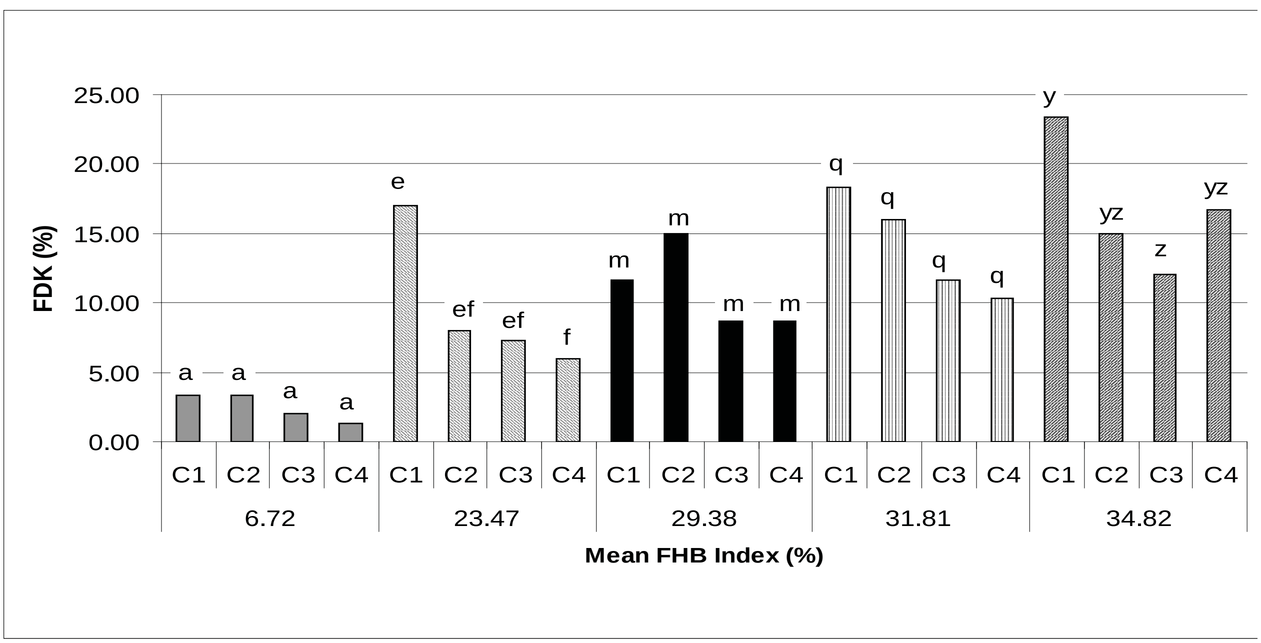

Fig. 1. Mean FDK in wheat grain harvested with four different combine configurations (C1, the default, and C2, C3, and C4 as described in the Materials and Methods) from wheat plots with different mean levels of FHB. Letters above the bars are for comparisons among configurations within, not across disease levels.

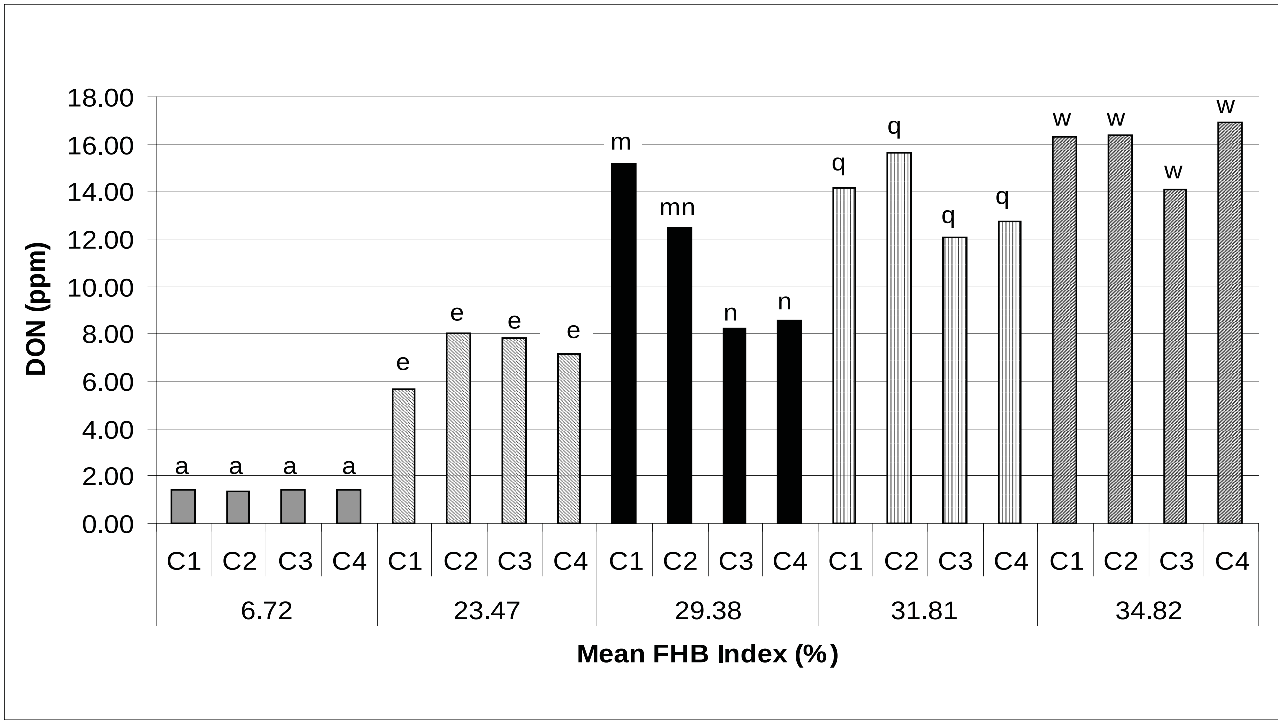

Fig. 2. Mean DON content of wheat grain harvested with four different combine configurations (C1, the default, and C2, C3, and C4 as described in the Materials and Methods) from plots with different mean levels of FHB. Letters above the bars are for comparisons among configurations within, not across disease levels.

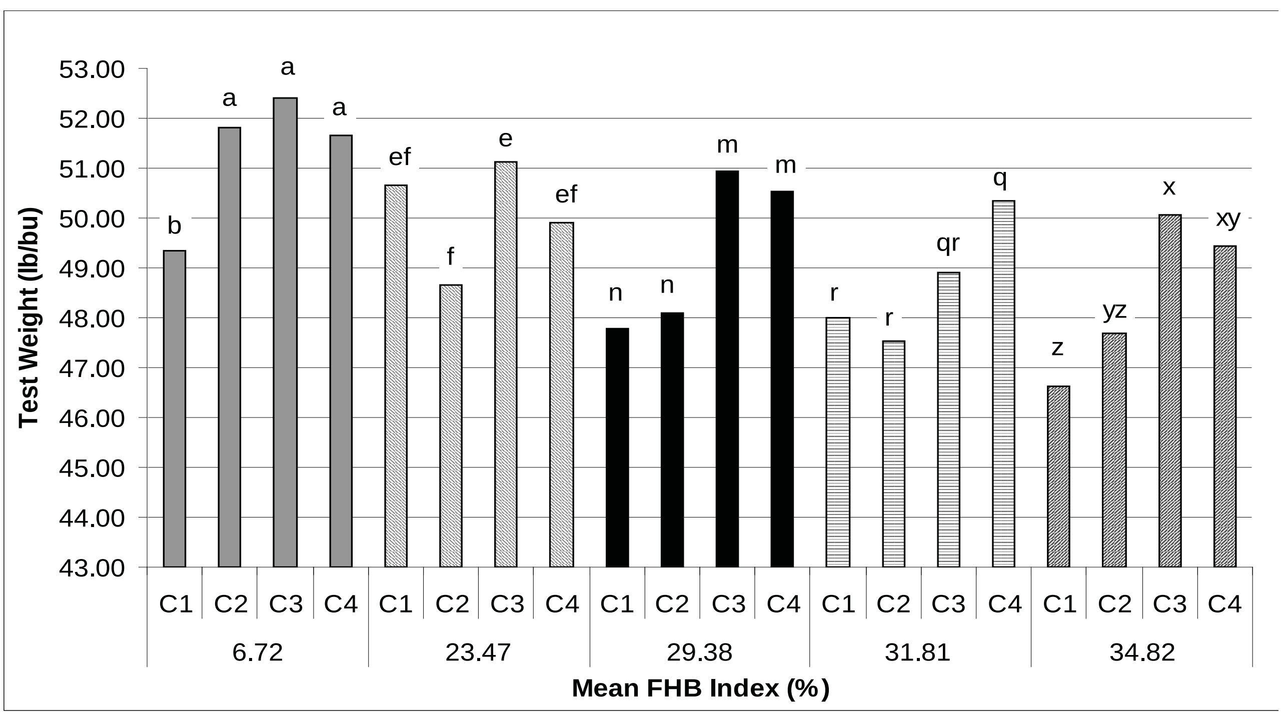

Fig. 3. Mean test weight (TW) of wheat grain harvested with four different combine configurations (C1, the default, and C2, C3, and C4 as described in the Materials and Methods) from plots with different mean levels of FHB. Letters above the bars are for comparisons among configurations within, not across disease levels.

COLONIZATION OF WHEAT HEADS BY FUSARIUM HEAD BLIGHT ANTAGONIST *CRYPTOCOCCUS FLAVESCENS* OH 182.9 WHEN APPLIED ALONE OR IN COMBINATION WITH PROTHIOCONAZOLE AND THE TREATMENT EFFECT ON FHB DISEASE DEVELOPMENT IN FIELD GROWN WHEAT

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OBJECTIVES

Quantify the colonization of infection court tissues of field-grown wheat by FHB biocontrol strain OH 182.9 in the presence or absence of field rates of prothioconazole and 2) determine FHB disease development for the same treatments applied in the colonization work.

INTRODUCTION

Research results to date from laboratories worldwide indicate that it is unlikely that any single control measure will reduce Fusarium head blight (FHB) of wheat to economically acceptable levels when conditions favor disease development. 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 considerable potential for significantly contributing to the reduction of FHB and deoxynivalenol (DON). We have isolated a prothioconazole-tolerant (PTCT) variant of OH 182.9 (OH 182.9 C3) that frequently exhibits enhanced biocontrol activity over its wild type progenitor strain (Schisler et al., 2009). This variant also is tolerant of tebuconazole. As part of an integrated control protocol, strain OH 182.9 could be applied to wheat and barley after flowering when fungicides are not approved for use. Alternatively, a tank mixed prothioconazole and OH 182.9 combination treatment applied at flowering would theoretically provide immediate protection from FHB and lasting protection due to OH 182.9 activity on wheat head infection courts after the fungicide component is no longer effective. The OH 182.9 component of this tank mix could be especially useful in limiting the total DON content in harvested grain by combating new DON producing infections by *F. graminearum* that can occur during early to late grain development (Del Ponte et al., 2007). 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 inadequately colonized infection courts and thereby improve biocontrol effectiveness.

MATERIALS AND METHODS

Selection of prothioconazole-tolterant (PTCT) variant C3

PTCT variant C3 of FHB antagonist *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) was generated by transferring log growth stage cells of wild type (WT) OH 182.9 into 10 ml of 1/5 strength Tryptic soy broth (TSB/5) containing 1 ppm of the fungicide prothioconazole (PTC) in 50 ml Erylenmeyer flasks, shaking flasks at 250 rpm for 5 days at 25 C, plating colonized broth onto 1/5 TSB agar (TSA/5) + 1 ppm PTC and repeating the process with increasing concentrations of PTC. Variant C3 was then evaluated for equivalence to the WT progenitor strain of OH 182.9 based on efficacy in reducing FHB of wheat in greenhouse tests, log growth rate in TSB/5, and carbon utilization profile.

Integrated OH 182.9 and prothioconazole treatments: colonization and efficacy FHB

Field trials were conducted in Peoria, IL in 2009. Soft red winter wheat cultivar Freedom (moderately resistant to FHB) was grown using standard agronomic conditions (Schisler et al., 2006). Corn kernels colonized by native *G. zeae* were scattered through plots (~25-40 kernels/m^2) three weeks prior to wheat flowering. Biomass of WT OH 182.9 and PTCT variant C3 (~3 x 10^8 cfu/ml and 40 gal/acre) 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., 2008). Treatments are shown in Table 1 and included OH 182.9 WT and PTCT variant C3 treatments, PTC at 6.5 oz/acre or 0.65 oz/acre, and combinations of PTC and OH 182.9 C3 applied at flowering (Feekes 10.5) or with OH 182.9 C3 applied 7 days after the PTC application at flowering. Sixteen, 88, 184 and 280 hours after treatment application to wheat heads at flowering, three replicate samples of glume and lemma tissues were taken from selected treatments (Figs 1 and 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 5 ppm prothioconazole to enumerate "total" microbial populations and populations of yeast OH 182.9 C3, respectively. Thirty hours after treatment applications, mist irrigation was applied for 4 minutes per hour from 9 PM to 7 AM for two weeks. Additionally, four rainfall events occurred during the course of the colonization study (Fig 1). Heads were scored for disease severity and incidence and grain evaluated for 100 kernel weight and deoxynivalenol content. Analysis of variance and Fisher's Protected LSD test ($P \leq 0.05$) 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 C3, only glume colonization data are presented. With no rainfall or mist irrigation after treatment application at 0 hours through the first monitoring of OH 182.9 C3 populations at 16 hours, log10 counts of OH 182.9 per gram of fresh glume tissue were low (<2) regardless of the presence or absence of PTC with cells of OH 182.9 C3 (Fig 1). Strain C3 made up approximately 10% or less of the total recovered microbial population (Fig 2). Initiation of misting irrigation prior to the 88 h sampling time corresponded with 1 log unit increases in OH 182.9 C3 that represented 40-70% of the total microbial population (Figs 1,2) though treatments did not differ. Substantial rainfall occurred immediately before application of the three treatments that included OH 182.9 C3 applied 7 days after flowering and in the 16 hours before the 184 hour assessment of OH 182.9 populations. The populations of OH 182.9 on glumes treated 16 hours before the 184 hour assessment were significantly higher in some cases, both on an absolute and percentage of the total population basis (Figs 1,2), than the glumes treated with OH 182.9 C3 seven days earlier. Yet all treatments except 1/10 PTC + OH 182.9 C3 applied at flowering (0 hours) supported OH 182.9 C3 populations that made up 60-95% of the total microbial population recovered (Fig 2) from glumes. Populations of OH 182.9 C3 dropped slightly by the 280 hour assessment for all treatments though even for treatments applied at 0 hours, OH 182.9 C3 made up 40-70% of the total recoverable microbial population (Fig 2). While additional studies are needed, these results support earlier work that demonstrated the competence of OH 182.9 in colonizing wheat head tissues and recovering from low populations once free moisture is available. There was no indication that field rates of PTC inhibited the colonization of OH 182.9 C3 compared to the treatment of OH 182.9 C3 alone. Preliminary results indicate that the hydrophobicity of the surfaces of glume and lemma tissues increase leading up to flowering and then are considerably lower after flowering (Dunlap and Schisler, this volume). Conducting colonization experiments under conditions of constant or controlled levels of moisture availability would help clarify if changes in the physiochemical nature of glume and lemma surfaces influence colonization of these surfaces.

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 and that treatments that contained both biocontrol and PTC provided the greatest arithmetic reduction in FHB symptoms and DON (Table 1). The discovery that PTCT variant C3 of OH 182.9 regularly exhibited enhanced efficacy in reducing FHB/DON and can be successfully combined with prothioconazole are key steps in the process of developing successful integrated FHB control strategies. We anticipate replicating these trials in two locations during the 2010 field season.

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DISCLAIMER

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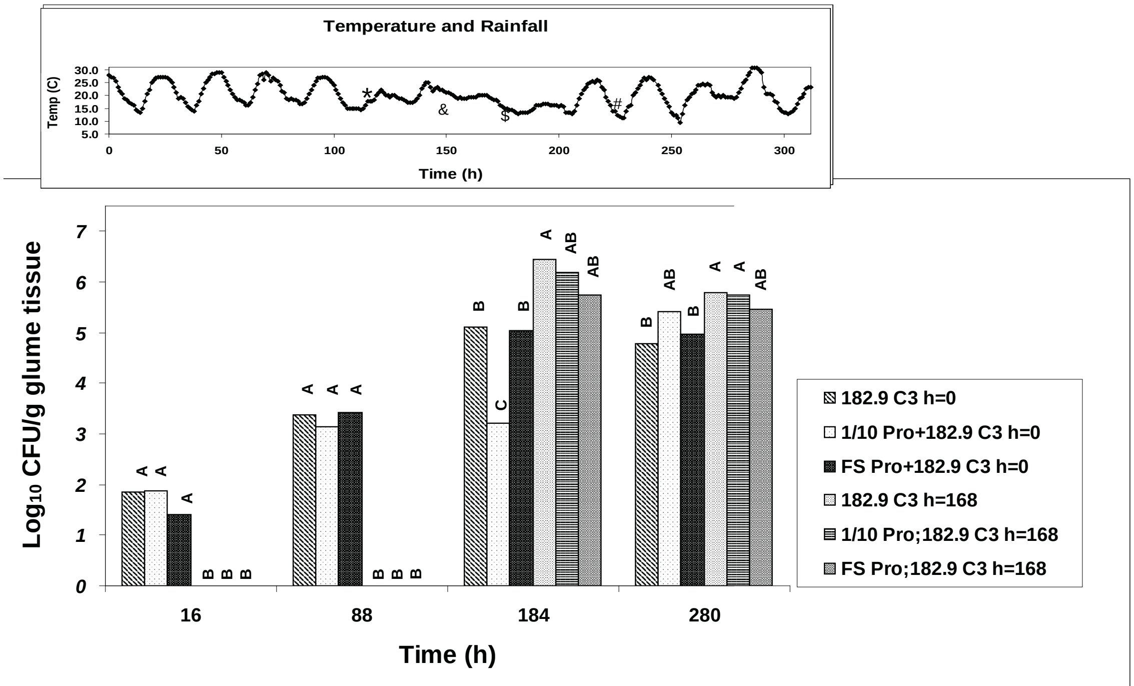

Fig 1 Log_{10} population of *Cryptococcus flavescens* OH 182.9 C3 on glume tissue when applied alone or in combination with prothioconazole at or seven days (168 hours) after wheat flowering. Rain events of 0.51 cm, 8.2 cm, 3.9 cm and 0.1 cm occurred between hours 120 and 144, 144 and 168, 168 and 216, and 216 and 240, respectively, and are designated on the temperature and rainfall graph with the symbols *, &, $, and #, respectively.

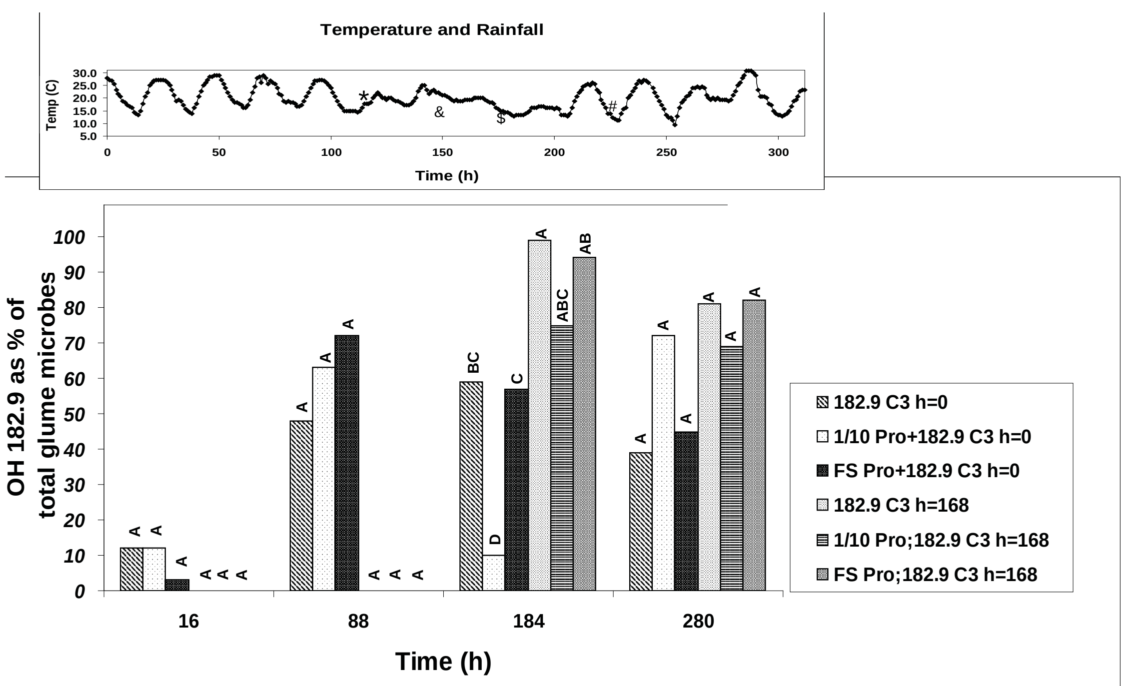

Fig 2 Population of *Cryptococcus flavescens* OH 182.9 C3 on glume tissue expressed as a percentage of the total recoverable microbial population when strain C3 applied alone or in combination with prothioconazole at or seven days (168 hours) after wheat flowering. Rain events are as described for Fig 1.

Table 1. 2009 field trial results at Peoria, IL: Influence of prothioconazole, yeast antagonist OH 182.9, prothioconazole tolerant variant C3 of OH 182.9 and combinations thereof on FHB disease parameters on winter wheat cultivar Freedom

	Wheat Cultivar Freedom			
Treatment[c]	DS (%)	DI (%)	100 KWT (g)	DON (ppm)
Untreated control	3.3[A]	30.7[A]	3.2[FG]	8.6[ABC]
182.9 C3 h=0	1.9[CD]	20.0[DE]	3.2[EFG]	8.5[ABC]
1/10 Pro+182.9 C3 h=0	1.3[DE]	14.9[EFG]	3.2[EF]	7.1[BCD]
FS Pro+182.9 C3 h=0	1.1[EF]	11.2[GH]	3.5[B]	2.1[F]
182.9 C3 h=168	1.9[CD]	18.9[DEF]	3.2[GH]	9.3[ABC]
1/10 Pro h=0;182.9 C3 h=168	2.7[AB]	27.2[AB]	3.2[FG]	9.6[ABC]
FS Pro h=0; 182.9 C3 h=168	0.9[EF]	10.4[GHI]	3.6[A]	3.3[EF]
1/10 Pro h=0	2.2[CD]	18.7[DEF]	3.3[D]	7.6[ABC]
FS Pro h=0	1.4[DE]	13.9[FG]	3.5[B]	3.5[DEF]
182.9 wild type h=0	2.5[BC]	23.7[BCD]	3.2[EF]	10.6[AB]
P value	0.05	0.05	0.05	0.05

[a]Within a column, means followed not followed by the same letter are significantly different ($P<0.05$, FPLSD mean separation)

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

[c]Pro (Prosaro)= Commercial fungicide formulation of prothioconazole applied at a rate equivalent to 6.5 oz/acre (FS=full strength) or at 1/10 this rate; 182.9 C3 (*Cryptococcus flavescens* variant C3)= prothioconazole tolerant variant of OH 182.9; 182.9 WT= Wild type strain of OH 182.9; h= time, in hours, of application of treatment (h= 0 represents application at flowering, h= 168 represents application seven days after flowering).

SPATIAL PATTERNS AND INCIDENCE-SEVERITY RELATIONSHIPS OF FUSARIUM HEAD BLIGHT EPIDEMICS ON WHEAT CROPS FOLLOWING SOYBEAN OR MAIZE IN RIO GRANDE DO SUL, BRAZIL

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ABSTRACT

A survey on Fusarium head blight (FHB) was conducted in 36 arbitrarily selected spring wheat fields in northern crop growing regions of Rio Grande do Sul State, southern Brazil. The fields varied in wheat cultivar and followed either corn or soybean. Surveys were made during October (approximately kernel soft dough stage). In each field, 20 sampling areas were randomly selected and 10 adjacent wheat heads were randomly sampled at the sampled area. In the laboratory, both incidence (I) and severity (S = FHB index) were visually estimated in each 10-head sample. Spatial pattern of FHB incidence was studied by calculating the mean incidence and the index of dispersion (D). The incidence-severity (I-S) relationship was studied by fitting empirical regression models to the data. FHB was present in all fields assessed with an overall mean incidence of 41.2% (0.6 – 90%). In most fields (30/36), the pattern of FHB was random. Three out of six fields where aggregation was detected were located in the same region that also had the highest mean incidence. Strong evidence of FHB aggregation among sampling areas (P<0.01) was verified in only three fields that followed soybean. However, fields following corn presented a slightly higher incidence levels. A model based on complementary log-log transformation of I and S performed well for the data set. Estimated slope from the fit of the model for the pooled data was 1.1. Our preliminary results confirm previous findings elsewhere and support our hypothesis that in areas where no-till system is intensive FHB spatial pattern is predominantly random given the abundance of regional inoculums levels. Once further incidence and severity data is collected, simple and robust models for predicting severity from incidence will be useful to perform faster and accurate FHB assessments.

INTEGRATED MANAGEMENT STRATEGIES FOR FUSARIUM HEAD BLIGHT OF SOFT RED WINTER WHEAT IN MISSOURI: SUMMARIZATION OF TRIAL DATA FOR THREE YEARS

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OBJECTIVE

To evaluate the importance of crop sequence, variety selection and fungicide application as components of an integrated management program for Fusarium head blight (FHB) of soft red winter wheat in Missouri.

INTRODUCTION

The severity of FHB or scab epidemics in the United States has caused enormous yield and quality losses in both wheat and barley over the last decade. The development of this disease is dependent on the genetics of the host, favorable environmental conditions, the prevalence of the causal fungus and the survival and spread of the causal fungus. Control of this disease has been difficult because of the complex nature of the host/pathogen interaction. Management of FHB and the associated mycotoxin DON have not been achieved by any single control measure. An integrated approach is critical to attaining the best possible management of FHB and DON in any given environment.

As a result of a workshop sponsored by the Chemical, Biological and Cultural Control Research Area of the U.S. Wheat & Barley Scab Initiative in 2006, a protocol for a multi-state project focusing on integrated management strategies for FHB was developed. The research portion of the project has been multi-state trials evaluating crop sequence, variety selection and fungicide application as an integrated management program for FHB.

The University of Missouri has participated in the multi-state integrated management project for the past three growing seasons. Results from the three years are summarized in this poster abstract.

MATERIALS AND METHODS

During the fall of 2006 two adjacent fields at the University of Missouri Bradford Research and Extension Center just east of Columbia, MO, were identified for this study. The fields had been in a corn/soybean rotation for at least five years prior to the initiation of the study and were separated by a small drainage ditch. The wheat trials were planted into standing corn residue or soybean residue on the same day. The remainder of each field was planted into the normal rotational crop of corn or soybeans. In subsequent years, the wheat trials were shifted to other areas of the same fields with the remainder of the fields planted to the normal rotational crop.

Five soft red winter wheat varieties with similar heading times and varying reactions to FHB were selected for the trial. The five varieties included the public varieties Bess and Roane which are widely grown in Missouri, the Agri-Pro variety Elkhart and the Pioneer varieties 25R47 and 25R54. The FHB resistance reactions for the five varieties are as follows: Bess is considered as tolerant, Elkhart as susceptible, Pioneer variety 25R37 as moderately susceptible, Pioneer variety 25R54 as moderately tolerant and Roane as moderately tolerant.

In the fall of 2006 the trials were planted no-tillage into either soybean residue or standing corn residue on the same day. Individual plots were 7 rows (~7.5” row spacings) by 30’ in length. Each trial was set up as a split plot trial with fungicide application as the main plot and variety as the sub-plot. There were 6 replicates in each trial. Sub-plots were separated by buffer plots. The foliar fungicide treatment Prosaro (6.5 fl oz/A) was applied at Feekes Growth Stage 10.51. A non-ionic surfactant was added to the fungicide at a rate of 0.125% v/v, and application was made using a CO2 pressurized backpack sprayer with TwinJet XR8002 nozzles mounted at an angle (30 and 60 degrees) forward and backward.

Plots were evaluated for incidence and severity of FHB, yield was taken, grain samples were submitted to North Dakota State University for DON analysis and grain samples were rated for percent of *Fusarium* damaged kernels (FDK). Data has been submitted annually to the regional coordinator for inclusion in the multi-state project report. Analysis of variance was used to determine the effects of variety, fungicide and their interactions on yield, DON levels, FHB index (average of 100 wheat heads per plot) and percent FDK for each residue type.

The trial was repeated following the same protocol during the 2007-2008 and 2008-2009 seasons.

RESULTS

Weather conditions during the 2006-2007 season were not conducive for the development of FHB at the Columbia, MO location. Conditions as the wheat crop was flowering were too dry for infection to occur and disease to develop. However, both the 2007-2008 and 2008-2009 seasons were quite conducive for the development of FHB. In both 2008 and 2009 weather conditions were unusually wet and cool as the wheat crop flowered and after flowering.

2007: Weather conditions were not conducive for the development of FHB. In both trials, the yield was statistically significantly different only by variety. For DON levels effects of residue type and residue type x variety were significant. All main and interaction effects were statistically significant for FHB index. Only residue type and variety were significant for % FDK. DON levels were slightly higher in all varieties in the corn residue trials than in the soybean residue trial.

2008: Weather conditions were quite favorable for the development of FHB and FHB developed in all five varieties in both residue types. In the corn residue trial all main and interaction effects were statistically significant for yield and DON levels. Overall, yields were higher and DON levels lower in the soybean residue trial than in the corn residue trial.

2009: Weather conditions again were very conducive for the development of FHB in all five varieties in both crop sequence trials. In the corn residue trial all main and interaction effects were statistically significant for both yield and DON. In the soybean trial main and interaction effects were statistically significant for DON. Although yields in the soybean residue trial tended to be lower than the yields of the same varieties in the corn residue trial, weed competition in the soybean trial may have been a factor. The DON levels for all varieties were lower in the soybean residue trial than in the corn residue trial.

Three Year Summary: Data from the three years for each crop residue type were analyzed using ANOVA. In corn residue, yields were statistically different for year, variety, year x variety and fungicide x variety but not for fungicide alone, year x fungicide or year x fungicide x variety. DON levels were statistically significantly different for all main and interaction effects. For both the FHB index and the percent FDK effects were statistically significant for all but year x fungicide x variety.

In soybean residue, yield was statistically significant for all main and interaction effects except year. DON levels and FHB index were statistically significant for all main and interaction effects. Percent FDK was statistically significant for year, fungicide, variety and year x variety but not for year x fungicide, fungicide x variety or year x fungicide x variety.

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DISCLAIMER

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

FUNGICIDES CONTROL OF FUSARIUM HEAD BLIGHT SYMPTOMS AND DEOXYNIVALENOL (DON) LEVEL CAUSED BY 15-ADON AND 3-ADON *FUSARIUM GRAMINEARUM* ISOLATES IN WHEAT IN ONTARIO

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ABSTRACT

Fusarium graminearum (Schwabe) causes Fusarium head blight (FHB), an important wheat disease. Deoxynivalenol (DON) is the most important mycotoxin produced by *F. graminearum*; 15-acetyl DON (15-ADON) and 3-acetyl DON (3-ADON) analogs are also produced. A shift in the presence of two *F. graminearum* chemotypes, 15-ADON and 3-ADON, has been reported in North America. The shift may influence current FHB management strategies including the use of fungicides. FOLICUR (tebuconazole) and PROLINE (prothioconazole) are two fungicides commonly used for FHB control in Ontario, while PROSARO has active ingredients from both fungicides. The objectives of this study investigated: 1) the effect of the fungicides on FHB symptoms and DON level after inoculation with 15-ADON and 3-ADON *F. graminearum* isolates in inoculated, misted wheat plots, and 2) the mycelium growth of different isolates of *F. graminearum* on PDA medium with and without fungicides. In 2008, both FHB index (%) and DON level were lower in cv. "Alsen" (moderately resistant) compared to cv. "Roblin" (highly susceptible) in all fungicide treatments and the untreated control, confirming that host resistance plays an important role in host-pathogen-fungicide interaction. Among all fungicide treatments, PROSARO and PROLINE produced the lowest FHB index DON concentration in the variety "Alsen", respectively. In addition, PROSARO resulted in the highest reduction of mycelium growth of both chemotypes compared to other fungicides.

EVALUATION OF INTEGRATED FHB MANAGEMENT METHODS UNDER MODERATE AND SEVERE EPIDEMICS IN NEW YORK

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars, foliar fungicide (Prosaro), and a biological control agent (*Bacillus subtilis*) on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) under two natural 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 2009, 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 winter wheat in the region. The two experimental wheat environments were characterized by the planting of winter wheat 1) no-till into soybean residue in late September 2008 and 2) no-till into corn residue in late October 2008. 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 a10 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) nonsprayed; 2) Prosaro 6.5 fl oz/A & Induce 0.125%; 3) *Bacillus subtilis* TrigoCor ca. 1.5 x 10^{14} cfu/A & Induce 0.125%; and 4) TrigoCor & Prosaro & Induce. Application was made with paired Twinjet nozzles mounted at an angle (30^{o} from horizontal) forward and backward and calibrated to deliver at 20 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 the USWBSI-supported mycotoxin laboratory of Dr. Schmale.

RESULTS AND DISCUSSION

Due to moist weather through grain maturation, FHB occurred in both experimental environments. A moderate FHB epidemic was observed in the timely-planted plot following no-till soy, and a severe FHB epidemic was observed in the late-planted plot following no-till corn. Difference in epidemic severity for the two experiments is best explained by differences in flowering dates and moisture conditions through flowering. Wheat cultivars reached Feekes GS10.5.1 on June 5 and June 12 for the timely and late-planted experiments, respectively, while rain occurred frequently from June 9 through early July. The impact of crop residue type on FHB development was apparently less important than weather conditions as an adjacent experiment on plowed ground with no corn residue planted to Jensen wheat on the same date had a similar incidence of FHB (40%) as compared to nontreated Jensen (49%) in the late-planted management experiment into no-till corn.

Foliar diseases, including leaf rust and leaf spots, were observed in both experimental environments and were reduced significantly by application of Prosaro. In the timely-planted experiment, no foliar spray treatment (fungicide, biological control, or combination) had a significant effect on the yield of any cultivar. In the late-planted experiment, significantly greater yields due to fungicide treatment were observed in the two white cultivars, Jensen and Richland (Figure 1). Under the lower disease pressure of the timely-planted experiment, the fungicide application decreased DON levels to below 2.0 ppm for the two red cultivars, Pioneer 25R57 and Truman. Under the higher disease pressure of the late-planted experiment, Prosaro did not consistently reduce DON contamination and, when reductions were observed, remaining DON levels still greatly exceeded the 2.0 ppm threshold for sale at flour mills. Therefore, under severe epidemic conditions, the combination of the best available cultivar and fungicide did not reduce DON to satisfactory levels (Table 1). While TrigoCor alone was not able to inhibit FHB, it neither impaired disease control of the fungicide when applied as a mixture nor reduced yield. Moderate FHB resistance was observed with the cultivar Truman, averaging the lowest FHB incidence and DON levels of all of the cultivars in both experimental environments. While Truman is a lower yielding cultivar, the benefit of resistance under high disease pressure was shown by the yield results of the no-till corn plot. Designation of moderate resistance status of cultivars including Jensen is based primarily on observations of FHB symptoms at soft dough stage. The finding of very high DON levels in a cultivar designated as moderately resistant suggests that DON production should be given greater weight in future designation of cultivar reaction.

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Table 1. Main effect of treatment on grain yield, FHB incidence, and deoxynivalenol contamination at Aurora, NY.

	Adjusted grain yield (bu/A)		
Treatment:	Moderate FHB epidemic	Severe FHB epidemic	Average
No treatment	79.1	47.3	63.2
TrigoCor	80.0	48.6	64.3
Prosaro	87.7	58.5	73.1
Prosaro & TrigoCor	89.5	57.4	73.5
LSD (*P*=0.05)	NS	7.8	

	FHB incidence (%)		
Treatment:	Moderate FHB epidemic	Severe FHB epidemic	Average
No treatment	4.7	50.6	28
TrigoCor	4.8	47.3	26
Prosaro	1.1	22.8	12
Prosaro & TrigoCor	2.4	25.1	14
LSD (*P*=0.05)	1.7	10.3	

	Contamination of grain by DON (ppm)		
Treatment:	Moderate FHB epidemic	Severe FHB epidemic	Average
No treatment	2.0	26.4	14.2
TrigoCor	2.7	25.9	14.3
Prosaro	1.2	17.2	9.2
Prosaro & TrigoCor	1.7	21.4	11.5
LSD (*P*=0.05)	0.8	NS	

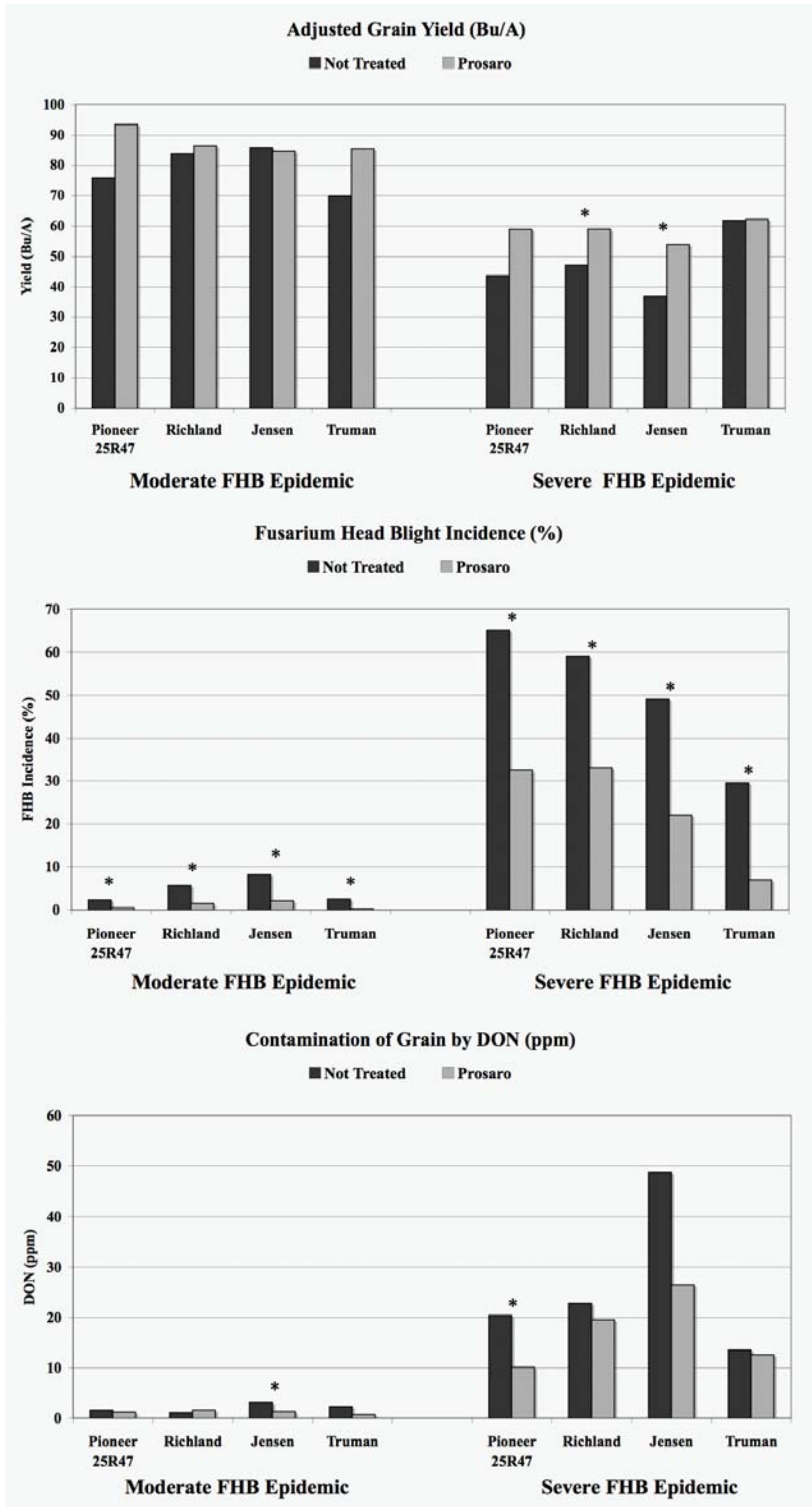

Figure 1. Effect of flowering stage application of Prosaro fungicide on yield, FHB incidence and DON contamination of four winter wheat cultivars in Aurora, NY. * denote treatment means that differ significantly at *P*=0.05.

INTEGRATED MANAGEMENT OF FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL IN WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat. In addition to lowering yield and grain quality, the causal fungus, *Fusarium graminearum*, also produces the mycotoxin deoxynivalenol (DON) which poses potential food and feed safety hazards. Integrating cultivar resistance and fungicide application is more effective in managing FHB than either strategy used alone. The objective of this study was to determine the effects of fungicide application and cultivar resistance on FHB and DON in winter wheat. Three cultivars differing in levels of resistance to FHB were planted following corn in the fall of 2008 at the University of Nebraska Agricultural Research and Development Center near Mead, NE. The cultivars were 2137 (susceptible), Jagalene (moderately susceptible), and Harry (moderately resistant). In the spring of 2009, corn kernels colonized by *F. graminearum* were applied to the soil surface in the wheat plots on May 20 at a rate of 50 g/m^2. Plots were not irrigated. The experimental design was a split plot in randomized complete blocks with six replications. Cultivars were the main plots and fungicide treatments (non-treated or treated with Prosaro at 6.5 fl. oz/acre + Induce non-ionic surfactant at 0.125% v/v) were the subplots. Plot size was 5 ft x 11ft. A CO_2-powered backpack sprayer and four Teejet 800-1 VS nozzles spaced 12 in. apart on a boom were used to apply fungicide to heads at early flowering. Fungicide was applied on May 28 (Jagalene and 2137) and on June 4 (Harry). Plots were inoculated with spores of *F. graminearum* (1 x 10^5 spores/ml) using a hand-pumped backpack sprayer. Inoculation dates were May 30 (Jagalene and 2137) and June 6 (Harry). Disease severity and incidence were assessed on 10 heads in each of five arbitrarily selected clusters in each plot and used to calculate FHB index. Disease assessment dates were June 20 (Jagalene and 2137) and June 27 (Harry). Plots were harvested with a small plot combine, which provided yield data. The percentage of *Fusarium*-damaged kernels (FDK) was measured by an automated single-kernel near-infrared system at the USDA ARS Grain Marketing and Production Research Center in Manhattan, KS. A grain sample from each plot was ground and sent to the North Dakota Veterinary Diagnostic Laboratory at North Dakota State University, Fargo, ND for DON determination. Disease levels were low due to dry weather in May. Differences in FHB index among cultivars were highly significant ($P < 0.0001$). FHB index in Harry (9.5%) was higher than that in either Jagalene (0.5%) or 2137 (1.0%). Fungicide application reduced FHB index, but not significantly ($P = 0.0748$). FHB index was 0.7, 1.3, and 10.3% for Jagalene, 2137, and Harry, respectively, in the non-sprayed treatment and 0.3, 0.7, and 8.7% for Jagalene, 2137, and Harry, respectively, in the Prosaro treatment. Fungicide treatment significantly ($P = 0.0051$) increased yield. Yield in the Prosaro treatment was higher (Jagalene, 34 bu/A; 2137, 29 bu/A; Harry, 36 bu/A) than that in the check treatment in all three cultivars. In the check treatment, yield of Jagalene (22 bu/A) was lower than that of 2137 (26 bu/A) or Harry (29 bu/A). Fungicide application significantly reduced FDK ($P < 0.0001$) and DON ($P = 0.0019$). FDK and DON in the Prosaro treatment were lower than in the check treatment for all three cultivars. In the check treatment, FDK (24%) and DON (0.52 ppm) in 2137 were lower than in Jagalene (41% FDK, 0.74 ppm DON)

and Harry (54% FDK, 5.36 ppm DON). In the Prosaro treatment, FDK (40%) and DON (2.86 ppm) in Harry were higher than in Jagalene (16% FDK, 0.44 ppm DON) and 2137 (21% FDK, 0.30 ppm DON). The winter wheat cultivars in this study differed in their reaction to FHB. Although fungicide application did not significantly ($P = 0.0748$) reduce FHB index, it reduced FDK and DON in all three cultivars. Late rains in early June coincided with flowering in Harry. Therefore, FHB index, FDK, and DON were all higher in Harry than in Jagalene or 2137.

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INTEGRATED MANAGEMENT OF FHB AND DON IN SMALL GRAINS: 2009 COORDINATED TRIALS

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OBJECTIVE

To evaluate the integrated effects of fungicide and genetic resistance on FHB and DON in all major grain classes in different cropping systems.

INTRODUCTION

Current FHB and DON control options include genetic resistance, cultural practices, chemical and biological control. However, when used individually, these control measures are not fully effective under environmental conditions favorable to disease development. Moderately-resistant wheat and barley cultivars may accumulate DON levels above critical thresholds for human and livestock consumption (Browne, 2009). Triazole fungicide efficacy varies among studies, with mean percent control between 40 and 60% for FHB index and 30 to 50% for DON accumulation (Paul *et al*, 2008). In general, more effective control is achieved when moderate resistance is combined with appropriate fungicide applications (Beyer *et al*, 2006). However, this control is variable among grain classes and cropping systems. In 2007 and 2008, coordinated trials in multiple states evaluated the effects of grain class, crop rotation, cultivar resistance, and fungicide application on the management of FHB and DON (Paul *et al*, 2007 and Paul *et al*, 2008). This report summarizes results from trials conducted in 2009.

MATERIALS AND METHODS

Plots were established in fields previously planted with wheat, corn or soybean. At least three commercial small grain cultivars were planted in four to six replicate blocks in each trial. The standard experimental design was a randomized complete block, with a split-plot arrangement of fungicide treatment (whole-plot) and cultivar (subplot). A few trials used a split-split-plot arrangement with previous crop as the whole plot. Fungicide (Prosaro, 6.5 fl. oz/A) was applied at anthesis, using CO_2 powered sprayers equipped with Twinjet XR8002 or paired XR8001 nozzles, mounted at a 30 or 60^o angle, forward or backward. Protocol did not include artificial inoculations or supplemental misting to stimulate disease development. FHB index (plot severity) was assessed during the dough stages of grain development. Following harvest, yield, test weight and percentage of *Fusarium*-damaged kernels (FDK) were determined for each sub-plot. Milled grain samples were sent to a USWBSI-supported laboratory for toxin analysis. Analysis of variance (linear mixed model) was used to evaluate the effects of fungicide, cultivar, (previous crop, when appropriate) and their interactions on FHB and DON. For severe epidemics, percent control was calculated to compare the effect of control measures to the untreated, susceptible check.

RESULTS AND DISCUSSION

Trials were conducted in 13 states (Arkansas, Illinois, Indiana, Kentucky, Maryland, Minnesota, Missouri, Nebraska, New York, North Dakota, Ohio, South Dakota and Wisconsin). FHB intensity and DON accumulation varied among locations. Trials with minimal disease and/or DON were not included in this summary (Arkansas, North Dakota, Ohio and Wisconsin). Data from Minnesota were forthcoming at publication time.

Illinois. Six soft red winter wheat (SRWW) cultivars were planted at four locations in a split-split plot design with previous crop (soybean or corn) as the main-plot, cultivar as the sub-plot and fungicide as the sub-sub-plot. FHB intensity varied greatly throughout the state (Table 1). In all locations, fungicide treatment had significant effects on index, while fungicide and cultivar had significant effects on DON. **Carbondale.** Prosaro-treated plots had significantly less disease than untreated controls, however FHB remained high (17.6 and 24.0%, respectively). Control measures provided minimal control of FHB index, but reduced DON levels, considerably (Table 1). In addition to cultivar and fungicide, previous crop and the cultivar x fungicide interaction had significant effects on DON accumulation. Generally, when moderately resistant cultivars were planted into soybean residue <2ppm DON accumulated in the grain, without the use of Prosaro (Table 1). **Dixon Springs**. Eight SRWW cultivars were used in this trial. The effects of cultivar and fungicide were significant for index at this location. Prosaro-treated plots had significantly lower mean index than the untreated checks, 3.9 and 12.0%, respectively. Cultivars Pro220, Exc5530 and Exc5170 had significantly lower index values than Cooper. Regardless of previous crop, Kaskaskia and P25R62 provided the highest levels of FHB control, compared to untreated Copper grown in corn residue (Table 1). DON data was unavailable for this site. **Monmouth**. Mean index values were <1% throughout this trial. Despite low disease intensity, DON levels were as high as 4.00ppm. Mean DON levels in Kaskaskia, P25R47and P25R54 were not significantly different from that of Cooper (mean 1.4, 2.3, 1.7 and 1.7ppm, respectively). **Urbana.** Three levels of previous crops (soybean, conventional corn and Bt corn) were used in this trial; however, this factor had no significant effect on index or DON. Kaskaskia had significantly higher levels of index (8.0%) and DON (3.4ppm) than Cooper (2.9%; 2.0ppm), despite providing some of the best disease control at the Dixon Springs location.

Indiana. Six SRWW cultivars were planted into corn residue. Index levels and grain DON content ranged from 0 to 6.5% and 0 to 1.7ppm, respectively. The effects of cultivar, fungicide treatment and their interaction were statistically significant for both index and DON, although mean DON values were <2ppm. P25R47 and P25R78 had significantly lower index than Hopewell, the susceptible check. Moderately resistant INW0412 and INW0801 had index levels that were not statistically different from Hopewell. Prosaro did not provide additional significant index reduction for Truman or P25R47.

Kentucky. SRWW cultivars were planted into corn residue near Princeton, KY. Index levels ranged from 0.3 to 6.5%, while DON ranged from 0.25 to 2.3ppm. The effects of fungicide, cultivar and their interaction were statistically significant for index and DON. AC9511 had significantly lower index and DON than Branson and P26R15 in untreated control plots. Combining AC9511 with Prosaro treatment did not provide any significant reduction in DON accumulation compared to the untreated control.

Maryland. SRWW cultivars were planted at two locations (Beltsville and Queensland) into both corn and soybean residues. Overall, FHB index was greater at Queensland (4.6%) than at Beltsville (1.8%) (DON data was unavailable at publication time). Index was slightly lower when the previous crop was soybean rather than corn. For both locations and previous crops, the effects of cultivar and fungicide were statistically significant for index. Bess, AC9511 and P26R15 had significantly less disease than susceptible SS8641 in all locations and cropping systems.

Missouri. Five SRWW cultivars were planted into corn and soybean residue. **Corn**. The effects of cultivar, fungicide and their interaction were statistically significant for FHB index and DON. Mean index and DON values were 25.7% and 5.6ppm, respectively. Moderately resistant cultivars, Roane and Bess had significantly lower disease and DON levels than other cultivars, however, mean index was still relatively high (Table 2). For each cultivar, the Prosaro treatment resulted in significantly lower DON than in the untreated check. Bess combined with Prosaro treatment was the only combination to achieve <2ppm DON. **Soybean**. The effects of cultivar, fungicide and their interaction were statistically significant for FHB index. Mean index and DON values were 29.1% and 2.8ppm, respectively. 'Roane' had significantly lower index than all other cultivars; however mean index for treated and untreated sub-plots was 17.10 and 19.97%, respectively (Table 2). Prosaro did not have a significant effect on index for Roane, compared to the untreated control. Only the effect of cultivar had a significant effect on DON. Roane, Bess and P25R54 accumulated significantly less DON than Elkhart and P25R47.

Nebraska. Three hard red winter wheat cultivars were planted into corn residue. For FHB index, only the effect of cultivar was statistically significant. The effects of cultivar, fungicide and their interaction were statistically significant for DON accumulation. Index and DON were significantly lower in Jagalene and 2137 than in the susceptible check, Harry. There was no significant reduction in DON between Prosaro-treated and untreated plots for Jagalene and 2137 (mean DON accumulation for these combinations were <1ppm).

New York. Two split-split plot trials were planted near Aurora, using two soft white and soft red winter wheat cultivars in each. Previous crop served as the whole plot factor, while cultivar and fungicide treatments served as sub- and sub-sub-plot factors, respectively. Overall, mean FHB index and DON levels were greater in SWWW than in SRWW (Table 3). **SWWW.** The effect of previous crop, fungicide, their interaction and the cultivar-fungicide interaction were statistically significant for index. All main and interaction effects, including the three-way interaction, were significant for DON accumulation. Jensen, the resistant cultivar, provided little control of FHB or DON; in fact, index and DON levels were often lower in Richland, the susceptible (Table 2). This seriously questions the resistance mechanisms Jensen may or may not possess. **SRWW.** The effects of previous crop, fungicide, cultivar and the crop x cultivar and crop x fungicide interactions were statistically significant for index. Only previous crop was statistically significant for DON in this trial, as levels were similar for both cultivars and treatments (Table 3).

South Dakota. A split-plot trial with 3 hard red winter wheat cultivars was planted into HRSW residue. Cultivar and fungicide treatment served as the whole plot and sub-plot factors, respectively, and were statistically significant for index and DON. The interaction between cultivar and fungicide was significant for DON. Overall, FHB index was significantly lower in Overland than Alice and Wesley. In all cultivars x fungicide interactions the difference between treated and untreated sub-plots was significant. Despite relatively low index (0.93%), Overland accumulated >2ppm DON without Prosaro.

CONCLUSIONS

In general, the greatest reductions in FHB intensity and DON accumulation were observed when moderately resistant cultivars were used. However, this coordinated effort demonstrated that cultivars, including Kaskaskia, P25R47, and P26R15, had variable FHB disease phenotypes at different locations. The effect of previous crop also had mixed results. In Illinois and Missouri, a non-host crop as the previous crop resulted in little control of FHB.

In Maryland and New York a non host crop resulted in reductions in index compared to a host crop as the previous crop. This warrants further study and suggests climate at these locations affects the efficacy of control measures. Under severe epidemic conditions, a three tier management approach of crop rotation with a non-host, moderately resistant cultivars and fungicide application was required to achieve < 2ppm DON and reduce index. Future work includes quantitative analysis of data from previous years' uniform trials, which will contribute to ongoing efforts to develop and disseminate "best management practices" for FHB and DON reduction.

AKNOWLEDGEMENT AND DISCLAIMER

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Table 1. Mean FHB Index, DON and estimated percent control for different previous crop–cultivar–fungicide combinations in two Illinois locations (DON data not available for Dixon Springs).

				FHB DISEASE		DEOXYNIVALENOL						FHB DISEASE	
Location (Grain Class)	Previous Crop	Cultivar	Prosaro	Mean Index (%)	% Control	Mean DON (ppm)	% Control	Location (Grain Class)	Previous Crop	Cultivar	Prosaro	Mean Index (%)	% Control
Carbondale, IL (SRWW)	corn	Cooper	NO	24.14	0.00	3.43	0.00	Dixon Springs, IL (SRWW)	corn	Cooper	NO	9.10	0.00
			YES	17.53	27.38	2.05	40.15				YES	4.17	54.21
		P25R47	NO	23.25	3.69	3.53	-2.92			P25R47	NO	20.40	-124.18
			YES	16.52	31.57	2.63	23.36				YES	4.03	55.68
		Kaskaskia	NO	20.75	14.04	2.18	36.50			Kaskaskia	NO	4.18	54.03
			YES	17.33	28.21	0.95	72.26				YES	0.93	89.74
		P25R54	NO	28.00	-15.99	3.00	12.41			P25R54	NO	5.63	38.10
			YES	18.61	22.91	1.13	67.15				YES	3.00	67.03
		E5530	NO	19.00	21.29	1.50	56.20			E5530	NO	13.45	-47.80
			YES	17.00	29.58	0.66	80.73				YES	7.87	13.55
		E5170	NO	28.25	-17.03	0.96	71.90			E5170	NO	17.03	-87.18
			YES	20.00	17.15	0.68	80.29				YES	11.02	-21.06
		…	…	…	…	…	…			Pro220	NO	9.13	-0.37
		…	…	…	…	…	…				YES	2.78	69.41
		…	…	…	…	…	…			P25R62	NO	5.32	41.58
		…	…	…	…	…	…				YES	1.93	78.75
	soybean	Cooper	NO	26.75	-10.81	2.03	40.88		soybean	Cooper	NO	23.00	-152.75
			YES	20.50	15.08	0.88	74.31				YES	4.53	50.18
		P25R47	NO	23.14	4.14	2.45	28.47			P25R47	NO	21.68	-138.28
			YES	14.31	40.72	1.38	59.78				YES	4.27	53.11
		Kaskaskia	NO	26.75	-10.81	1.09	68.18			Kaskaskia	NO	2.38	73.81
			YES	16.21	32.85	0.48	86.06				YES	0.07	99.27
		P25R54	NO	26.50	-9.78	1.50	56.20			P25R54	NO	5.23	42.49
			YES	18.25	24.40	0.75	78.10				YES	2.83	68.86
		E5530	NO	22.10	8.45	1.16	66.28			E5530	NO	18.45	-102.75
			YES	17.82	26.18	0.32	90.66				YES	7.52	17.40
		E5170	NO	19.76	18.14	0.52	84.74			E5170	NO	25.40	-179.12
			YES	17.00	29.58	0.17	95.11				YES	4.18	54.03
		…	…	…	…	…	…			Pro220	NO	8.30	8.79
		…	…	…	…	…	…				YES	2.77	69.60
		…	…	…	…	…	…			P25R62	NO	2.73	69.96
		…	…	…	…	…	…				YES	0.93	89.74

Table 2. Mean FHB Index, DON and percent control for different previous crop–cultivar–Prosaro combinations in Missouri.

				FHB DISEASE		DEOXYNIVALENOL	
Location (Grain Class)	Previous Crop	Cultivar	Prosaro	Mean Index (%)	% Control	Mean DON (ppm)	% Control
Missouri (SRWW)	corn	Elkhart	NO	47.07	0.00	15.98	0.00
			YES	30.11	36.03	6.03	62.25
		P25R47	NO	36.30	22.87	9.70	39.31
			YES	19.29	59.02	3.70	76.85
		P25R54	NO	32.73	30.46	6.22	61.11
			YES	15.86	66.31	2.15	86.55
		Roane	NO	22.30	52.63	4.52	71.74
			YES	17.12	63.62	2.18	86.34
		Bess	NO	21.76	53.76	3.78	76.33
			YES	14.13	69.99	1.42	91.14
	soybean	Elkhart	NO	48.57	-3.18	5.20	67.47
			YES	37.99	19.28	5.30	66.84
		P25R47	NO	28.74	38.94	4.78	70.07
			YES	29.99	36.29	3.60	77.48
		P25R54	NO	38.90	17.36	1.65	89.68
			YES	21.31	54.72	1.67	89.57
		Roane	NO	19.97	57.58	1.10	93.12
			YES	17.10	63.67	1.22	92.39
		Bess	NO	23.34	50.40	1.20	92.49
			YES	25.19	46.48	2.03	87.28

Table 3. Mean FHB index, DON and percent control for different previous crop-cultivar-Prosaro combinations in New York.

				FHB DISEASE		DEOXYNIVALENOL	
Location (Grain Class)	Previous Crop	Cultivar	Prosaro	Mean Index (%)	% Control	Mean DON (ppm)	% Control
New York (SRWW)	corn	Pioneer	NO	9.05	0.00	20.54	0.00
			YES	4.08	54.97	10.09	50.88
		Truman	NO	2.95	67.40	13.46	34.47
			YES	0.70	92.27	12.60	38.66
	soybean	Pioneer	NO	0.24	97.31	1.62	92.14
			YES	0.05	99.45	1.23	94.04
		Truman	NO	0.25	97.21	2.31	88.75
			YES	0.03	99.62	0.67	96.76
New York (SWWW)	corn	Richland	NO	6.38	0.00	22.79	0.00
			YES	3.43	46.27	19.53	14.31
		Jensen	NO	9.23	-44.71	48.77	-114.04
			YES	4.13	35.29	26.39	-15.82
	soybean	Richland	NO	0.78	87.71	1.13	95.06
			YES	0.22	96.57	1.65	92.78
		Jensen	NO	1.46	77.11	3.11	86.35
			YES	0.41	93.49	1.34	94.14

INHIBITION OF DEOXYNIVALENOL ACCUMULATION BY PREINOCULATION WITH NONTOXIGENIC *FUSARIUM GRAMINEARUM* - PRELIMINARY TESTS OF A NOVEL STRATEGY

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ABSTRACT

According to biological control theory, the best agents are those that share the same ecological niche and environmental requirements as the target. Many examples exist for hypovirulent pathogen isolates being used to control a virulent pathogen. This concept is based on the theory that hypovirulent isolates can compete with virulent isolates for limited niches and substrates and potentially can induce host resistance mechanisms. Extending this theory to Fusarium head blight (FHB), the ideal agent would be an isolate of the pathogen that does not produce deoxynivalenol (DON) and is hypovirulent. A naturally-occurring isolate of *Fusarium graminearum* (WG-9) was isolated from a wild grass from a remote, nonagricultural area in northern Minnesota by L.R. Gale. WG-9 has never produced detectable amounts of DON or other derivatives in inoculated spikelets in greenhouse experiments. Spread of WG-9 on point-inoculated wheat heads, however, varied between experiments from low to moderate compared to standard isolate PH-1. In this study, we are testing the concept that preapplication of a nontoxigenic (Tox-) hypovirulent strain, such as WG-9, to wheat heads can inhibit floret infection by a toxigenic (Tox+) virulent pathogen resulting in reduced DON accumulation in the grain. WG-9 was sprayed at 10^5 spores/ml onto flowering heads of a scab-susceptible spring wheat and then PH-1 was inoculated at the same spore concentration 1 day later. Scab severity was determined 7 to 9 days after pathogen inoculation. Upon seed maturation, the proportion of kernels infected by WG-9, PH-1, or both strains were determined using a multiplex PCR system with primers based on TRI3 and TRI12 gene sequences which reliably distinguished between the strains. In addition, kernels were assayed for DON content. When WG-9 and PH-1 were sequentially inoculated onto wheat at the high spore concentration, there was no reduction in total disease severity as WG-9 alone caused substantial scab. All of the seed was infected and shriveled. Consequently, DON content in kernels from PH-1 inoculated wheat heads pretreated with water were extremely high (average over 100 ppm). Pretreatment of wheat heads with WG-9 prior to PH-1 inoculation reduced the DON content by 10%. A lower proportion of seed was infected with PH-1 when the spikelets were pretreated with WG-9 as compared to pretreatment with water. These results support the hypothesis that a Tox- strain might compete with or exclude a Tox+ strain. The high disease severity caused by the Tox- strain alone is an obvious drawback. We will be exploring applications of WG-9 at much lower spore concentrations and the use of scab resistant cultivars as possible solutions. Field experimentation also is essential to confirm the benefits of pretreatment with Tox- strains.

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RESULTS OF 2009 UNIFORM BIOLOGICAL CONTROL TRIALS

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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 and barley across a range of environmental conditions.

INTRODUCTION

Various strains of biological control agents (BCA) have been demonstrated to be effective in separate studies (DaLuz et al., 2003; Jochum et al., 2006; Khan et al., 2004) in reducing FHB and deoxynivalenol (DON) in wheat and barley. But no one strain has shown to be consistently effective as a stand-alone treatment across diverse environments (Jochum et al., 2008; Yuen et al., 2007). Mixtures of BCA strains have improved the consistency of biocontrol in other pathosystems (Cruz et al. 2006). When this strategy was evaluated for FHB control using mixtures of bacterial strains, no benefits from applying the organisms in mixtures were found, presumably because the organisms were mutually antagonistic (Yuen and Jochum, 2004). The Uniform Biocontrol Trials in 2009 evaluated 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). Schisler et al. (2007) showed that formulations containing both of these individually-effective, compatible strains had potential to reduce FHB symptoms on wheat in greenhouse trials. Despite the apparent advantages of applying strain mixes, the disadvantages for the manufacturer are capital costs, operation, maintenance, registration and management of a different fermentation for each strain used in a mix. One solution to this obstacle is to co-culture the strains together in one fermentor. Thus, for the current investigation, cultures containing both strains were produced in a fermentor in SDCL medium, and after the cells were concentrated, they were shipped frozen to cooperator field sites. At application, expected cell concentrations were 5 x 10^7 and 8 x 10^8 CFU/ml for strains OH 182.9 and OH 71.4, respectively.

As part of an integrated control protocol with fungicides, a tank mixed combination of BCA and fungicide applied at flowering could theoretically provide immediate protection from FHB and lasting protection due to BCA activity after the fungicide component is no longer present or effective. Alternatively, biocontrol agents could be applied separate from the fungicide after kernel development begins, a stage of development when fungicides are not approved for use. In either case, BCA could be especially effective in limiting the total DON content in harvested grain by combating new infections by the pathogen that can occur during early to late grain development (Del Ponte et al., 2007). In earlier investigations, tank-mixed combinations of bacterial strains with tebucanozole resulted in better performance than the organism or fungicide alone (DaLuz et al., 2003; Jochum et al., 2006; Khan et al., 2004). Subsequent studies with bacterial BCA tank-mixed with Prosaro 421 SC (a formulation of prothioconazole and tebucanozole; Bayer CropScience), however, revealed no improvement (Yuen et al., 2007; Jochum et al., 2008). The sequential application of a fungicide followed by a BCA has not been tested. Therefore, another focus of the 2009 uniform biocontrol trials was the integration of BCA with a fungicide by combining the components as a tank mix or applying BCA as a follow-up treatment at late bloom.

MATERIALS AND METHODS

Six trials were conducted across four states on a range of wheat market classes (Table 1). The biological materials tested were the double yeast, supplied by D. Schisler, and Taegro (Novozymes Biologicals, Salem, VA), a commercial product containing *Bacillus amyloliquefaciens* FZB24. Treatments tested in these trials are listed in Table 2. 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. Pre-application samples of BCA at some locations were sent to G. Yuen for analysis of populations using dilution plating. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum*-infested corn kernels and utilized mist irrigation systems to stimulate infection. 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 and pooled together using ProcMixed (SAS), with LSmeans separated by the LSD test at the 95% confidence level.

RESULTS AND DISCUSSION

Moderate to high FHB levels were recorded at Missouri and North Dakota trials. Dry weather conditions in Nebraska and Michigan hindered FHB development despite misting being provided. Nevertheless, significant treatment effects were found in Nebraska and Michigan for some in-field disease parameters. While none of the treatments reduced in-field disease measurements compared to the control in all trials, treatments involving Prosaro alone or combined with a BCA were efficacious in the majority of cases. The double yeast and Taegro applied alone were efficacious in Missouri on 'Elkhart' and in Michigan. When results from all trials were pooled, head severity and index in these biological treatments were significantly lower than the control. In only two instances did any treatment exhibit better efficacy than Prosaro alone; both involved a combination of Prosaro with Taegro. Interestingly, the treatment with Prosaro followed by the double yeast reduced DON by 34% and was the only treatment to significantly reduce DON when averaged across all locations. FDK was reduced only in North Dakota where all treatments had a significant effect. There were no differences in test weights and yields between any treatments at any location (data not shown).

The results with the biological treatments in these trials are promising. The treatments with the BCA alone or in combination with the fungicide were comparable in consistency to the standard fungicide and in a few instances provided higher levels of control than the fungicide. The reduction of DON by combining Prosaro and the double yeast may have resulted from the biocontrol agents inhibiting late infections by *F. graminearum* when reduced fungicide activity would be expected. Some of the instances in which the BCA were not effective could have been related to population levels of the organisms declining during shipment or storage. The viable cell concentration in the double yeast inoculum applied in North Dakota, in particular, was considerably lower than expected levels. Formulation to improve shelf life might provide more consistent performance in the future. In addition, populations of the two yeast strains in the double yeast co-culture differ by more than a log unit. Further tests may clarify whether efficacy improvements would be realized with a product containing equivalent populations of these two strains.

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Table 1. 2009 uniform biological control 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 '2137'	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. 2009 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	55	66	77	58	81	24	60
Pro	50	58	71*	38*	74*	20	52*
Tae	58	59	79	48*	79	21	57
Tae late	46	63	87	45*	78	20	56
Pro + Tae	38*#	59	63*	40*	74*	20	49*
Pro early/Tae late	49	54	54*#	40*	83	19	50*
DYs	47	52	83	43*	84	19	54*
Pro early/DYs late	No data	No data	63*	43*	71*	20	53*
P	0.0248	Ns	0.0001	0.0121	0.0008	Ns	0.0001
$LSD_{0.05}$	11	-	13	5	5	-	5
SEVERITY (%)							
Control	8	14	21	79	38	9	28
Pro	8	9	14*	55*	36	4*	21*
Tae	11	9	20	63*	39	4*	24*
Tae late	9	9	21	63*	41	3*	24*
Pro + Tae	7	10	15*	63*	38	4*	23*
Pro early/Tae late	9	14	11*	58*	33	4*	21*
DYs	9	10	20	63*	34	4*	23*
Pro early/DYs late	No data	No data	13*	61*	36	5*	23*
P	0.0831	0.0823	0.0013	0.0402	Ns	0.0010	<0.0001
$LSD_{0.05}$	-	-	5	12	-	2	3

Table 3 (continued)

Treatment	NE Mead	NE Lincoln	ND Langdon	MO 'Elkhart'	MO 'Roane'	MI Clarksville	LS means
INDEX (%)							
Control	4	9	13	45	31	2	17
Pro	4	5	8*	21*	27	1*	11*
Tae	6	5	13	30*	31	1*	14*
Tae late	3	6	16	28*	32	1*	14*
Pro + Tae	3	6	6*	25*	28	1*	11*
Pro early/Tae late	4	7	3*	24*	27	1*	11*
DYs	4	5	14	27*	28	1*	13*
Pro early/DYs late	No data	No data	5*	27*	26	1*	12*
P	0.0437	0.0806	<.0001	0.0011	Ns	0.0017	<0.0001
$LSD_{0.05}$	2		5	9		1	2
FDK (%)							
Control	1	1	5	23	12	12	9
Pro	1	1	1*	22	9	7	7
Tae	2	2	4*	18	9	9	7
Tae late	2	2	4*	27	7	9	8
Pro + Tae	2	<1	1*	19	9	6	7
Pro early/Tae late	1	2	1*	21	7	10	7
DYs	1	1	3*	24	9	5	7
Pro early/DYs late	No data	No data	2*	18	10	6	7
P	Ns	0.0995	<.0001	Ns	Ns	Ns	Ns
$LSD_{0.05}$			1				
DON (ppm)							
Control	<0.5	<0.5	Tbd	6.6	1.2	0.9	2.9
Pro	<0.5 - 0.5	<0.5	Tbd	5.2	0.7	1.8	2.6
Tae	<0.5	<0.5 - 0.7	Tbd	7.5	1.1	2.0	3.5
Tae late	<0.5 - 0.6	<0.5	Tbd	6.8	1.1	1.0	2.9
Pro + Tae	<0.5	<0.5	Tbd	4.8	1.4	0.5	2.2
Pro early/Tae late	<0.5 -0.5	<0.5	Tbd	4.8	0.7	1.5	2.3
DYs	<0.5	<0.5 - 0.5	Tbd	6.1	0.9	1.2	2.7
Pro early/DYs late	No data	No data	Tbd	3.9*	0.9	1.0	1.9 *
P	Ns	Ns		0.0155	Ns	Ns	0.0164
$LSD_{0.05}$				1.9			0.8

* = 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
Tbd = to be determined