Integrated Approaches to a Solution

Proceedings of the 2008 National Fusarium Head Blight Forum

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Photos on the cover starting at the top center and moving clockwise:

- Severe Fusarium head blight infection and perithecia of *Gibberella zeae* on six-rowed barley; photo submitted by Brian Steffenson, Unversity of Minnesota.
- Wheat-L. racemosus translocation stock with a new gene for FHB resistance; photo submitted by Bernd Friebe, Kansas State University
- Field of Glenn wheat developed by North Dakota State University; photo submitted by Marcia McMullen, North Dakota State University
- Winter wheat planted into a no-till corn field; photo submitted by Dave Van Sanford, University of Kentucky.
- USDA-ARS scientists from College Station, TX applying Folicur® to field scale test plots near Crookston, MN; photo submitted by Charla Hollingsworth, University of Minnesota

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SESSION 1:

FHB MANAGEMENT

Co-Chairpersons: Erick DeWolf and Don Hershman

EFFECTS OF HOST RESISTANCE LEVEL AND INOCULATION TIMINGS ON FUSARIUM HEAD BLIGHT (FHB) DEVELOPMENT AND DEOXYNIVALENOL (DON) PRODUCTION IN THE FIELD IN NORTH DAKOTA.

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INTRODUCTION

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* (teleomorph: *Gibberella zeae*), is one of the most important diseases of wheat and other cereals worldwide. The disease affects both yield and quality by reducing grain fill and by contaminating grains with various mycotoxins, especially deoxynivalenol (DON). The disease has caused billions of dollars losses to wheat industry in the USA due to multiple disease epidemics (Nganje et al., 2001). FHB is managed primarily through integrating multiple strategies including use of resistant varieties, fungicide applications and cultural practices. A reliable disease forecasting/decision support system is essential to predict FHB epidemic during the season for the regional wheat growers to know if fungicide applications are needed for their crop. Some fungicides with new chemistry such as "PROLINE" have proved effective in FHB management, yield increase, and DON reduction. However, it has also been observed that not all wheat cultivars respond to fungicide applications in similar manner for yield increase (Marcia McMullen, *personal communication*).

Knowledge of level of host resistance, plant growth stage crucial for infection, and relationship between FHB severity and DON production in wheat is important in the development of FHB management strategies including a precise decision support system. Collaborative efforts of epidemiologists located at seven land grant states universities including North Dakota State University have resulted in the development of a FHB forecasting system with more than 80% accuracy, and it has been deployed successfully for the forecasts in ND and other regions of the US. Research work is still underway to make the support system more accurate for forecasting of FHB severity and DON level.

OBJECTIVES

1. Determine the effect of wheat genotypes with different levels of resistance on the development of FHB and DON production

2. Explore the effect of inoculation timings (plant growth stage) on FHB development and DON production

3. Assess the correlation if any between FHB severity and DON level

MATERIALS AND METHODS

Three wheat cultivars Glenn (FHB resistant), Steele-ND (moderately susceptible) and Trooper (susceptible) were planted on May 4 and May 14 and May 9 and 15, in 2007 and 2008, respectively in a field plot located at North Dakota State University Experimental area at Fargo. The experimental design was a split-split plot, with 3 replicates. Planting date (early and late) served as the whole plot; wheat variety (susceptible, moderately susceptible, and moderately resistant) as the sub plot; and inoculation timing [no inoculation, inoculation at early flowering (Feekes GS 10.51), and inoculation at mid flowering (GS10.52) as the sub-sub plot].

Strips of 20 feet wide of wheat cultivar Alsen (moderately FHB resistant) were planted to separate main

and subplots from each other serving as buffer. The sub-sub plot size was 20 ´ 10 feet with total 54 plots.

The plots were spray-inoculated with *F. graminearum* spores suspension @ 100,000 spores/ml, with a CO_2 backpack type sprayer equipped with nozzles mounted at 60 degrees angle forward and backward to provide maximum head coverage. Two hundred-twenty-five heads (45 heads/spot) from five spots in each subplot (treatment) were examined for FHB incidence and severity (Stack and McMullen, 1995) at dough stage (Feekes GS 11.2).

Twenty to Forty heads from each subplot depending on the availability, with FHB severity of 0%, 7-21%, 22-50%, 51-79%, and 80-100% were tagged in 2007; whereas 60 heads of each FHB severity category of each cultivar planted on May 9 were tagged in 2008. The heads were hand clipped and kept heads of each category separately for DON analysis and correlation between FHB severity and DON production.

RESULTS

The cultivars differed significantly ($P < 0.05$) in FHB severity but not in disease incidence and DON concentration in both years. Glenn has the lowest level (20.60% and 25.03) in both 2007 and 2008. Trooper has the highest level (28.12%) of FHB severity regardless of planting dates in 2007. Steele-ND has the highest level (46.56%) of disease severity in 2008; however, DON levels differed significantly between the two planting dates. Inoculation timings had significant affect on FHB incidence, severity, and DON concentration in 2007 and 2008 (Table 1 and 2).

All the three disease components: incidence (12.75% and 56.85%), severity (41% and 46.56%), and DON (2.45 ppm and 2.17 ppm) were higher when the cultivars were inoculated at mid flowering stage (GS 10.52) in both years (Table 1 and 2). A positive correlation was observed between FHB severity and DON concentration in all three cultivars Glenn ($r = 0.9865$ and 0.9872), Steele-ND (0.9893 and 0.9354), and Trooper (0.9844 and 0.9928). Overall, Trooper (FHB susceptible) had more DON concentration in all five disease severity categories (range: 1.06-75.68 ppm and 1.10-51.30 ppm) as compared to Steele-ND (1.39-56.86ppm and 0.50-48.50 ppm) and Glenn (0.91-64.63ppm and 0.50-23.4) in 2007 and 2008 (Fig. 1 and 2).

The results indicate that infection at mid flowering growth stage is crucial in FHB incidence, severity, and DON production. Additionally, incorporation of FHB severity level into the FHB disease forecasting system would help in DON level prediction prior to the harvest.

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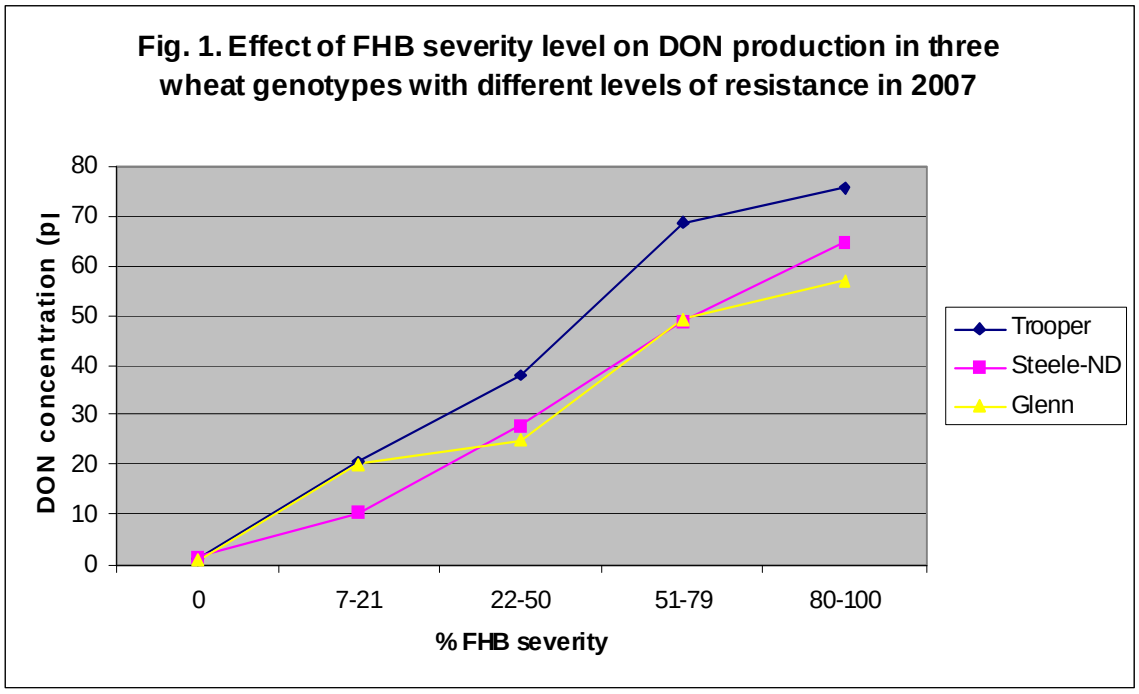
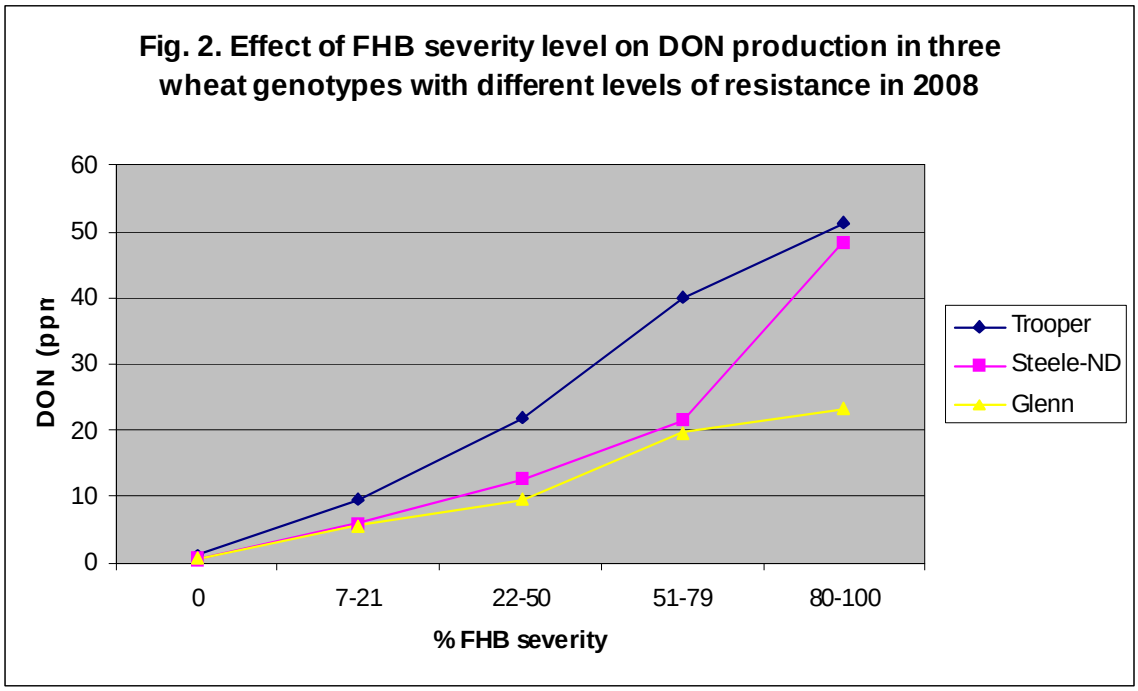
Table 1. Effect of wheat cultivars, planting date, and inoculation timings on FHB development and DON production in 2007.

Source	FHB Incidence	FHB Severity	DON
	P>F	*P>F*	*P>F*
Cultivar	0.76	0.03	0.64
Planting date	0.07	0.19	0.007
Inoculation timings	0.001	0.001	0.001

Table 2. Effect of wheat cultivars, planting date, and inoculation timings on FHB development and DON production in 2008.

Source	FHB Incidence	FHB Severity	DON
	P>F	*P>F*	*P>F*
Cultivar	0.25	0.004	0.06
Planting date	0.71	0.19	0.001
Inoculation timings	0.001	0.001	0.001





MICROPLOTS IN COMMERCIAL WHEAT FIELDS FOR QUANTIFYING THE LOCAL CONTRIBUTION OF GIBBERELLA ZEAE FROM NATURAL CORN DEBRIS TO FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION.

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OBJECTIVES

To determine the relative contribution of inoculum of *Gibberella zeae* from naturally over-wintered corn debris to local spike infection and deoxynivalenol (DON) accumulation in commercial wheat fields using a debris microplot experimental system.

INTRODUCTION

A quantitative understanding of the contribution of within-field inoculum sources of *Gibberella zeae* to infection of wheat and barley is important for developing and/or excluding strategies for managing Fusarium head blight (FHB) in individual fields. In another report in these Proceedings, Keller et al. document the contribution of released clonal inoculum from concentrated sources of *G. zeae*-inoculated debris to Fusarium head blight in cereal fields in New York and Virginia. Concentrated clonal inoculum sources contributed significantly to FHB infection at the source, but background populations of *G. zeae* contributed an even greater percentage. The contribution of clonal inocula to spike infection fell off sharply to background levels within 10-20 feet of the concentrated sources, thus validating the use of debris microplots, spaced at 100 ft, as a tool for estimating the contribution of an inoculum source to local spike infection. The next logical question is "What does inoculum from naturally over-wintered cereal debris contribute to FHB infection of local cereal spikes?" To begin to answer this question, we conducted debris microplot experiments in six, geographically diverse, commercial fields of winter wheat in New York in 2008.

MATERIALS AND METHODS

Wheat in each field was planted following harvest of a non-cereal crop such as pea, dry bean, or soybean. Grower cooperators refrained from foliar fungicide use in the experimental portion of each field. Locally overwintered, natural corn stalks were collected in April from a location close to each wheat field by placing a 33 in. diameter plastic 'Hoola Hoop' onto two 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. We also utilized microplots with no corn debris to serve as check plots, and microplots with differing quantities of clone-inoculated corn stalks to calibrate the inoculum contribution from natural corn debris. Ten microplots were set out prior to stem elongation in each wheat field in a randomized design of five treatments and two reps. Microplots were separated by a minimum of 100 ft. Treatments were 1) no debris, 2) natural corn debris, 3) 3 g of clone-inoculated corn stalks, 4) 30 g of clone-inoculated corn stalks, and 5) 300 g of clone-inoculated corn stalks. Inoculum substrates were secured within microplots fashioned of 2 ft high hardware cloth and shaped with a 33 in. diameter 'Hoola Hoop', fastened with plastic zip-ties, and secured to the soil with metal ground staples. Wheat spikes above each microplot were scored for FHB at soft dough stage. At grain maturity, all spikes above the 2 ft cages were harvested and dried. Grain was threshed from a subsample of spikes and sent to Virginia Tech for DON analysis. Fifty intact spikes from each microplot were surface-disinfested and plated onto a *Fusarium* selective medium; candidate colonies were confirmed as *G. zeae* on potato dextrose agar and the incidence of spike infection was calculated.

RESULTS AND DISCUSSION

Local release of concentrated clonal inoculum (300 g, approximately the amount of stalk dry weight encountered in a 33 in. diameter area of natural corn debris) resulted in increased spike infection incidence (Table 1) and DON (Table 2) over background levels and in two fields resulted in grain contaminated with DON at levels in excess of 2 ppm (Table 2). Release of naturally-overwintered corn stalks resulted in significantly lower levels of local spike infection than did the concentrated clonal source in five of the six experiments (Table 1), and significantly lower levels of DON in three of the experiments (Table 2). Although natural corn stalks as well as clonal inoculum at one-tenth and one one-hundreth of concentrated strength showed numerical increases in spike infection and DON over background inocula in most locations, these differences were generally not statistically significant. This suggests that the six different local sources of natural corn stalks contributed only small incremental levels of inoculum over that contributed by spores in the atmospheric background on each farm. If within-field sources of *G. zeae* (i.e., infested residues of corn, wheat, or barley) contribute a significant proportion of local inoculum for FHB, then management of those residues should lead to significant reductions in FHB and DON in those fields. These results from six locations in one non-epidemic year in New York suggest that the FHB/DON management benefits of tillage, rotation, or debris treatments in a single field may be limited. This question bears expanded testing under variable environments and production systems. To that end, corn debris microplot experiments are planned for a USWBSI Management Project in commercial cereal fields in Illinois, Missouri, Nebraska, New York, and Virginia in 2009 and 2010.

ACKNOWLEDGEMENTS

This material is based upon work supported by Cornell University Hatch Project NYC153433 and mycotoxin analysis supported by the U.S. Wheat and Barley Scab Research Initiative. We thank Dr. David Schmale and staff at Virginia Tech for quantifying DON in our samples. We are indebted to the New York farmers who allowed us to conduct experiments in their wheat fields.

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Table 1. Contribution of corn residue inoculum sources in microplots to infection of local wheat spikes by *Gibberella zeae* in six commercial New York wheat fields in 2008. Each 33 in. diam. caged microplot in a wheat field contained either 1) no corn debris, 2) naturally overwintered corn debris collected in a 33 in. diam. sample from the nearest corn field, or 3) 3, 30, or 300 g dry weight (prior to autoclaving) of corn stalks inoculated with a clone (Gz014) of *Gibberella zeae*.

	Percent of spikes infected by *G. zeae* (St. Dev.)					
Corn residue in plot >	**None**	**Natural**	**Inoc. 3g**	**Inoc. 30g**	**Inoc. 300g**	**LSD (*P*=0.05)**
Cayuga Co. - Aurora	4 (3)	11 (4)	14 (6)	18 (3)	43 (16)	20
Livingston Co. - LeRoy	1 (1)	2 (0)	5 (4)	6 (3)	39 (4)	8
Monroe Co. – Hilton	2 (3)	12 (8)	11 (4)	33 (10)	57 (16)	24
Monroe Co. - Scottsville	0 (0)	0 (0)	2 (0)	5 (7)	40 (17)	21
Seneca Co. - Waterloo	11 (1)	8 (0)	7 (1)	29 (13)	49 (24)	NS
Steuben Co. - Bath	4 (2)	17 (1)	3 (1)	20 (6)	62 (3)	8
Average	**4**	**8**	**7**	**19**	**48**	

Table 2. Contribution of corn residue sources of *Gibberella zeae* in microplots to accumulation of deoxynivalenol in local wheat spikes in six commercial New York wheat fields in 2008. Treatments were the same as in Table 1.

	Deoxynivalenol in grain in ppm (St. Dev.)					
Corn residue in plot >	**None**	**Natural**	**Inoc. 3g**	**Inoc. 30g**	**Inoc. 300g**	**LSD (*P*=0.05)**
Cayuga Co. - Aurora	**0.14** (0.19)	**1.12** (0.98)	**0.65** (0.23)	**0.96** (0.45)	**5.14** (0.43)	1.38
Livingston Co. - LeRoy	**0.30** (0.08)	**0.37** (0.27)	**0.19** (0.26)	**0.32** (0.05)	**2.13** (2.31)	NS
Monroe Co. – Hilton	**0.23** (0.11)	**0.21** (0.08)	**0.16** (0.02)	**0.41** (0.17)	**1.17** (0.66)	NS
Monroe Co. - Scottsville	**0.00** (0.00)	**0.18** (0.06)	**0.08** (0.11)	**0.26** (0.01)	**0.98** (0.06)	0.16
Seneca Co. - Waterloo	**0.11** (0.16)	**0.00** (0.00)	**0.23** (0.32)	**0.29** (0.41)	**0.90** (0.72)	NS
Steuben Co. - Bath	**0.23** (0.00)	**0.63** (0.16)	**0.24** (0.12)	**0.58** (0.37)	**1.88** (0.29)	0.59
Average	**0.17**	**0.42**	**0.26**	**0.47**	**2.03**	

HOST RESISTANCE CORRELATED WITH THE AMOUNT OF DON REDUCTION ACHIEVED WITH FUNGICIDES.

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ABSTRACT

Fusarium head blight (FHB) is a serious disease of wheat. The best controls for FHB are planting of resistant cultivars and application of fungicides to heads. However integrating these strategies may provide additional benefits for small grain producers including reduced disease losses and lower levels of the toxin deoxynivalenol (DON). This research was conducted to determine if resistant cultivars show higher benefits (greater reductions in DON) from fungicide application than susceptible ones. A winter wheat field experiment was established and infested with corn grains colonized by *Fusarium graminearum* applied to the soil surface in three applications about 2 wk apart beginning 4 wk prior to heading (100 g/m^2 total applied). During heading and flowering, plots were sprinkler irrigated (3 min/hr) from 9:00 p.m. until 6:00 a.m. Six winter wheat cultivars were selected based upon their reaction to FHB on a 1-9 scale where 1=resistant and 9=susceptible. The six cultivars, followed by their reactions, were Truman (3), Heyne (4), Roane (5), Karl 92 (6), Overley (9), and Tomahawk (9) and were arranged in a split-plot design with cultivars as main plots and presence or absence of fungicide as sub-plots. There were four replications and sub-plots were 5' by 15'. The fungicide Prosaro (6.5 fl oz/A plus Induce spreader at 0.125%) was applied at the fully headed growth stage using flat-fan nozzles angled forward about 30°. FHB index (% blighted florets) was determined for each sub-plot on May 30, June 2, June 4, and June 9. Sub-plots were harvested with a small-plot combine to determine yields and percentage Fusarium-damaged kernels (FDK). Ground grain samples were sent to the North Dakota State University Veterinary Diagnostic lab for analysis of DON). Severe FHB developed at the site as evidenced by the non-sprayed susceptible cultivar Tomahawk yielding only 7.9 bu/A; however, the non-sprayed moderately resistant cultivar Truman yielded 56.2 bu/A, so yield potential at the site was good. There were significant (P=0.0133, 0.0041, 0.0002, respectively) correlations between grain yields, average FHB index, and FDK and the reduction in DON achieved by fungicide application. These correlations indicate that a cultivar's resistance reaction to FHB can help predict the degree of DON reduction by the fungicide Prosaro. More resistant cultivars show higher reductions. Although there were significant correlations between DON reductions from fungicide and three resistance parameters (yields, disease index, FDK), the R^2 values indicate that a cultivar's resistance reaction to FHB explained only 26-48% of the reduction. Clearly, there are other factors that influence DON reduction from fungicide application. However, if these findings are confirmed for naturally occurring FHB epidemics, it may be possible to reduce the impact of the disease and toxin in Kansas by combining fungicides with more resistant cultivars.

MODELING FUSARIUM HEAD BLIGHT AND DON IN BARLEY.

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ABSTRACT

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae*, continues to be a serious problem for barley producers in the U.S. Northern Great Plains. *G. zeae* causes direct economic loss through a reduction in grain yield and also because it produces mycotoxins that can impact the marketability of a crop, e.g. deoxynivalenol (DON). Management of FHB is primarily accomplished with agronomic practices that limit in-field inoculum (e.g. rotation) and through the application of fungicides. The timing of application is critical and therefore a need exists for a risk-advisory system that growers could use to make management decisions. The objective of this research was to develop model(s) for such a system that predicts FHB and/or DON based on weather conditions.

Varieties of regionally adapted barley (both 2- and 6-row types) were grown at multiple locations in the Northern Great Plains during the 2005-8 growing seasons. Crop stage was monitored regularly and no additional inoculum was applied. The incidence and severity of FHB was measured and environmental variables recorded. Correlation analysis and regression techniques were used to identify the variables that were associated with high disease and/or DON events and then predictive models were developed with logistic regression. Models were evaluated based on their sensitivity, specificity, deviance R-square, etc.

Simple weather variables that explained general trends (e.g. mean hourly temperature) tended to have the highest correlation coefficients and were most predictive of high FHB/DON instances. In general, high levels of disease and DON occurred at a location when the mean hourly temperature and relatively humidity were both greater than 22°C and 75%, respectively, for the 10 days prior to full head emergence. A preliminary model was developed that combined these variables. This model had true positive and negative rates of ~90% when tested with the 2005-7 data sets and was able to predict low disease in all of the 2008 South Dakota testing locations. Further analysis of model accuracy is ongoing.

FUNGICIDE CONTROL OF FUSARIUM HEAD BLIGHT ON SOFT RED WINTER WHEAT IN ILLINOIS.

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ABSTRACT

Like many other states in the Midwest, when the conditions are favorable for Fusarium head blight (FHB; caused by *F. graminearum*) in Illinois, losses can be devastating. With the large acreage of corn grown in Illinois, *F. graminearum* inoculum is all around, and it is important for Illinois wheat growers to use integrated management to control FHB and the associated mycotoxin, deoxynivalenol (DON). Because different fungicide products are available for use on wheat, the identification of the most efficacious fungicides that growers can use in an integrated management program for control of FHB and DON is important. Fungicide trials were conducted on winter wheat grown at five locations in Illinois (Brownstown, Carbondale, Dixon Springs, Monmouth, and Urbana). Soft red winter wheat cultivars susceptible to FHB were planted at each location ('Madison' – Brownstown, Dixon Springs, and Monmouth; 'Pioneer 25R78' – Carbondale; and 'Cooper' – Urbana). To increase the likelihood of getting adequate FHB disease pressure, trials were planted into corn stubble, and *F. graminearum* spawn was spread throughout the experimental area. Trials at Carbondale and Urbana were irrigated prior to heading through soft-dough to provide a favorable environment for *F. graminearum* infection and FHB development. In addition to an untreated control, six "core" treatments were applied at Feekes 10.5.1 and evaluated at all locations, which were: Folicur at 4 fl oz; Proline at 5 fl oz; Prosaro at 6.5 fl oz; Caramba at 10 and 14 fl oz; and Topguard at 14 fl oz. At Carbondale, Dixon Springs, and Urbana, Headline at 6 fl oz applied at Feekes 9.0, 10.0, or 10.5 and Proline at 5 fl oz applied at Feekes 10.5 or 5 days after Feekes 10.5.1 were evaluated in addition to the "core" treatments, and at Carbondale and Urbana, Proline 5 fl oz + Headline 6 fl oz applied at Feekes 10.5 was evaluated in addition to the "core" treatments. Overall, the fungicides that provided the most consistent reduction in FHB and DON across the locations were Prosaro at 6.5 fl oz and Caramba at either 10 or 14 fl oz. In general, at the locations where FHB and/or leaf diseases were at moderate to high levels, these fungicides provided significantly greater grain yields compared to the untreated controls. At Carbondale, Headline applied at Feekes 10.5 significantly increased DON levels compared to the untreated control, indicating that strobilurin fungicide applications made to wheat at the Feekes 10.5 growth stage or later could increase the risk of a spike in DON levels.

FUNGICIDES FOR FHB MANAGEMENT: PAST, PRESENT, AND FUTURE.

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ABSTRACT

The best plan for managing Fusarium head blight (FHB), caused by *Fusarium graminearum*, includes an integrative approach that utilizes many different tools. An effective foliar fungicide is one of the crucial tools needed to help make an integrative management plan work successfully. Unfortunately, this "crucial tool" was difficult to identify early on. Results from fungicide tests conducted from the 1970s into the 1990s showed that a few fungicides provided some control of FHB; however, results were often inconsistent, especially with reductions in mycotoxins, such as deoxynivalenol (DON). Few fungicides were registered for use on small grains in the U.S. during this period, and the only one that had good efficacy on FHB (benomyl; Benlate®) was difficult to apply because of its formulation and had limited activity against important leaf diseases that also impacted wheat. Epidemics of FHB in the 1990s in major small grain production regions of the U.S. sparked a multi-state effort to evaluate fungicides for control of FHB. Results of this multi-state effort indicated that the triazole fungicide tebuconazole (Folicur®) was the best of the group tested in reducing both FHB and DON. The process of registering Folicur for use on small grain crops with the U.S. Environmental Protection Agency (EPA) began, but registration was delayed. From 1998 to 2007, Folicur was available for use as a section 18 emergency exemption for wheat growers in some, but not all, states affected by FHB. In 2001, manufacturing of Benlate was discontinued by DuPont. Results of continued, multi-state testing of fungicides indicated that the triazole fungicides prothioconazole (Proline®) and metconazole (Caramba®) provided good control of FHB, and perhaps a better reduction of DON than tebuconazole. In additional fungicide tests, the mixture of tebuconazole + prothioconazole (Prosaro®) was shown to provide better control of FHB and DON than either tebuconazole or prothioconazole alone. In 2007, Proline was registered by the U.S. EPA, and in 2008, Folicur, Caramba, and Prosaro all became registered with the U.S. EPA to control FHB and other wheat diseases. For the first time ever, in the 2009 season, wheat growers in most of the United States will have access to multiple fungicide products that have been proven to reduce FHB and DON. Even though these fungicides have been proven to reduce FHB and DON, there is still much room for improvement. Future fungicide evaluations for control of FHB and DON should include different mixtures of the most efficacious triazole fungicides, mixtures of fungicides with different modes of action, and experimental fungicides.

MULTI-STATE UNIFORM FUNGICIDE TRIALS TO CONTROL FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL.

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OBJECTIVE

To identify the most efficacious foliar fungicides for control of Fusarium head blight and deoxynivalenol with multi-state uniform fungicide trials.

INTRODUCTION

Foliar fungicide application is one of the key components of an integrated disease management system for Fusarium head blight (FHB; caused by *Fusarium graminearum*) and the associated mycotoxin deoxynivalenol (DON). Although different fungicides are available for use on small grain crops, applying the most efficacious fungicide available is critical in reducing FHB and DON. Reviewing the results of many fungicide trials, Mesterhazy (2003) concluded that tebuconazole was the best active ingredient out of the tested fungicides, but that new fungicides should be developed with better efficacy than tebuconazole. Paul et al. (2008) using a multivariate meta-analysis, reported that prothioconazole, metconazole, and tebuconazole + prothioconazole reduced FHB by an additional 14 to 20% and DON 25 to 29% compared to tebuconazole. Progress has been made in identifying new fungicides with better efficacy against FHB and DON, but additional levels of control could potentially be achieved with new fungicide active ingredients and mixtures. The objective of this study was to identify the most efficacious foliar fungicides for control of FHB and DON.

MATERIALS AND METHODS

The uniform fungicide treatment list was comprised of "core" treatments which were evaluated at nearly all of the locations, and "optional" treatments which were evaluated at fewer locations (Table 1). The core treatments were designed to compare different fungicides applied at Feekes 10.5.1 for control of FHB and DON. The optional treatments were designed for different reasons. The Headline treatments applied at different timings (Feekes 9, 10, or 10.5) were designed to determine the latest that a strobilurin fungicide, such as Headline, could be applied and not increase DON levels. Strobilurin fungicides applied to wheat heads have been reported to increase DON levels compared to untreated heads (Blandino et al., 2006; Mesterhazy et al., 2003), and questions on how late strobilurin fungicides can be applied to wheat and not raise DON levels are unanswered. The Proline + Headline (2 fl oz) treatment was designed to determine if a low rate of Headline could be applied with a triazole to decrease FHB and DON, without a spike in DON due to the strobilurin component. The Proline applied at different timings was designed to help determine the window of application.

These tests were conducted on one hard red winter wheat cultivar and a range of soft red winter wheat cultivars, hard red spring wheat cultivars, and spring barley cultivars in multiple states (Table 2). Each site used different techniques to help increase FHB disease pressure, such as plant susceptible cultivars, plant

into corn or small grain stubble, spread *F. graminearum* spawn across plot areas, inoculate heads with *F. graminearum*, and/or irrigate. All fungicides were applied with hand booms that were calibrated to deliver the treatments at recommended pressures and volumes. A non-ionic surfactant at 0.125% v/v was included with all fungicide treatments. Disease ratings were collected at soft dough, and plots were harvested with small plot combines to determine yield. Grain samples were sent to a laboratory to determine DON levels (DON analysis was not completed for samples from all locations at the time this article was written). Each location had a minimum of 3 replications and appropriate statistical designs were used.

RESULTS AND DISCUSSION

FHB index values and DON levels. Two locations, Columbia, MO and Urbana, IL, had the highest FHB index values in the untreated controls which were 32 and 25, respectively. Three locations, Butlerville, IN, Langdon, ND (hard red spring wheat trial), and Monmouth, IL, had FHB index values in the untreated controls that ranged from 5.0 to 8.8. All other locations had FHB index values less than 5.0 in the untreated controls. At the time this article was written, DON analysis on samples from all locations had not been completed; however, in the trials where DON analysis had been completed, DON levels ranged from 0 to 10.68 ppm in untreated controls. Locations with DON levels over 1 ppm in the untreated control occurred at Carbondale, IL, Columbia, MO, Monmouth, IL, and Urbana, IL.

Effect of "core" treatments on FHB index. At the locations with the highest FHB index (Columbia, MO and Urbana, IL), all of the "core" treatments except Topguard significantly reduced the FHB index compared to the untreated control on FHB-susceptible cultivars. At the locations with FHB index values in the untreated controls ranging from 5.0 to 8.8 (Butlerville, IN, Langdon, ND, and Monmouth, IL), results differed at each location. At Butlerville, IN, only Folicur and Prosaro were evaluated, and only Prosaro significantly reduced the FHB index compared to the untreated control. At Langdon, ND on hard red spring wheat, all of the core treatments significantly reduced the FHB index compared to the untreated control, and Folicur, Proline, Prosaro, and Caramba (both rates) significantly reduced the FHB index compared to Topguard. At Monmouth, IL, no significant differences occurred among treatments for FHB index.

Effect of "core" treatments on DON. At the locations with DON levels over 1 ppm (Carbondale, IL, Columbia, MO, Monmouth, IL, and Urbana, IL), results differed at each location. At Carbondale, IL, only Prosaro and Caramba (both rates) significantly reduced DON levels compared to the untreated control. At Columbia, MO and Monmouth, IL, none of the core fungicide treatments significantly reduced DON levels compared to the untreated controls. At Urbana, IL, Folicur, Proline, Prosaro, and Caramba (both rates) significantly reduced DON levels compared to the untreated control. In these trials, even when fungicides did significantly reduce DON levels, DON levels may still have been too high, even from fungicide-treated plots, for elevators to accept the grain; thus, showing the need for more efficacious fungicides and integrated management.

Effect of "core" treatments on grain yield. At nine locations, core fungicide treatments significantly affected yield. These locations were: Dixon Springs, Monmouth, and Urbana, IL; West Lafayette, IN; Columbia, MO; Fargo, ND (hard red spring wheat trial); and Brookings (hard red spring and hard red winter wheat trials) and Watertown (hard red spring wheat trial), SD. At Dixon Springs, IL, plots treated with Proline, Prosaro, Caramba (14 fl oz rate), and Topguard had significantly higher yields than the untreated control, which was at least partially due to leaf rust control at that location. At Monmouth, IL, plots treated with Folicur and Caramba (both rates) had significantly greater yields compared to the untreated control. At Urbana, IL, plots treated with Prosaro, Topguard, and Caramba (both rates) had significantly greater yield than the untreated control due to FHB and leaf rust control. At West Lafayette, IN, only Folicur and Prosaro were tested, and plots treated with Prosaro had significantly greater yield than the untreated control. At Columbia, MO, all of the core treatments significantly improved yield compared to

the untreated control on cultivar Roane; however, only Proline, Prosaro, and Caramba (10 fl oz rate) significantly improved yield on cultivar Elkhart. The yield improvement at Columbia was likely due to control of FHB and leaf diseases. At Fargo, ND, all core treatments significantly improved yield compared to the untreated control on the hard red spring wheat trial due to control of FHB and leaf diseases. All core fungicide treatments significantly improved yield compared to the untreated control in the hard red winter wheat trial at Brookings, SD due to leaf disease control. Fungicide treatments did not improve yield of the hard red spring wheat cultivar Briggs at Brookings or Watertown, SD; however, all core treatments improved yield of cultivar Oxen at Brookings, and Folicur and Caramba (both rates) improved yield of cultivar Oxen at Watertown due to control of leaf diseases.

Effect of "optional" treatments. At locations with DON analysis completed and where Headline was applied at Feekes 9, 10, and 10.5 (Carbondale, Dixon Springs, and Urbana, IL; and Columbia, MO), only the Carbondale, IL location showed a significant spike in DON levels compared to the untreated control. At this location, Headline applied at Feekes 10.5 significantly increased the DON level compared to the untreated control (3.62 vs. 1.93 ppm). The Proline + Headline (2 fl oz) treatment did not show an increase in DON levels, but also did not seem to provide any added control of FHB. Proline applied at either Feekes 10.5 or 5 days after the 10.5.1 application timing tended to provide similar results to Proline applied at the Feekes 10.5.1 timing. Additional research should be conducted to confirm that the timing of Proline application between 10.5 and 10.5.1 will not cause different results.

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DISCLAIMER

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

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Table 1. Fungicide treatments evaluated in the 2008 uniform fungicide trials.

Core treatments	Application timing (Feekes)	Active ingredient(s)	No. of locations tested
Untreated			20
Folicur 4 fl oz/A	10.5.1	tebuconazole	20
Proline 5 fl oz/A	10.5.1	prothioconazole	18
Prosaro 6.5 fl oz/A	10.5.1	tebuconazole + prothioconazole	20
Caramba 10 fl oz/A	10.5.1	metconazole	18
Caramba 14 fl oz/A	10.5.1	metconazole	18
Topguard 14 fl oz/A	10.5.1	flutriafol	18
Optional treatments			
Proline 5 fl oz + Headline 2 fl oz	10.5	prothioconazole + pyraclostrobin	4
Headline 6 fl oz	9.0	pyraclostrobin	6
Headline 6 fl oz	10.0	pyraclostrobin	5
Headline 6 fl oz	10.5	pyraclostrobin	5
Proline 5 fl oz	10.5	prothioconazole	4
Proline 5 fl oz	5 days after 10.5.1	prothioconazole	4

Table 2. Small grain classes and cultivars evaluated by each state.

Small grain class	States tested	Cultivars tested
Soft red winter wheat	IL, IN, MO	Cooper, Elkhart, Madison, Pioneer 25R78, Roane
Hard red winter wheat	SD	Wesley
Hard red spring wheat	MT, ND, SD	Alsen, Briggs, Hank, Oxen, Trooper
Spring barley	ND, SD	Robust, Stellar, Tradition

EFFECT OF WINTER WHEAT HARVEST TIMING ON DEOXYNIVALENOL (DON).

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ABSTRACT

When a moderate to severe Fusarium head blight (FHB) epidemic develops, few tools are currently available for managing deoxynivalenol (DON) levels in wheat. At times, growers have been advised to conduct an early harvest of wheat fields with severe FHB, and dry the grain. However, studies of DON concentrations in wheat grain over time have suggested that DON levels decrease during grain-fill and up until normal harvest time. Our objective was to measure DON levels in wheat heads during and after the normal harvest period and under different moisture regimes.

In a misted field nursery in Kinston, North Carolina, we inoculated plots of four wheat cultivars (three moderately resistant to FHB and one susceptible) with 10^5 spores/ml at anthesis. To create two levels of epidemic severity, one block was misted daily for 21 days starting at anthesis, while the other block received no mist during that period. In addition, we manipulated moisture levels in the time window corresponding to a normal or late harvest: within the above-mentioned blocks, plots were misted for 0, 7, or 14 days, starting 1 wk before normal harvest time. All treatment combinations had four replicates. Starting 2 wks before normal harvest time, 30 spikes were chosen randomly from each plot at 7-day intervals for 6 wks. In addition, winter wheat samples were collected from growers' fields in six locations in eastern North Carolina where a natural FHB epidemic occurred in 2008. Collections were made from each field on three dates: around the beginning of normal harvest time, or 7 or 14 days later. On each occasion, all spikes were harvested from two arbitrarily chosen rows in each of four or five replicate "plots" that comprised 10-foot subsections of 40- or 50-foot strips, with each strip six rows wide. All spike samples were threshed and analyzed for DON content.

At Kinston, FHB incidence and severity were significantly greater in the plots misted for 21 days starting at anthesis than in the plots not misted during that period (mean DI 40% and 22%, mean DS 10% and 7%, respectively, $P \leq 0.002$). Mean DON across all sampling dates at Kinston was significantly higher in the block receiving 21 days of mist following inoculation than in the block not misted during that period (5.0 vs. 2.3 ppm, $P < 0.0001$). In the block receiving 21 days of post-anthesis mist, DON was higher 2 wks before normal harvest (the first sampling date) than on any other date. In the block receiving no mist during that period, DON was higher 1 wk before normal harvest (the second sampling date) than on any other date. In the growers' fields, DON levels stayed the same or declined over time. Relevant data on rainfall and temperature are being obtained and analyzed. The objective is to determine whether delay of harvest could be a tool for management of DON in severe FHB epidemics.

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ADVANCES IN THE EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT AND APPLICATIONS IN PREDICTION MODELS.

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ABSTRACT

Fusarium head blight (FHB) has been an important disease of wheat and barley for more than a century. Both historical accounts and current experience provide some valuable insights into the epidemiology of the disease and suggest weather patterns and crop production practices that generally favor the development of severe FHB epidemics. However, the quantification of many critical aspects of Fusarium biology and details of disease epidemiology has remained elusive for many years. The past decade has brought a tremendous global effort to better understand the epidemiology of this disease, and develop practical tools for management. Critical advancements were made that have helped researchers understand spore dispersal and survival during transport, as well as the processes of infection, colonization and toxin accumulation. These research results have stimulated a new generation of prediction models using both mechanistic and empirical modeling approaches. The focus of the U.S. modeling effort has also been expanded to predict the risk of unacceptable levels of deoxynivalenol (DON) in addition to the severe disease epidemics. Preliminary results indicate that it may be possible to predict the risk of DON greater than 2 ppm with more than 75% accuracy using either mechanistic or empirical modeling approaches. The most accurate predictions are likely to require weather information from anthesis and early stages of kernel development. Further research is needed to maximize the accuracy of models using only pre-flowering weather information and increase the potential use of the models as decision tools for making timely fungicide applications. Candidate models for DON prediction will be tested during the 2009 growing season. If these tests are successful, a model will be released for public deployment as early as 2010.

CULTURAL CONTROL PRACTICES IN THE MANAGEMENT OF FUSARIUM HEAD BLIGHT.

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ABSTRACT

Reduced tillage practices have been adopted worldwide in agriculture over the past twenty-five years. Although the implementation of conservation tillage has been essential to protect vulnerable soils, it has resulted in unanticipated changes in the prevalence of cereal diseases, notably Fusarium head blight (FHB). *Fusarium spp*. survive saprophytically on a range of crop residues including corn, small grain cereals and numerous other grasses. Ascospores released during the late spring and early summer, from perithecia that have developed on crop residues on the soil surface, provide the primary inoculum for FHB epidemics. The link between conservation tillage and FHB is evident if one examines the historical records of FHB in the Upper Midwest. The pattern of epidemics indicate that the only period when FHB was of minor importance in wheat and barley was from the end of World War II until the mid-1980's. This period spans the years from the introduction of tractors with sufficient power to invert the top layer of soil, until the time when the use of the moldboard plough was largely abandoned in favor of tillage systems that provided protection against soil erosion. Further exacerbating the situation in the United States is the increasing corn acreage. Corn production has been promoted by incentives for the increased utilization of corn ethanol as a non-petroleum-based fuel. In Minnesota there has been a significant increase in the acreage of corn in the Red River Valley since the early 1990's. While fungal diseases are generally only of minor importance in corn, *Fusarium* can readily infect the corn plant, inciting stalk and ear rots. Corn breeders have largely prevented the problem of stalk rot by breeding varieties with sufficiently sturdy stems to avoid lodging, even when damaged by a *Fusarium* infection. Ear rot is frequently associated with insect damage to developing corn ears and this damage has been reduced, in part by the introduction of transgenic corn carrying the gene that codes for the *Bacillus thuringiensis* (Bt) toxin. Interestingly, the residues of Bt-corn decompose more slowly than the residues of corn not carrying the Bt gene. Thus, while *Fusarium* poses a limited threat to corn, the increase in corn acreage and the reduced rate of decomposition of Bt-corn undoubtedly exacerbate to the problem of FHB in wheat and barley. We seem unlikely to be able to reduce the threat of FHB epidemics, the attending damage to grain from DON, or the financial devastation to the wheat and barley industries, without addressing the underlying origin of the problem, *Fusarium*-infested crop residues. Given the limitations of our current agricultural practices we are challenged to find ways to reduce the inoculum potential of *Fusarium*-infested residues without removing residues from the soil surface. Host resistance, crop rotation, tillage, residue destruction and chemical and/or biological control, specifically targeting *Fusarium* spp. within the crop residues may play important roles in an integrated approach to the management of FHB. These approaches will work by reducing the initial level of residue colonization, accelerating residue decomposition, and/or reducing the survival or inoculum production potential of the pathogen.

DISCLAIMER

INTEGRATED MANAGEMENT FOR FUSARIUM HEAD BLIGHT OF WINTER WHEAT IN WISCONSIN.

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ABSTRACT

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is a sporadic disease of wheat in Wisconsin, however, when it has occurred, losses have been significant. Furthermore, management guidelines for controlling Fusarium head blight in WI do not exist. As such, it is important to further our understanding and develop a disease management system for wheat in WI that compares the yearly risk of FHB with other diseases known to cause yield loss in the state, especially Powdery mildew and the Septoria-Stagonospora leaf blotch complex. During 2007-2008, foliar fungicide experiments were conducted at the Arlington Agricultural Experiment Station (Arlington, WI) and the West Madison Agricultural Experiment Station (Verona, WI). These experiments examined the effect of wheat variety and fungicide timing on grain yield and development of foliar diseases of winter wheat. The experimental design was a randomized complete block split plot with two soft red winter wheat varieties (Public variety Kaskaskia and Pioneer 25R47) on the whole plot level and six fungicide timings on the subplot. Kaskaskia and P 25R47 are two common winter wheat varieties grown in Wisconsin. Both have mid-ratings for resistance or tolerance to FHB. Subplot treatments included an untreated control, Quilt applied at one of four timings ranging from Feekes 7 to Feekes 10.5, and Proline applied at Feekes 10.5.1. Experimental units measured 3 m by 8 m and plots were planted at a rate of 3.71 million seeds per hectare. Disease assessments were taken prior to first fungicide application in late May and after the last fungicide application in late June. At Arlington, a significant variety by fungicide interaction for the late June disease assessment was found, with applications of foliar fungicides applied at Feekes 7 reducing Powdery mildew in Kaskaskia (20% vs. 0%), while no Powdery mildew was observed in the P 25R47. Interestingly at Arlington, the highest yields were observed with the applications of Proline at Feekes 10.5.1 (6% to 13% higher). At West Madison, disease incidence was higher for diseases that occur at the Feekes 7 growth stage, such as Powdery mildew, compared to Arlington. While grain yield was significantly higher in P 25R47 compared with Kaskaskia at West Madison, yield was not affected by fungicide application. To further quantify the incidence and severity FHB at both locations, a calculation of FHB index was made in the field and the percentage of diseased kernels was assessed after harvest by counting the number of diseased kernels out of a sample of 200 kernels. At Arlington, FHB index values were higher for Kaskaskia (difference of 0.4046), but there was no observed difference at West Madison. Conversely though, the percentage of diseased kernels was higher in P 25R47 compared to Kaskaskia at both locations (29.0% for Kaskaskia and 37.5% for P 25R47 at West Madison, and 29.4% for Kaskaskia and 37.1% for P 25R47 at Arlington). These results indicate that the risk and effects of FHB in WI are complex and often underestimated. Further research to determine the most important risk factors and control options would be beneficial for WI growers.

IMPACT OF EXTENDED PERIODS OF MIST-IRRIGATION ON DEOXYNIVALENOL ACCUMULATION IN *FUSARIUM*-INFECTED WHEAT.

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ABSTRACT

Two field experiments were conducted to examine the effect of extended periods of moisture on the accumulation of deoxynivalenol (DON) in *Fusarium graminearum*-infected wheat. The experiments, conducted in 2007 and 2008, were a split-split-plot design with five replications. Main plots were the duration of mist-irrigation after inoculation [14, 21, 28 and 35 d after inoculation]. Sub-plots were wheat cultivars. Three wheat cultivars used were Alsen (moderately resistant with the resistance derived from Sumai 3), 2375 (moderately susceptible) and Wheaton (susceptible). Sub-sub-plots were *F. graminearum* isolate. Five isolates were used (49-3, 81-2, B45A, B63A, and Butte86ADA-11) in addition to a mock-inoculated water control. The *F. graminearum* isolates used were selected as they differed for relative aggressiveness and DON production capacity. The two-rowed plots (1.8 m long) were inoculated twice, at anthesis and 3 d after anthesis (DAA) with macroconidial inoculum (1 x 10^5 conidia ml^{-1}) at a rate of 30 ml per meter of plot row. The inoculum was applied using a CO_2-powered backpack sprayer. FHB severity was assessed 21 DAA by counting the total and visually symptomatic spikelets in 20 arbitrarily selected heads per plot. Visually scabby kernels (VSK) and DON were determined on grain harvested at maturity. In addition to the assessment of FHB and DON analyses on mature grain, DON was also determined in heads (10 per plot) sampled 0, 7, 11, 14, 21, 28 and 41 DAA. These heads were dried and the entire head ground and analysed for DON. Severity, VSK and the DON for mature grain, were significantly higher (P - 0.05), across all isolates, in the susceptible wheat cultivar Wheaton than in the other cultivars examined. FHB severity and VSK were significantly lower (P = 0.05) in the treatments receiving the least amount of mist-irrigation (14 d) than for treatments receiving additional mist-irrigation, suggesting that extended periods of moisture promote disease development. DON was however significantly lower (P = 0.05) in the 35 d misting treatment than those treatments receiving less water. In the irrigation treatment receiving the longest misting period (35 d) the DON concentration in heads peaked at 14 DAA, and then declined till harvest. However, in the 14, 21 and 28 d mist-irrigation treatments, DON was observed to increase again after the cessation of mist-irrigation, with these increases being most pronounced for the treatments with shorter mist-irrigation periods. DON in head tissues were significantly lower in treatments with increased durations of irrigation in heads sampled 21, 28 and 41 dai, and these difference were greatest in the treatment receiving the longest mist-irrigation period (35 d). The largest reduction in DON observed in the 35 d mist-irrigation treatment was seen in the susceptible wheat cultivar Wheaton. Our results suggest that longer durations of moisture after inoculation, either from mist-irrigation or rainfall, may increase the FHB severity and VSK, although DON concentrations may be concomitantly reduced. Leaching may explain the reduction of DON observed in increased misting duration treatments.

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HOW APPLICATION TECHNOLOGY FOR FHB HAS CHANGED OVER THE DECADE.

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ABSTRACT

The terminology 'application technology' has been used to describe methods for applying compounds that reduce the effects of Fusarium head blight (FHB). The primary objective of fungicide application technology research has been to increase the efficacy of fungicides for FHB control. The first research focused on ground application systems and the sprayer components that could effectively improve the efficacy of the fungicides. Two major types of ground application sprayers have been and are being studied. The first type uses hydraulic pressure to generate spray drops and deliver them to the target which on small grains is the grain head or spike. This pressure is either generated by pumps, used by most growers, or pressurized CO_2 which is quite common in the research community because of convenience. The spray solution is pressurized and pushed through a nozzle orifice. The nozzle creates a configuration of small drops and the orientation of the orifice directs the spray toward the target. A second ground system, that is not quite as common, uses an air stream to deliver the drops to the target. This system offers some advantage over the hydraulic system in that the air stream can be adjusted to carry the drops greater distances than the hydraulic system. A hydraulic nozzle typically would be able to push the drops about 50 cm before the drops freefall or are carried by the wind. Aerial application of fungicides has also been researched although not as extensively as ground application. Reports from North Dakota show that in some years about half the fungicide applied to small grains is applied by aerial methods and half by ground methods. The components of these systems that have been studied include drop size, which can be changed by altering pressure and nozzle type, orifice delivery angle, spray volume and spray system travel speed. Several other components have been studied on limited basis because they have not shown to improve currently adopted technologies. These include a rotary atomizer for drop formation, a static electricity system for delivering the drops to the head, and an air induction nozzle that creates a pulsating combination of various size drops. More recently the research field has evolved to include the many classes of adjuvants. Adjuvants work in many different ways and include many types of compounds. One type encapsulates the fungicide molecule to avoid evaporation and movement to areas other than the target.

EFFECTS OF FHB SEVERITY AND CULTIVARS ON DON ACCUMULATION IN WINTER WHEAT.

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, can cause significant losses resulting from yield reduction, kernel damage, and presence of deoxynivalenol (DON), an important mycotoxin with serious food safety implications. In 2008, an experiment was conducted to identify relationships between visual assessments of FHB and DON. Three winter wheat cultivars (Jagalene, Harry, and 2137) were planted following corn on 27 October 2007. Plots were inoculated with conidia and ascospores of *F. graminearum* (1×10^5 spores/ml) at early anthesis and were not irrigated. There also was heavy natural inoculum. Cultivars were arranged in randomized complete blocks with three replications. FHB severity was determined 21 days after inoculation and 20 heads were tagged in each of 13 disease severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 70, and 90% in each cultivar. There was a significant positive correlation between FHB severity in the 13 severity categories and DON for all the cultivars: Jagalene ($r = 0.92$, $P = .0001$), Harry ($r = 0.64$, $P = 0.0176$) and 2137 ($r = 0.88$, $P = 0.0001$). However, DON levels were highest in Harry (32 ppm) followed by Jagalene (29 ppm) and 2137 (19 ppm). Similar data were reported in 2007 comparing two cultivars; Harry had a higher level of DON than 2137. This study demonstrated (i) a positive association between DON levels and FHB severity, and (ii) differences among cultivars in the levels of DON they accumulated. For the second year Harry, a moderately resistant cultivar, accumulated higher levels of DON than the susceptible 2137.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-7-080. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

REACTON OF WINTER WHEAT CULTIVARS TO FHB AND DON.

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is an important disease due the 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 2008, an experiment was conducted to study the reaction of winter wheat cultivars to FHB and DON. Twelve cultivars (Jagalene, Harry, 2137, Hondo, Alliance, Infinity, Goodstreak, Karl 92, Wahoo, Millennium, Wesley, and Overley) were planted following corn on 27 October 2007 at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Plots were inoculated with conidia and ascospores of *F. graminearum* (1×10^5 spores/ml) at early anthesis and were not irrigated. There also was heavy natural inoculum. Experimental design was a randomized complete block with four replications. FHB severity and incidence were determined 21 days after inoculation on 10 heads in each of 10 arbitrarily selected locations in each plot and used to calculate disease index. Plots were harvested with a small plot combine which provided yield data. 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. One thousand kernel weight (1000kwt) was determined by counting 1,000 kernels from each plot with an Agriculex ESC-1 electronic seed counter and weighing the sample on an Ohaus electronic balance. Linear correlation analysis was used to determine relationships between FHB index, FDK, DON, yield, 1000kwt, and test weight. Development of severe FHB was favored by excessively wet weather before and during anthesis. Differences among cultivars were highly significant for FHB index ($P < 0.0001$), yield ($P = 0.0068$), 1000kwt ($P < 0.0001$), FDK ($P < 0.0001$), and DON ($P < 0.0001$). Overley had the highest FHB index (64%) followed by Jagalene (35%) and Wesley (30%). Harry had the lowest FHB index (13%) followed by Hondo (14%) and Goodstreak (14%). FHB index in the rest of the cultivars ranged from 17% to 22%. FDK ranged from 21% (2137) to 42% (Harry and Wahoo). Harry had the highest concentration of DON (9.9 ppm) followed by Overely (8.8 ppm) and Jagalene (8.0 ppm). Karl 92 had the lowest concentration of DON (3.7 ppm) followed by Hondo (3.8 ppm) and Alliance (4.1 ppm). DON concentration in the rest of the cultivars ranged from 4.5 ppm to 6.7 ppm. Yield was generally low due to high disease pressure (FHB and Septoria leaf blotch) and ranged from 11 bu/acre (Wahoo) to 20 bu/acre (Karl 92). One thousand kernel weight ranged from 25 g (Wahoo to 31 g (2137).

There was a significant positive correlation between FDK and DON ($r = 0.59$, $P = 0.0442$). There was a significant negative correlation between FDK and yield ($r = -0.74$, $P = 0.0061$), FDK and test weight ($r = -0.64$, $P = 0.0238$), FDK and 1000kwt ($r = -0.84$, $P = 0.0007$), index and test weight ($r = -0.69$, $P = 0.0121$), and DON and test weight ($r = -0.74$, $P = 0.0060$). All other correlations were not significant at $P = 0.05$. This study demonstrated differences among winter wheat cultivars in their reaction to FHB and DON. It was interesting to note that Harry had the lowest FHB index but the highest DON concentration, implying that cultivars with resistance to FHB may be susceptible to DON accumulation. When selecting cultivars, resistance to both FHB and DON accumulation should be considered.

ACKNOWLEDGEMENT AND DISCLAIMER

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DETERMINING POTENTIALS FOR DON ACCUMULATION FROM PRE-HEAD TIMING OF FUNGICIDE APPLICATION ON SPRING WHEAT AND 6-ROWED MALTING BARLEY IN MINNESOTA.

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ABSTRACT

The primary objective of this experiment was to determine whether fungicide-based leaf disease management strategies of wheat and barley promote increased levels of deoxynivalenol (DON) in Fusarium head blight (FHB) diseased grain compared with the nontreated control.

The 2007 wheat and barley experiments included three replicates planted into two years of corn residue on 8 May 07 at the Northwest Research and Outreach Center in Crookston, Minnesota. Wheat and barley cultivars/lines were selected based on their resistance levels to Fusarium head blight (FHB). Wheat cultivars were Reeder (MS), Knudson (MS-MR), and Glenn (MR) while 6-rowed barley cultivars were Tradition, Robust, and M122 (University of Minnesota advanced germplasm line). Six fungicide treatments [nontreated, Folicur (tebuconazole) 4 fl oz/A, Headline (pyraclostrobin) 7 fl oz/A, Quilt (propiconazole & azoxystrobin) 13.88 fl oz/A, Stratego (propiconazole & trifloxystrobin) 13.88 fl oz/A, Tilt (propiconazole) 4 fl oz/A] began on 15 June 07 and were repeated on a weekly basis until 2 July 07 (four weeks) to determine whether fungicide product or application timing influenced DON levels. Barley was harvested 7 August and the wheat on 16 August 07. The tests received neither misting nor pathogen inoculum. Data were analyzed with PROC GLM in SAS using LSD mean comparisons. Log transformations were conducted on DON data. Values reported here are not transformed.

FHB development and associated losses from disease were minimal on cereal crops in the Red River Valley during 2007. Mean FHB index values ranged from 3.15% to 0.05% on wheat and from 0.88% to 0.03% on barley. Yield means ranged from 87.23 bu/A to 58.50 bu/A for wheat and from 109.19 bu/A to 79.53 bu/A for barley. While DON levels were numerically very low, ranging from 0.48 to 0.04 ppm in wheat and from 0.49 to 0.07 ppm in barley, differences were significant between treatments. Reeder had higher DON levels from an application of Quilt (0.42 ppm) or Headline (0.35 ppm) at Feekes growth stage (FGS) 2 compared with the no fungicide control (0.16 ppm; $P<0.0001$). Reeder again responded with elevated DON levels from a FGS 10.0 to 10.4 timing application of Quilt (0.48 ppm) compared with the nontreated control (0.28 ppm; $P<0.0001$). Neither Knudson, nor Glenn responded to fungicide application with significantly increased DON levels. A similar outcome was identified from an application of fungicide on barley. Tradition showed significantly higher DON levels from an application of Quilt (0.41 ppm) at FGS 10 to 10.4 compared with the no fungicide control treatment (0.16 ppm; $P=0.0311$). Neither Robust, nor M122 had significantly elevated DON levels from a fungicide treatment. Increased DON levels in wheat and barley from an early growth stage application of fungicide was unexpected and needs additional study.

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UNDERSTANDING PRACTICAL OUTCOMES FROM IMPLEMENTING INTEGRATED FHB MANAGEMENT STRATEGIES ON MALTING BARLEY IN MINNESOTA.

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OBJECTIVE

The objective of this two year experiment was to determine grain yield and kernel quality benefits from treating four commercially-available 6-rowed malting barley cultivars and four advanced 6-rowed malting germplasm lines with different Fusarium head blight (FHB) disease management strategies.

INTRODUCTION

This research represents Minnesota's participation in the multi-state, multi-year integrated disease management cooperative research effort which was organized to identify the most practical means for managing FHB across states.

MATERIALS & METHODS

The 2007 experiment included four replicates planted on 9 May 07 into soybean residue near Warren, in northwest Minnesota. Cultivars planted were Drummond, Legacy, Robust, and Tradition while barley germplasm entries were 6B01-2218 (Celebration) and 6B01-2513 [Busch Agricultural Resources, Inc. (BARI)], M122 (University of Minnesota), and ND20448 (North Dakota State University). All entries were exposed to a fungicide treatment (Table 1). Likewise, the 2008 experiments had four replicates of the same entries listed above at field experiment sites near Warren and Mahnomen, Minnesota. The Warren site was planted on 30 April 08 and Mahnomen on 8 May 08. None of these tests received misting or pathogen inoculum. Data from three experiment years (1-2 sites x 2 years) were analyzed using PROC MIXED of SAS. Best linear unbiased estimate or prediction values (BLUEs or BLUPs) were calculated for factors and their interactions. Fungicide and cultivar were considered fixed while environment and its interactions were considered random.

RESULTS & DISCUSSION

FHB development and associated losses from disease were minimal on cereal crops during both production years in the Red River Valley. FHB incidence means were significantly less for germplasm entry (6.48%) compared with cultivar (11.17%; P=0.0096), while no differences were detected for FHB severity. FHB indexes were numerically low, but differences were detected. The germplasm index mean was lower than that of cultivar (0.31% vs 0.62%; P=0.0296) when averaged over four fungicide treatments. Moreover, germplasm was less than cultivar when disease management Strategy 1 was compared to Strategies 2 through 4 (no fungicide: germplasm 0.43%, cultivar 0.79%, P=0.0178; fungicide: germplasm 0.27%, cultivar 0.56%, P=0.0397). Environment promoted yield and kernel quality during the second year of our test. Thousand kernel weight (TKW) means were significantly greater during 2008 (39.09 g) compared with 2007 (35.35 g; P<0.0001). No differences in TKW were detected between entries or fungicide treatment. Test weight averages were also greater in 2008 (48.64 lb/bu) than 2007 (44.57 lb/bu; P<0.0001). No differences in test weight were detected between entries or fungicide treatment. Protein means were slightly increased in germplasm (12.82%) compared with cultivar (12.61%) in the absence of fungicide (P=0.0332). A similar response was not detected from entries in the presence of fungicide. Yields were greater in 2008 (110.81 bu/A) compared with 2007 (30.71

bu/A; $P<0.0001$) when saturated soils became a production issue at the test site. Disease management strategy had no effect on yield with one exception. Cultivars responded well to fungicide Strategy 2 resulting in an increase of 5.81 bu/A ($P=0.0279$) over the germplasm mean. Deoxynivalenol and malt quality data for 2008 are not yet available. This information will be critical in determining whether malting barley can once again be produced in Minnesota.

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DISCLAIMER

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Table 1. Disease management strategies tested on four commercially-available 6-rowed malting barley cultivars and four advanced 6-rowed malting barley germplasm lines at a total of three locations during 2007 and 2008 in the Red River Valley.

Trt	Product	Active ingredient	Application Rate*
1	Nontreated control……	- - -	
2	Folicur……...	tebuconazole	4.0 fl oz/A
3	Prosaro……...	tebuconazole & prothioconazole	6.5 fl oz/A
4	Prosaro……...	tebuconazole & prothioconazole	8.2 fl oz/A

*Treatments 2 through 4 included 0.125% Induce, a nonionic surfactant. Fungicide applications made at Feekes growth stage 10.5 = early heading

UNDERSTANDING PRACTICAL OUTCOMES FROM IMPLEMENTING INTEGRATED FHB MANAGEMENT STRATEGIES ON SPRING WHEAT IN MINNESOTA.

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OBJECTIVE

The objective of this two year experiment was to determine grain yield, kernel quality, and economic benefits from treating a total of 19 hard red spring wheat cultivars with different disease management strategies.

INTRODUCTION

This research represents Minnesota's participation in the multi-state, multi-year integrated disease management cooperative research effort which was organized to identify the most practical means for managing Fusarium head blight (FHB) across states.

MATERIALS AND METHODS

The 2007 experiment included four replicates of 13 cultivars at each of two locations. Planted into soybean residue within commercial fields, a site was located near Oklee in northwest Minnesota and another near Fergus Falls in west central Minnesota. The Oklee site was planted on 27 April 07 and the Fergus Falls site on 2 May 07. Spring wheat cultivars included Ada, Alsen, Banton, Bigg Red, Briggs, Freyr, Glenn, Knudson, Oklee, Samson, Steele-ND, Ulen, and Walworth. Cultivars were treated with one of six disease management strategies (Table 1). The 2008 test included three replicates of 15 cultivars at two commercial field experiment sites. Cultivars included Ada, Alsen, Bigg Red, Breaker, Briggs, Faller, Freyr, Glenn, Hattrick, Knudson, Kuntz, RB07, Samson, Steele-ND, and Tom. Cultivars were treated with the same disease management strategies as before. Second year sites were planted into soybean residue near Fisher and St. Hilaire, Minnesota, both locations situated in northwest Minnesota. The Fisher site was planted on 1 May 08 and St. Hilaire on 5 May 08. No tests received misting or pathogen inoculum. Stand count data were collected at Feekes growth stage 2. Data from four experiment years (2 sites x 2 years) were analyzed using PROC MIXED of SAS. Best linear unbiased estimate or prediction values (BLUEs or BLUPs) were calculated for factors and their interactions. Fungicide and cultivar were considered fixed while environment and its interactions were considered random.

RESULTS AND DISCUSSION

Data indicated that early stands were not significantly influenced by environment, cultivar, or fungicide. Thousand kernel weight means (TKWs) were significantly greater during 2008 (38.79 g) than 2007 (32.65 g; $P<0.0001$). Cultivars with moderate resistance (MR) to FHB had greater TKWs when compared with moderately susceptible cultivars (MS; $P=0.0154$). Three parameters (yield, test weight, and protein) will be reported separately, but when combined contribute to grain grade which determines disease management strategy economic outcome. Test weight averages were greater in 2008 (64.25 lb/bu) than 2007 (61.94 lb/bu; $P<0.0001$). No significant differences were detected between cultivars grouped by FHB resistance [MR: 63.22 lb/bu, moderately resistant-moderately susceptible (MR-MS): 63.06 lb/bu, MS: 62.96 lb/bu] or fungicide strategy. Protein means were only marginally higher in 2007 (14.23%) compared with 2008 (13.96%; $P=0.0476$). MR-MS cultivars had higher protein (14.47%) than MR (14.03%) or MS (13.87%), while MR and MS were statistically similar. Fungicide strategy did not influence protein.

Yield average was greater in 2008 (91.83 bu/A) compared with 2007 (72.21 bu/A; $P<0.0001$). When grouped by resistance levels, cultivars yielded similarly (MR: 81.33, MR-MS: 80.78, MS: 84.13 bu/A). Fungicide strategy had no effect on yield with one exception. When fungicide Strategy 2 was compared with Strategies 4 and 5, yields from the later were significantly greater. Overall treatment net return mean (economic market benefits derived from combined yield, test weight, and protein minus costs of fungicide and application) was not different between years (2007: $561.63/A, 2008: $585.62/A), and was not significant between cultivar resistance groups or fungicide strategies.

Disease development and associated losses were minimal on spring wheat grown in the Red River Valley during 2007 and 2008. This research demonstrates that prophylactic use of fungicide in environments that do not support disease development does not result in significant yield, kernel quality, or economic benefits when compared to the nontreated control.

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DISCLAIMER

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Table 1. Disease management strategies tested on a total of 19 cultivars of hard red spring wheat at four locations during 2007-08 in the Red River Valley.

			Application	
Strategy	Product	Active ingredient	Rate*	Timing**
1	Nontreated control..			
2	Dividend Extreme..	difenoconazole and mefenoxam	3 fl oz/100 lbs	Seed applied pre-plant
3	Headline.....	pyraclostrobin	3 fl oz/A	FGS 2
	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl oz/A	FGS 10.51
4	Dividend Extreme..	difenoconazole & mefenoxam	3 fl oz/100 lbs	Seed applied
	Headline.....	pyraclostrobin	3 fl oz/A	FGS 2
	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl oz/A	FGS 10.51
5	Dividend Extreme..	difenoconazole & mefenoxam	3 fl oz/100 lbs	Seed applied
	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl oz/A	FGS 10.51
6	Folicur/Proline.....	tebuconazole & prothioconazole	3 + 3 fl oz/A	FGS 10.51

*Treatments 3 through 6 included 0.125% Induce, a nonionic surfactant

** Feekes growth stage (FGS)

2008 RESULTS FROM THE UNIFORM EVALUATION OF BIOLOGICAL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT ON WHEAT AND BARLEY.

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OBJECTIVE

To evaluate, using standardized methodology, a set of biological control agents applied alone and in combination with a fungicide for effectiveness in managing Fusarium head blight (FHB) caused by *Fusarium graminearum* and deoxynivalenol (DON) accumulation in wheat and barley across a range of environmental conditions.

INTRODUCTION

Since 2004, experimental microbial agents have been compared for efficacy in controlling FHB and DON as part of the USWBSI-funded program for standardized evaluation of biological control agents. Among them are strains of bacteria *Bacillus subtilis* Trigocor 1448 (da Luz et al., 2003) and *Bacillus* sp. 1BA (Draper et al., 2001). Bacteria in the genus *Bacillus* are attractive candidates for development into commercial biocontrol products because of their ability to produce endospores that are resistant to environmental extremes and their potential to express a number of biocontrol mechanisms (McSpadden Gardener, 2004; Schisler et al., 2004). One strain to recently gain EPA registration is *B. amyloliquefaciens* (=*B. subtilis* var. *amyloliquefaciens*) FZB24 and is the active ingredient in the product Taegro (Novozymes Biologicals). While this strain has been shown to control a wide range of plant pathogens (Krebs et al., 1998), it had not been evaluated previously for control of FHB and DON. Combinations of biological control agents with the fungicide tebuconazole were reported to be more effective in controlling FHB than the microorganisms or the fungicide alone (da Luz et al., 2003; Khan et al., 2004; Jochum et al., 2006). In uniform fungicide trials for FHB control in 2006, the fungicide formulation Prosaro 421 SC (Bayer CropScience) that combines tebuconazole and prothioconazole was demonstrated to be more effective than tebuconazole in enhancing yield and reducing levels of DON (Paul et al., 2006). Therefore, the 2008 uniform biocontrol trials were designed to compare the three *Bacillus* strains, applied alone or in combination with Prosaro, for efficacy in controlling FHB and DON.

MATERIALS AND METHODS

Seven trials were conducted across three states on barley and a range of wheat market classes (Table 1). In each trial, three *Bacillus* biological agents (Table 2) were tested alone or in tank mix with the fungicide Prosaro 421 SC (6.5 fl oz/A). There also was a treatment of Prosaro alone and a non-treated control. A broth culture or formulation of each organism was provided by the originating laboratory/company and sent to the researcher in each location. The pre-application population of each organism strain in the treatment suspensions were determined by the local researcher using dilution plating. All treatment liquids were amended with 0.125% Induce (v/v). In all locations, one application of each treatment was made at early flowering (Feekes 10.51) in 20 gal/acre using CO2-pressurized sprayers (approximately 40 psi) equipped

with flat-fan nozzles oriented at a 30° downward angle forward and backward. The size and number of replicate plots varied among trials. Pathogen inoculum was provided in some of the trials in the form of spore suspensions or inoculated corn grain. In addition, mist irrigation systems were utilized at some locations during flowering to stimulate infection. In all trials, FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (% plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. The incidence of Fusarium-damaged kernels (%FDK), as well as yield of seed and test weight, were determined after harvest. Samples from each plot were sent to the North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND for analysis of DON content. Analysis of variance was performed on results from each trial separately. Data from all trials were pooled and analyzed together using ProcMixed (SAS). The LSD test was used for means separation.

RESULTS AND DISCUSSION

Dry weather conditions in South Dakota during the flowering period inhibited disease and DON production, while wet weather in Missouri, Nebraska, and North Dakota resulted in higher FHB development. In Missouri, viral diseases barley yellow dwarf and wheat streak mosaic were quite widespread, impacting plant vigor and eventually yield.

None of the *Bacillus* strains applied alone had any effect on disease parameters measured in the field, while Prosaro applied alone or in combination with a biological agent was effective in reducing FHB measures in multiple trials (Tables 3A and 3B). All of the treatments reduced the incidence of Fusarium diseased kernels and most increased test weight in the North Dakota trial (Table 3B). DON was reduced by TrigoCor 1448 in only one trial, while treatments involving Prosaro reduced DON levels in most of the trials (Table 3B). In locations with high disease pressure, however, DON levels in those treatments still exceeded acceptable levels. None of the treatments increased yields over the control except in the North Dakota trial where Prosaro alone and in combination with every biocontrol agent increased plot yields (data not shown).

The collective results from this year's multistate trials indicated no single *Bacillus* strains to be superior in performance across a range of environments or crops. While Prosaro alone reduced the LS mean of incidence of Fusarium diseased kernels (Table 3B), the treatment was not consistently effective across all trials and there was no benefit from combining the biocontrol agents with Prosaro 421 SC. Therefore, it may be desirable to explore combinations of biocontrol agents with less efficacious fungicides, or with moderately resistant varieties as a means to broaden the selection of tactics that can be used to protect florets from Fusarium infection.

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DISCLAIMER

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Table 1. 2008 uniform biological control trial locations, crop cultivars, and researchers

State (location)	Crop market class and cultivar	Researcher and Institution
MO	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
MO	Soft red winter wheat 'Elkhart'	L. Sweets, University of Missouri
NE-1 (Mead)	Hard red winter wheat '2137'	C. Jochum & G. Yuen, University of Nebraska
NE-2 (Lincoln)	Hard red winter wheat '2137'	C. Jochum & G. Yuen, University of Nebraska
SD	Hard red spring wheat 'Briggs'	K. Ruden, South Dakota State University
SD	Six-rowed barley 'Robust'	K. Ruden, South Dakota State University
ND	Hard red spring wheat 'Howard"	S. Halley, North Dakota St. Univ., Langdon, ND

Table 2. Biological control agents tested in 2008 uniform trials.

Organism	Supplier
Bacillus sp.1BA	Bruce Bleakley, South Dakota State University
Bacillus subtilis TrigoCor 1448	Gary Bergstrom, Cornell University
Taegro *Bacillus amyloliquefaciens* FZB24	Novozymes Biologicals, Inc., Glen Allen, VA

Table 3A. 2008 results across seven uniform biocontrol trials denoted by state and crop

Treatment	MO 'Roane'	MO 'Elkhart'	NE-1 2137	NE-2 2137	SD Wheat	SD Barley	ND 'Howard'	LS mean
INCIDENCE (% heads infected)								
Control	*11*	*40 ab**	*95 a*	*96*	*6*	*79 a*	*88 a*	**66**
Prosaro	6	29 c	80 c	88	3	63 ab	54 bc	54
1BA	9	40 ab	91 ab	92	11	64 a	78 a	63
1BA + Prosaro	9	31 bc	82 bc	88	5	50 b	54 bc	53
TrigoCor 1448	15	43 a	91 ab	90	8	63 ab	83 a	61
TrigoCor 1448 + Prosaro	9	26 c	88 abc	95	2	59 ab	42 c	52
Taegro	13	35 abc	91 ab	91	9	71 ab	81 a	63
Taegro + Prosaro	10	28 c	89 abc	87	5	50 b	56 b	52
P	NS [§]	0.011	0.0771	NS	NS	0.0598	<.0001	NS
SEVERITY (% spikelets infected)								
Control	23	66	21	27	10	6	21 a	23
Prosaro	20	74	19	21	7	5	12 b	21
1BA	19	66	25	28	14	5	21 a	25
1BA + Prosaro	20	73	20	19	13	5	14 b	21
TrigoCor 1448	29	75	26	22	10	5	21 a	26
TrigoCor 1448 + Prosaro	20	58	19	24	5	5	12 b	19
Taegro	23	66	23	20	10	6	22 a	23
Taegro + Prosaro	25	78	20	20	10	6	13 b	22
P	NS	NS	NS	NS	NS	NS	0.0002	NS

*Means separation (P=0.05) shown only when treatment effect was significant; [§] NS = Not significant.

Table 3B. 2008 results across seven uniform biocontrol trials denoted by state and crop.

Treatment	MO 'Roane'	MO 'Elkhart'	NE - 1 2137	NE - 2 2137	SD Wheat	SD Barley	ND 'Howard'	LS Mean
INDEX (plot severity)								
Control	3	27 ab*	19	27	0.56	5	17 a	15
Prosaro	1	21 bc	15	19	0.35	3	4 b	10
1BA	2	27 ab	23	26	1.47	4	13 a	15
1BA + Prosaro	2	23 bc	16	17	0.77	2	4 b	10
TrigoCor 1448	4	32 a	24	20	0.87	3	15 a	15
TrigoCor 1448 + Prosaro	2	15 c	17	23	0.11	3	2 b	9
Taegro	3	23 bc	21	18	0.84	4	16 a	13
Taegro + Prosaro	2	21 bc	17	18	0.72	3	4 b	9
P	NS	0.04	NS	NS	NS	NS	<0.0001	NS
FDK (%)								
Control	17	29	28	12	2	nd[#]	15 a	16 a
Prosaro	17	25	18	9	3	nd	4 cd	11 c
1BA	16	30	24	15	3	nd	7 bc	14 ab
1BA + Prosaro	14	24	18	9	2	nd	8 b	12 b
TrigoCor 1448	17	31	28	13	4	nd	7 b	15 a
TrigoCor 1448 + Prosaro	12	25	21	11	1	nd	3 d	10 c
Taegro	12	24	27	13	3	nd	6 bc	13 b
Taegro + Prosaro	17	23	20	10	2	nd	4 cd	11 c
P	NS	NS	NS	NS	NS		<0.0001	0.08
DON (ppm)								
Control	1.98 a	12 a	6	4.76 a	1.68 abc	2.63 abc	nd	3
Prosaro	1.23 d	9 bcd	8	2.02 c	1.15 abc	1.88 bc	nd	3
1BA	1.85 ab	12 a	5	3.71 ab	1.95 ab	2.25 abc	nd	3
1BA + Prosaro	1.7 abcd	10 bc	7	2.72 bc	1.33 abc	1.13 c	nd	3
TrigoCor 1448	1.4 bcd	11 ab	8	4.33 ab	2.08 a	3.5 a	nd	3
TrigoCor 1448 + Prosaro	1.83 abc	7 de	9	2.95 bc	0.9 c	1.38 c	nd	3
Taegro	1.85 ab	12 a	8	3.76 ab	1.88 abc	3 ab	nd	3
Taegro + Prosaro	1.35 cd	7 e	7	2.44 bc	1.0 bc	1.65 bc	nd	2
P	0.03	<0.0001	NS	0.08	0.01	0.003		NS
TEST WEIGHT (pounds/bu)								
Control	59	50 c	60	60	53 ab	38	31 d	55
Prosaro	58	54 ab	60	60	55 a	39	35 a	57
1BA	58	52 bc	60	60	52 ab	40	33 bc	56
1BA + Prosaro	59	51 bc	60	60	54 ab	40	35 a	56
TrigoCor1448	57	53 ab	60	60	52 ab	40	32 cd	56
TrigoCor1448 + Prosaro	59	54 ab	60	60	55 ab	40	36 a	57
Taegro	58	50 c	60	60	51 b	39	33 cd	55
Taegro + Prosaro	59	55 a	60	60	55 ab	39	35 ab	57
P	NS	0.0075	NS	NS	0.0132	NS	0.0007	NS

*Means separation (P=0.05) shown only when treatment effect was significant.
#nd = no data; [§]NS = not significant.

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 *Bacillus* on wheat florets over critical infection periods in greenhouse and field settings. Using dilution plating, we quantified *Bacillus* populations on wheat heads at 0h, 4-5h, 7d, and 14d after *Bacillus* application. In greenhouse and field experiments, *Bacillus* populations survived at significantly high levels (10^7 and 10^6 per head, respectively) that were consistent throughout the sampling period. The persistence of *Bacillus* on wheat florets suggests this BCA, applied at anthesis, is present in sufficient numbers to protect plants against *Fusarium* infections through flowering and early grain development. In addition to viable bacterial populations, the production and persistence of antifungal metabolites relative to biological control also need to be assessed in controlled and field environments. *Bacillus subtilis* produces several antifungal compounds that appear to contribute significantly to its biocontrol efficacy. Among these compounds are lipopeptides in the fengycin, iturin, and surfactin families, which we have identified through HPLC and mass spectrometry. In a greenhouse experiment, filtered *Bacillus* supernatant enriched for these lipopeptides conferred a 49% reduction in DON and a 30% reduction in FHB severity. The reductions in DON and disease severity were significantly greater than those caused by application of washed *Bacillus* cells, but were less than those caused by a *Bacillus* whole broth culture. These results indicate that lipopeptides may be a critical component in the biocontrol arsenal of *Bacillus*, and suggest that optimization of biocontrol should involve maximizing production of these important compounds under field conditions. We are currently investigating the relative contributions of each family of lipopeptides to biocontrol, and we hope to determine what, if any, threshold levels of these families are necessary for optimal biocontrol. These findings will direct optimization of BCA preparation and application for enhanced biological control.

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RELEASED CLONES AND BACKGROUND INOCULA OF *GIBBERELLA ZEAE* CONTRIBUTED TO FUSARIUM HEAD BLIGHT IN WINTER CEREALS IN NEW YORK AND VIRGINIA.

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ABSTRACT

An increased understanding of the relative contribution of within-field inoculum sources of *Gibberella zeae* to infection of wheat and barley is important for developing and/or excluding strategies for managing Fusarium head blight (FHB). Clonal isolates of *G. zeae* containing rare alleles (relative to background populations) were released in replicated 33 in. diameter circular plots in eight commercial winter cereal fields (seven wheat and one barley) in New York and Virginia over 2007 and 2008. Each field was planted following harvest of a non-cereal crop in order to eliminate potential with-in field sources of *G. zeae*. The eight field environments were categorized as six 'nonepidemic' (all wheat), one 'moderate epidemic' (wheat), and one 'severe epidemic' (barley) based on the incidence of FHB symptoms in non-inoculated plots. Mature wheat spikes were collected at the released inoculum source, at a radius of 10 feet from the source, at a radius of 20 feet from the source, and from non-inoculated (control) sites separated at least 100 feet from a released source. Spikes were observed for symptoms of FHB, disinfested, and plated onto a *Fusarium* selective medium. Nearly 1,500 isolates of *G. zeae* were recovered from spikes among the eight fields. Amplified fragment length polymorphisms (AFLPs) were used to genotype isolates recovered from these spikes and to determine the contribution of released isolates to FHB at various distances from those sources. The clonal isolates released in our field experiments had unique AFLP haplotypes; therefore we were able to observe these clones in a mixed/diverse background population containing numerous AFLP haplotypes. AFLP data demonstrated that locally released clones as well as background inocula contributed to FHB. In 2007, a minority of the recovered isolates in VA (23%; 94/401; moderate epidemic-wheat) and NY (16%; 11/66; nonepidemic-wheat) had AFLP profiles that were identical to our released clones, with the majority of the recovered isolates coming from background sources. Preliminary AFLP data from 2008 experiments showed that 53% (102/191) of the recovered isolates in VA and 66% (180/272) in NY had AFLP profiles that were identical to our released clones, with a considerable percentage coming from background sources. Released clones were recovered at their highest frequencies at the sources, at greatly reduced frequencies at 10 and 20 feet from sources, and only occasionally from non-inoculated sites. Spike infection percentages approached background levels within 10-20 feet of clonal inoculum sources for both fields. FHB symptom incidences, infected spike incidences, and deoxynivalenol (DON) levels (NY fields only) fell off sharply to background levels within 10-20 feet of clonal sources in the fields under study in 2008. Our work has important implications for the management of FHB/DON in wheat and barley. If within-field sources of *G. zeae* (i.e., infested residues of corn, wheat, or barley) contribute a significant proportion of local inoculum for FHB, then management of those residues should lead to significant reductions in FHB and DON in those fields.

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MORE THAN 40 YEARS OF OBSERVATIONS FROM OHIO CONFIRM THE IMPORTANCE OF RELATIVE HUMIDITY AND PRECIPITATION FOR FUSARIUM HEAD BLIGHT EPIDEMICS.

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ABSTRACT

For each of 44 years, an ordinal assessment of FHB in Ohio was performed, based on the general magnitude of disease symptoms, DON in grain, and yield-loss estimates. Each year was given a rating between 0 and 9, and quantitative relationships between the ratings and weather and climatic variables were investigated for the period from 1965 to 2008. Weather data were gathered from weather stations near Wooster, Ohio, and summary variables (such as average RH or ambient air temperature) were calculated for a wide range of time windows and starting times of the windows during the wheat growing season. The windows ranged from 5 to 270 days in duration, beginning at June 20 (around growth stage 11.3) and proceeding back to September 24 of the previous year (about the time of planting). Spearman rank correlation coefficients were calculated to identify the time periods in which weather variables showed significant effects on the FHB rating. This overall protocol is commonly known as 'Window Pane' analysis, which was first introduced by Coakley and colleagues in 1982. Effects of both long-term climatic and short-term weather variables on FHB rating were found. FHB rating and average relative humidity were significantly ($P < 0.01$) correlated for short time windows (e.g., 5-30 days in duration) in both early and late spring, covering the period for spore production, infection, spike colonization and DON production. Average relative humidity for long time windows (e.g., 210 days, beginning at growth stage 11.3) was also significantly correlated with FHB rating, demonstrating the climatic effects of moisture on disease. FHB rating and precipitation were significantly correlated ($P < 0.01$), but only for short time windows in late spring. There was no significant effect of temperature for any time window. In conclusion, results confirm that the impact of FHB on winter wheat in Ohio is dependent, at least in part, on the short- and long-term atmospheric moisture conditions and the short-term amount of precipitation.

RELATIONSHIP BETWEEN FHB AND DON AMONG SRWW CULTIVARS WITH DIFFERENT LEVELS OF TYPE II RESISTANCE.

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ABSTRACT

Deoxynivalenol (DON) is among several mycotoxins produced by *Fusarium* species in infected wheat spikes. DON reduces grain quality and commercial value, leading to price discount and dockage if contamination exceeds 2 ppm. As both a consequence of, and a virulence factor for Fusarium head blight (FHB) development, grain DON content is in general positively correlated with FHB visual estimates, however, this association varies among studies. Difference in cultivar resistance to FHB and DON is one of several factors likely responsible for variation in the functional relationship between FHB and DON. Resistance in the *F. graminearum*/DON/wheat system is a complex trait, with several different types reported (Types I, II, II, IV and V), but not completely characterized. Resistance to fungal spread within the spike, known as type II, is widely adopted in breeding programs, with high levels of Type II generally corresponding to high levels of resistance to DON accumulation. Our goal was to characterize the FHB-DON functional relationship and how it is modified by cultivar resistance. Three experiments were conducted in two years using three soft red winter cultivars with different levels of Type II resistance (Truman, moderately resistant; Hopewell, moderately susceptible; and Cooper, susceptible). At anthesis, plots were spray-inoculated with a 50,000 spores/ml suspension consisting of equal proportions of ascospores and macroconidia. Prior to physiological maturity, FHB head severity was assessed and 20 spikes were tagged in each of 11 severity categories: 0, 1, 2, 3, …10 diseased spikelets per spike. Spikes in each category were hand-harvested and tested for DON. Linear mixed model covariance analysis was used to evaluate the influence of cultivar on the relationship between FHB and DON. In all three experiments, there was a significant linear relationship between FHB and DON, with significantly difference regression slope among the cultivars. For a given level of FHB severity, DON concentration varied among the three cultivars. Hopewell consistently accumulated more DON than the other two cultivars at most of the 11 disease categories, and the DON accumulation rate (FHB-DON regression slope) was significantly higher for Hopewell than for Truman in all three experiments. At various levels of severity, DON differences between Truman and Cooper were smaller than the differences between Hopewell and Truman, but generally, Cooper accumulated more DON than Truman for a similar level of FHB. We found that DON accumulation did not always parallel Type II resistance levels. Hopewell, considered moderate susceptible, accumulated significantly more DON than Cooper, considered susceptible, and based on the FHB-DON regression slope, the rate of DON increase with FHB increase for Cooper was similar to that of Truman, the moderate resistant cultivar, in two of the three experiments. Our data suggest that Type III resistance (resistance to DON accumulation) should be considered separately from Type II resistance when evaluating cultivars and visual estimates of FHB should not be the only standard for evaluating resistance.

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STUDY OF FUNGICIDE EFFECT AND ITS COMBINATION WITH WHEAT CULTIVAR RESISTANCE ON THE RELATIONSHIP BETWEEN FHB AND DON AND THE ACCUMULATION OF DON IN ASYMPTOMATIC WHEAT SPIKES.

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ABSTRACT

Deoxynivalenol (DON) accumulation in *Fusarium graminearum*-infected wheat reduces grain quality, resulting in economic losses for wheat producers. Generally, DON levels are positively correlated with disease intensity, however, in some instances, DON accumulation may exceed 2 ppm (threshold grain buyers adopt when purchasing wheat) in visually disease-free grain. The association between FHB and DON may be influenced by several factors including weather condition, cultivar resistance, fungicide effect on FHB and DON, and pathogen aggressiveness and DON producing ability. Studies have shown that fungicides with similar modes of action may have similar effects on FHB index (visual symptoms) but different effects on DON. This may be due in part to differential effects of the fungicides on grain colonization, and consequently, DON accumulation. Since DON response to fungicide treatment is often confounded by disease response to the fungicide, it is difficult to ascertain the direct effect of fungicide on DON based on mean DON levels from research plots. Our goal was to evaluate fungicide effects on the FHB/DON relationship and the combined effects of fungicide and cultivar on the accumulation of DON in asymptomatic grain. A field experiment was conducted using a split plot design, with six SRWW cultivars of different levels of FHB resistance and three fungicide treatments as whole- and sub-plot factors, respectively. The sub-plot treatments were Folicur (4 fl.oz./A) and Prosaro (6.5 fl.oz./A) applied at anthesis, as well as an untreated check. Plots were spray-inoculated during anthesis with a spore suspension containing 25,000 spores / ml. Fifteen (and in one case ten) asymptomatic spikes were tagged in plots of each treatment combination, and in plots of the susceptible cultivar Cooper, four additional sets of spikes with 1, 2, 3 and 4 diseased spikelets per spike, respectively, were tagged. All tagged spikes were hand-harvested and analyzed for DON. There was a significant linear relationship between FHB and DON for the fungicide treatments and the untreated check. However, the regression slopes were not significantly different among the treatments, suggesting that fungicides did not alter the rate of increase in DON with increase in FHB, relative to the untreated check. The height of the FHB/DON regression line was lower for Prosaro than Folicur, but the difference between the lines was not statistically significant. The DON content of asymptomatic grain ranged from 0.06 to 3.6 ppm. The main effects of fungicide and cultivar on DON (on as square-root transformed scale) in asymptomatic grain were statistically significant. The moderately resistant cultivars (Truman and McCormick) had significantly lower asymptomatic grain DON content that the moderate susceptible cultivars (Hopewell and AGI101). Averaged across cultivars, mean DON in asymptomatic grain was significantly lower in Prosaro-treated plots than in the untreated check.

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MANAGEMENT OF SCAB IN WHEAT USING RESISTANT VARIETIES AND FUNGICIDE.

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ABSTRACT

A study was conducted to assess the effectiveness of fusarium head blight (FHB) resistance, Proline® fungicide, and a combination of both control measures in reducing losses in grain yield and quality. 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 2007-2008. 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 was applied at 5.5 oz/ac before flowering time of each variety 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 and fungicide treatment had a significant effect on all traits. Treatment and location interaction effects were common except for yield. All of the scab assessment parameters, except for severity, were significantly and highly correlated ($r = 0.4$ to 0.96, $P < 0.001$) with each other. All of the scab parameters, except for FHB severity, had a significant ($r = -0.6$ to -0.9, $P < 0.001$) negative effect on test weight and grain yield. Results of this study indicate that a single Proline fungicide application in eight winter durum varieties significantly reduced FHB incidence (5 varieties), severity (1), index (4), FDK (2) and DON (2). Fungicide application resulted in a significantly higher test weight and yield in two and five durum varieties, respectively. Fungicide application had less effect on FHB infection in moderately resistant versus susceptible winter durum wheat varieties. Non-treated resistant durum varieties, such as VA05WD-12 and VA05WD-16, had less infection than treated susceptible varieties. Four of the non-treated durum varieties had higher test weight and one had higher yields than one of the treated susceptible durum varieties.

The fungicide treatment had less effect on FHB in the moderately resistant SRW wheat versus durum wheat varieties. Only the susceptible SRW cultivar Coker 9835 benefited from the fungicide treatment, which resulted in a significant reduction in scab infection and development and higher test weights and grain yields. FHB resistance in the SRW wheat cultivars was greater than in the winter durum varieties at reducing scab infection and development and losses in grain yield and quality. Results of this study indicate that utilization of SRW and winter durum 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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INFECTION TIMING AND MOISTURE DURATION EFFECTS ON FHB AND DON DEVELOPMENT IN SPRING WHEAT AND DURUM, ND.

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ABSTRACT

We continue to investigate how moisture duration and growth stage at inoculation affect Fusarium head blight (FHB) development and DON production in hard red spring wheat and durum wheat. FHB susceptible and more resistant cultivars of hard red spring wheat and durum wheat were grown in the greenhouse. At one of four growth stages (Feekes 10.5 = full head emergence, 10.51 = early flowering, 10.54 = kernel watery ripe, or 11.2 = early soft dough), grain heads were inoculated with spores of a mix of isolates of *Fusarium graminearum* (20,000 spores/ml; 20 ml per pot) and subsequently placed under one of three intermittent misting treatments. The misting treatments were 1) two days in an enclosed mist chamber with intermittent mist for 30 seconds for every 5 minutes, 2) misting treatment one followed by an additional 3 days of misting on the greenhouse bench, under an overhead RainBird sprinkler system, misting for 15 minutes for four times per night, or 3) misting treatment one followed by 8 days under the bench misting system described for misting treatment two. Each combination of growth stage treatment plus misting treatment was replicated six to eight times per cultivar/trial and each trial was done three times. FHB incidence, head severity and index ([incidence x head severity])/100 were determined 14 to 21 days after inoculation. DON, 15ADON, and 3ADON values were determined on grain that was hand-threshed at maturity, ground, and then analyzed by the NDSU Toxicology Lab using gas chromatography and electron capture techniques. Results indicated that both the susceptible durum (Monroe) and the susceptible spring wheat (Trooper) had considerably more FHB and DON than their more resistant counterparts (Divide durum and Glenn spring wheat), for most inoculation timings and moisture treatments. The 5 day and 10 day misting treatments resulted in greater disease and DON than the 2 day duration, indicating duration of moisture does affect disease severity and DON, similar to previous findings. The two durum cultivars had a long window of vulnerability to infection, from flowering (Feekes 10.51) through early soft dough stage (Feekes 11.2), while severe infection occurred primarily at one growth stage in spring wheat - at kernel watery ripe (Feekes 10.54) in the susceptible spring wheat, and at flowering (Feekes 10.51) in Glenn, the more resistant cultivar. Inoculations at full head emergence but before flowering (Feekes 10.5) in both grain classes resulted in no or very low FHB and DON. 15ADON was only detected in one experiment, and nivalenol was not detected. 3ADON detection also was limited and occurred almost exclusively in the susceptible cultivars when misting durations were 10 days and only when the DON levels were very high (average > 25 ppm).

PHYSIOLOGIC PROFILING AND CARBON SOURCE UTILIZATION OF FOUR *BACILLUS* STRAINS USED AS BIOLOGICAL CONTROL AGENTS OF FHB.

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ABSTRACT

Understanding use of plant nutrients and specific carbon sources by plant pathogens such as *Fusarium graminearum* is important for designing biological control methods to control the fungus. It is important in biological control agent (BCA) spray application to avoid adding carbon sources to the spray mix that would stimulate *F. graminearum.* Four strains of *Bacillus amyloliquefaciens* (designated 1D3, 1BC, 1BE, and 1BA), used as biocontrol agents antagonizing FHB, have not been previously assayed for xylanase and carboxymethylcellulase (CMCase) activity. Plate assays were conducted with two CMC-containing media and one xylan-containing medium, with incubation at both 27 Celsius and 45 Celsius. Medium CMC-1 had a high C/N ratio and yeast extract as nitrogen source. Medium CMC-2 had a much lower C/N ratio and ammonium sulfate as nitrogen source. Ratios of zone of clearance diameter to colony diameter were compared. On CMC-1, the ratio for strain 1BA was about the same at both temperatures, but for the other three strains, ratios were about twice as large at the higher temperature, with values up to 4.7. On CMC-2, the ratio value of 4.6 for strain 1BA was about 1.4 times as large at the higher temperature; while ratios for the other three strains were approximately the same at both temperatures. At 27 Celsius, ratios for strains 1BC and 1BE were twice as large on CMC-2 as on CMC-1. For xylanase activity at 27 Celsius, strain 1D3 had the lowest ratio of 2.8, while all other strains had ratios near or above 4. At 45 Celsius, no zones of clearance were present for any strain besides that below the colonies. The xylanase activity of the strains appeared to be much more temperature sensitive than the CMCase activity. Ability of the BCAs to use CMC and xylan may correlate with their originally being isolated from wheat residues. BioLog GEN III microplates were also used to determine which carbon sources each strain used; and which substances were inhibitory to each strain. The strains all shared ability to grow well on one or more organic acids. Two of the strains (1BA and 1BC) grew well on pectin. Analysis of stimulatory and inhibitory substances affecting growth of these bacilli is important for developing their use as biocontrol agents of FHB. (Parts of the poster were presented at the Society for Industrial Microbiology Annual Meeting and Exhibition, August 10-14, San Diego, CA).

USE OF MOST PROBABLE NUMBER AND PCR METHODS TO ESTIMATE POPULATIONS OF *BACILLUS* STRAIN 1BA APPLIED TO WHEAT AND BARLEY FOR BIOLOGICAL CONTROL OF FHB.

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ABSTRACT

After spray application of biological control agents (BCAs) onto grain heads for control of FHB, it is important to understand how populations of the BCAs change over time. We are focusing on a *Bacillus* 1BA strain for use as a BCA. In the 2008 uniform biocontrol trials in Brookings, using most probable number methodology to assay BCA populations, control plots that did not receive spray application of BCAs had very low numbers, in the hundreds per gram fresh plant mass, showing there is a small number of native bacteria that can tolerate the high salt and temperature conditions used in recovering and counting BCA bacteria from grain heads. Bacterial numbers recovered from barley were low in both control and treated plots, again indicating that 1BA does not grow well on barley heads.

In the Brookings trial, endospore numbers (in the heat-pasteurized count) did not increase dramatically until about day 16 after spraying. There was probably a large increase in vegetative cells between days 9 and 16 that was not detected in the sampling dates used. Results from 2006 and 2007 showed an increase in numbers around day 10 that was much greater than the one for 2008. Inclusion of Prosaro with Induce and *Bacillus* 1BA resulted in an early increase of 1BA numbers (around day 3) followed by a decline and no futher increase in numbers. This apparent effect of Prosaro in causing a more rapid increase in 1BA numbers on wheat heads was also noted in 2007 field data.

In Brookings, plots, wheat heads that had been sprayed with 1BA mutant strains (spontaneous mutant having rifampicin resistance) were processed for extraction of bacterial DNA, and using primers specific for the surfactin gene, PCR was carried out on the extract to see if there was evidence of surfactin genes on the grain heads. PCR product for surfactin was detected on inoculated wheat heads, but not on control (uninoculated) heads. This shows that PCR methodology is able to detect presence of the surfactin-producing BCAs on treated grain heads. PCR analyses of grain heads will continue in FY09, and primer sets for the lipopeptide iturin will also be used in addition to the surfactin primers.

THE INFLUENCE OF FUNGICIDES FOLIAR TREATMENTS ON THE WHEAT YIELD AND QUALITY.

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Wheat crops are damaged by numerous diseases which caused quantitative and especially qualitative yield losses in Transylvania conditions.The complex of foliar diseases : powdery mildew (*Blumeria graminis* f. sp. *trtrici),* leaf and glume blotch (*Septoria trtrici* and *Stagonospora nodorum*), rusts (*Puccinia striiformis, Puccinia recondita and Puccinia graminis*) and tan spot (*Pyrenophora tritici – repentis*) as well as head blight (*Fusarium* spp.) and ears blackening (*Alternaria* and *Cladosporium*) are the most frequent in wheat crops. Yield losses reaching 30% from yield value depend on climatic conditions and wheat cultivar.

METHODOLOGY

The effect of fungicide foliar treatments and winter wheat was studied at ARDS Turda during two years. It was organized by factorial trials after block split type with 3 treatments variants: untreated (T_0), 1 Treatment (T_1) applied at through early flag leaf emergence (ZGS38) and 2 treatments (T_2) applied through early flag leaf emergence (ZGS38) and in the end of flowering (ZGS73). The fungicides used contain: spyroxamine 250 g/l+tebuconazole 167g/l+triadimenole 43g/l at dose 0,6 l/ha, for the first treatment, respectively prothioconazole 125g/l+ tebuconazole 125g/l at dose 0,9 l/ha for the second treatment. In the field, attack degree for main diseases (%) and yield (kg/ha) and in the laboratory, baking parameters protein and wet gluten content (%) were determined. It also evaluated, thousand kernels weight (TKW), volumetric weight and percentage of diseased kernels.

RESULTS

The weather conditions from April, May, June months of 2 years is characterized by high temperature associated with weather deficit, were not very favorable of the diseases occurrence, it know that is essentially weather- dependent. Foliar diseases: powdery mildew, tan spot, leaf blotch and brown rust and ears diseases: Fusarium head blight (FHB) were presented in wheat crops. By applying of one single fungicide treatment, attacked leaf area by foliar diseases was significantly reduced in average with 50% and quite more at Turda 2000 and Apullum cultivars. Applying of 2 treatments diminished substantially diseased leaf area (3,8%) and the FHB attack (2,6%), with positively effect on the yield capacity. Applying one foliar treatment increases yield with 5,4-13,8 %, average being 9,5% and for two treatments with 14,0-20,1%, average being 16,4 %, in the two ears. For Turda 95 and Dumbrava wheat cultivars, the highest yield by 6436 kg/ha respectively 6462 were registered.(Fig 1.)

Between spikes and disesased kernels a positive and semnifictive correlation exists, defined by equation: $y=1,0447x+5,7327$; $R^2 = 0,6268$*.(Fig.2.)

Besides substantially significant yield gains were really improved the quality in term baking due to gluten content. Applying two treatments with fungicides determined an evident increase reach up to 30,7 % of the wet gluten and to 11,7% of the protein conten (Fig.3). Tested fungicides were presented a good efficacy in controlling of foliar and ear disease, remarked Soprano (0,75l/ha), Tango Super(1.0l/ha), Artea (0,4l/ha), Caramba(1,0l/ha), Falcon (0,7l/ha), Amistar Extra (0,5l/ha), Nativo 0,8 (l/ha), Prosaro (0,9l/ha).

CONCLUSIONS

Realizing of wheat performed and quality yield could not possible without a corresponding protection against foliar and ear diseases in humide and semi-humide area, like Transilvania-Romania.

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Fig.1.Yied (kg/ha) at five wheat cultivars, in relathionships with fungicide treatments number (Turda, 2006-2007)

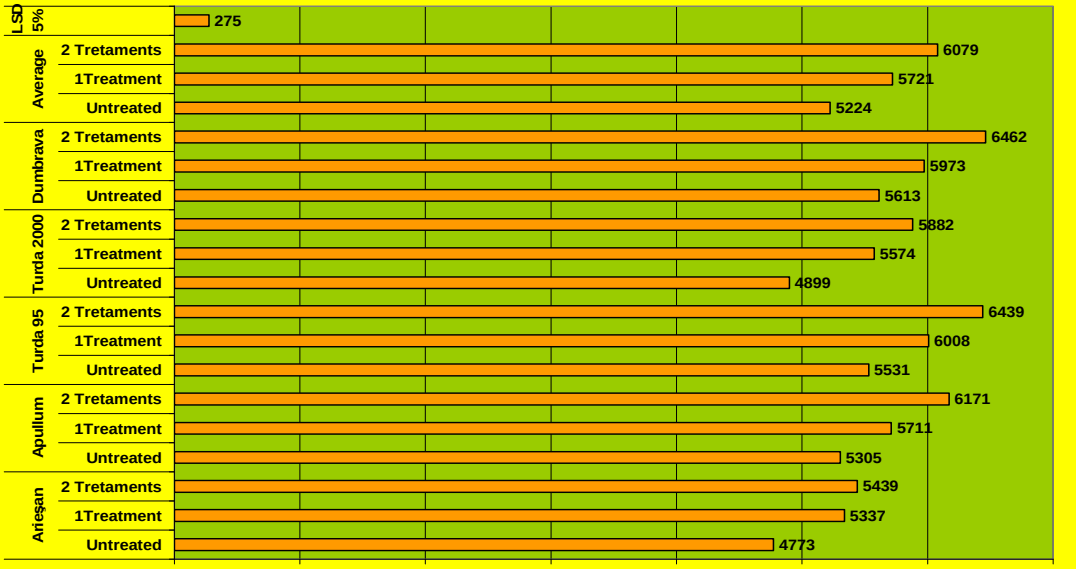


Fig.2.The relation between spikes diseased (%) and grain diseases(%) l at five wheat cultivars testated in three experimental conditions

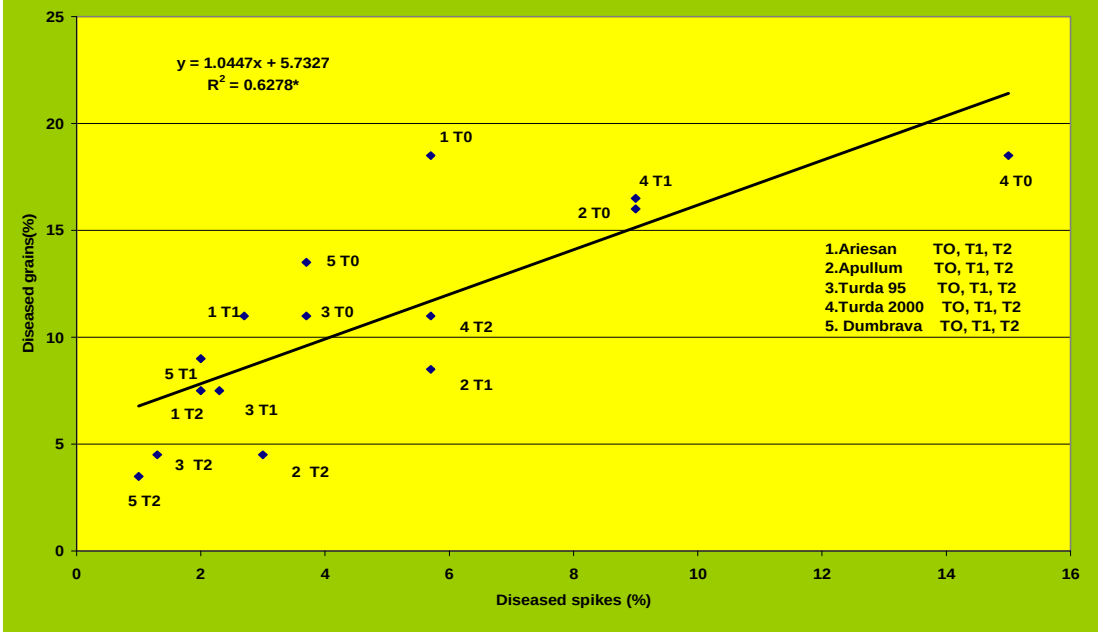
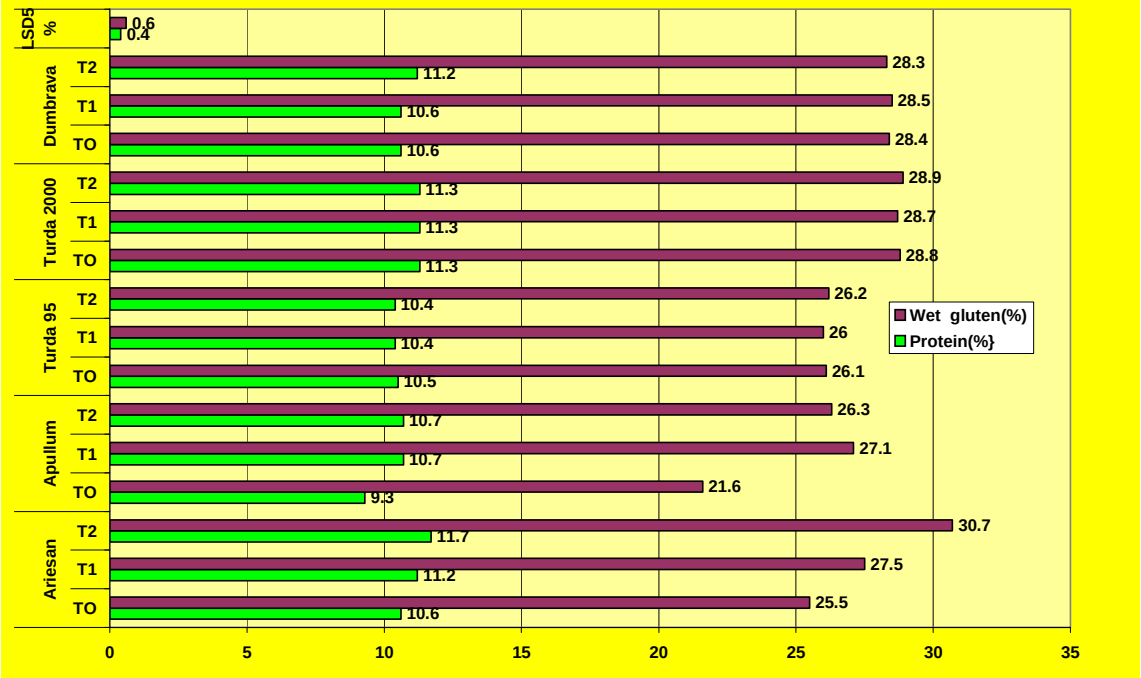


Fig.3.The influence of fungicide tratements number on the protein and wet gluten content at five wheat cultivars (ARSD, 2006-2007)



PREDICTION MODELS FOR DEOXYNIVANENOL ACCUMULATION RISK USING EMPIRICAL AND MECHANISTIC MODELING APPROACHES.

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ABSTRACT

The focus of Fusarium Head Blight (FHB) risk assessment tool (online: www.wheatscab.psu.edu) is to predict the risk of FHB epidemics with greater than 10 percent field severity (FHB index). This level of disease severity is strongly correlated with yield losses from FHB and generally associated with high levels of deoxynivalenol (DON) in harvested grain. However, there are cases when the disease symptom is not a good representation of DON accumulation, and recent research continues to indicate complex relationships between disease symptoms and accumulation of DON. Thus, in the effort of developing FHB forecasting models with better accuracy for disease severity and DON, we have employed mechanistic and empirical modeling approaches, and adjusted our focus to predict both risk of disease and DON accumulation. In the mechanistic modeling approach, simulation models were developed using a computer language STELLA (isee systems, NH) where critical steps in disease development were described by a series of differential equations. In the empirical modeling approach, potential predictor variables, which were based on weather conditions, were examined for their relationship with DON using non-parametric correlation coefficients and binary logistic regression. In both methods, weather information from up to 10 days before and 7 days after 50% anthesis was utilized. Several candidate models have been developed, and preliminary results indicated that accuracy of models for predicting the risk of DON accumulation greater than 2 ppm ranges from 65-75%. The accuracy of models developed using either mechanistic or empirical modeling approaches was similar. Models using weather information during anthesis or early stages of kernel development provided higher accuracy indicating that conducive weather during early stages of kernel development is critical to the prediction of DON contamination. Future research will focus on developing models that use only pre-anthesis weather.

INFLUENCE OF CULTIVAR RESISTANCE, INFECTION TIMING, AND INOCULUM DENSITY ON FHB DEVELOPMENT AND DON ACCUMULATION IN ASYMPTOMATIC WHEAT SPIKES.

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ABSTRACT

Deoxynivalenol (DON) is a mycotoxin that accumulates in wheat spikes infected by *Fusarium graminearum*. In general, DON levels are positively correlated with visual symptoms of Fusarium head blight (FHB), but DON also accumulates in infected wheat spikes that display no symptoms of FHB. These asymptomatic infections may result in DON contamination that exceeds the critical threshold of 2 ppm. This study was conducted to evaluate the effects of host, pathogen, and environmental factors on FHB development and DON accumulation in asymptomatic wheat grain. Three soft red winter wheat cultivars with different levels of resistance to FHB (Cooper, susceptible; Hopewell, moderately susceptible; and Truman, moderately resistant) were inoculated at four different growth stages with different inoculum densities. Inoculations were done at anthesis, one week post-anthesis, two weeks post-anthesis, and three weeks post-anthesis using spore densities of 0, 10,000, 20,000, and 30,000 spore/mL. The inoculum suspension consisted of a 1:1 mixture of macroconidia and ascospores from ten Ohio isolates of *F. graminearum*. The experimental design was a split-split plot, with cultivar as the whole-plot factor and inoculation timing and inoculation density as the sub and sub-sub plot factors, respectively. FHB index (IND) was rated at soft dough, and prior to physiological maturity, asymptomatic spikes were tagged in each plot and later individually harvested. Samples of both symptomatic and asymptomatic grain from each plot were analyzed for DON. IND and DON data were analyzed using Proc Mixed of SAS. The main and interaction effects of cultivar, inoculation timing, and inoculum density on IND, DON, and DON in asymptomatic grain were statistically significant. As expected, for all cultivars, at all inoculum concentrations, DON and IND were greatest when inoculations were done at anthesis. Both disease and DON increased with increasing spore concentrations so that the highest levels of DON and IND were observed for each cultivar when 30,000 spores/mL were applied at anthesis. For both samples from the entire plot (including diseased and diseased-free spikes) and samples of asymptomatic spikes, the critical threshold for DON (2 ppm) was exceeded in Cooper and Hopewell inoculated at anthesis, but not in Truman. Of the cultivars, Hopewell consistently had the highest mean IND and DON contamination. Hopewell was the only cultivar to accumulate DON in excess of 2 ppm in grain harvested from asymptomatic spike inoculated after anthesis. This occurred when inoculation was done one week after anthesis using 30,000 spores/mL. DON accumulation and disease development were influenced by cultivar, infection timing, inoculum density, and resulting interactions, and understanding the dynamics of these effects under different environmental conditions is crucial for predicting DON, particularly in connection to asymptomatic infections.

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INFLUENCE OF WITHIN-PLOT FHB VARIABILITY ON THE RELATIONSHIP BETWEEN FHB AND DON.

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ABSTRACT

Visual disease symptoms and accumulation of the mycotoxin deoxynivalenol (DON) in wheat grain are highly variable in Fusarium head blight (FHB) infected plots and fields. In part, this variability is the result of variability in anthesis and inoculum density within wheat fields. Wheat is most susceptible to infection at anthesis, but the extrusion of anthers occurs at different times for wheat planted in the same field and even for spikelets on the same wheat spike, resulting in heterogeneity of infection and consequently in heterogeneity of visual disease development and DON accumulation. This affects the accuracy with which DON is estimated, and understanding FHB and DON variability is critical for the development of sampling protocols and prediction of DON from FHB. This study was conducted to (1) evaluate the variability of FHB symptoms and DON within artificially inoculated plots, and (2) to explore the relationship between FHB and DON variances and mean FHB and DON. Six soft red winter wheat cultivars with varying levels of resistance to FHB infection (Cooper and 25R47, susceptible; Hopewell and AGI101, moderately susceptible; Truman and McCormick, moderately resistant) were planted in 10-ft x 30-ft plots and inoculated at anthesis using a backpack sprayer with a concentration of 50,000 spores/mL. There were three replicate plots of each cultivar. The inoculum suspension consisted of a 1:1 mixture of macroconidia and ascospores from ten Ohio isolates of *Fusarium graminearum*. At soft dough, 600 spikes (30 clusters with 20 spikes each) were tagged and rated for visual disease symptoms in each plot. After physiological maturity, clusters were harvested separately and tested for DON. Mean index (IND) and DON ranged from 0.91 to 31.58 % and 1.14 to 17.00 ppm, respectively, and index and DON variances among clusters within plots ranged from 1.43 to 140.40 and 0.51 to 39.05, respectively. Mean and variance data for index and DON were log-transformed and subjected to regression analyses using Minitab 15. There were significant linear relationships between log-transformed variances and log-transformed means for both IND and DON. This implies that, on a log-transformed scale, as mean IND and DON increased their respective variances also increased. The rate of increase in log-transformed IND variance with increase in the log of mean index was 1.33, with 96% of the variation in transformed index variance explained by the variation in the log of mean IND. For DON, the rate of increase in log-transformed DON variance with increase in the log of mean DON was 1.41 and 91% of the variation in transformed DON was explained by variation in the log of mean DON.

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INTEGRATED MANAGEMENT OF FHB AND DON IN SMALL GRAIN: 2008 UNIFORM TRIALS.

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OBJECTIVE

Evaluate the combined effect of fungicide and genetic resistance on FHB and DON in small grain.

INTRODUCTION

Fusarium Head Blight (FHB) and its associated toxin (deoxynivalenol, DON) continue to be a concern in every sector of the wheat and barley industries. Chemical, biological, and cultural management approaches, as well as genetic resistance, all contribute to FHB and DON reduction. However, when used individually, none of these approaches have been fully effective against this disease and toxin. The effects of fungicide application, genetic resistance, and residue management are highly variable and strongly influenced by the environment. Under favorable weather conditions, moderately resistant varieties may become infected and DON contamination may exceed critical threshold levels. In the case of fungicides, efficacy varies from one trial to another, with overall mean percent control between 40 and 60% for index and between 30 and 50% for DON (for the most effective fungicides). Fungicides are generally most effective at reducing FHB and DON when used in combination with moderate resistance; however, the magnitude of this interaction effect seems to vary from one cropping system to another. Beginning in 2007, coordinated studies were initiated to evaluate the integrated effects of multiple management strategies on FHB and DON in all classes of small grain. Results from trials conducted during the 2008 growing season are summarized herein.

MATERIALS AND METHODS

Field experiments were conducted to investigate the effects of fungicide and genetic resistance and FHB and DON accumulation under natural conditions. The standard experimental design was a split plot with 3 to 6 replicate blocks. Wheat variety and fungicide application served as the whole-plot and sub-plot factors, respectively. Trials were established both in fields previously planted with *Fusarium graminearum* host (corn and wheat) and non-host (canola and soybean) crops. Plot dimensions and cropping practices varied among trials (see individual trial reports for details). In general, between three and six locally adapted and commonly cultivated cultivars, with different levels of resistance to FHB, were planted. There were two adjacent plots of each cultivar in each block. Sub-plot treatments were established by applying Proline + Folicur (as a tank mix of 3 fl. oz of each, called Proline 3+3) or Prosaro (6.5 fl. oz/A) to one plot of each cultivar (at Feekes' growth stage 10.5.1) and leaving the other plot untreated. A non-ionic surfactant was added to the treatment at a rate of 0.125% v/v, and applications were made using CO_2-pressurized sprayers, equipped with Twinjet XR8002 nozzles or paired XR8001 nozzles, mounted at an angle (30 or 60^o) forward and backward.

In each plot, percent FHB incidence (INC), diseased-head severity (SEV), index (IND; also known as field or plot severity), and *Fusarium*-damaged kernels (FDK) were quantified. Plots were harvested and yield and test weight determined. Milled grain samples from

each plot were sent to one of the USWBSI-funded DON Testing Laboratories for DON analysis.

Analysis of variance (linear mixed model) was used to evaluate the effects of cultivar, fungicide and their interaction on FHB intensity and DON content.

RESULTS

A total of 23 trials were conducted in nine states (Kentucky, Louisiana, Minnesota, Missouri, Nebraska, New York, North Dakota, Ohio, and South Dakota). FHB intensity and DON varied from one location to another, with some trials having zero or nominal disease development and DON contamination. Trials with zero or nominal levels of disease (New York, Ohio, Minnesota, and Kentucky), were not included in this summary.

Louisiana. FHB intensity was very low in the trial conducted at Crowley, LA. Mean index and incidence ranged from 0.6 to 4.8 and 8.8 to 25.0%, respectively. Proline and Prosaro treated plots had significantly lower levels of disease than the untreated check. Averaged across all cultivars, mean index was 1.9% for Proline and 2.2% for Prosaro, compared to 3.4% for the Check.

Missouri. Two trials were conducted in Missouri to evaluate fungicide and variety effects on FHB and DON. In the first, plots were planted no-till into corn residue and in the second, no-till into soybean residue. *In the trial planted into corn stubble,* mean index, incidence and DON ranged from 2.0 to 29%, 10.0 to 47.5% and 3.7 to 18.7 ppm (Fig. 1), respectively. For index, the effects of fungicide and cultivar, but not the interaction, were statistically significant. Averaged across all cultivars, Proline 3+3 resulted in a significant reduction in index (26%) relative to the untreated check. The moderately resistant cultivars, Bess and Roane, with mean index of 2.6% percent, had significantly lower disease than the susceptible cultivar Elkhart, which had a mean index of 26.7%. Averaged across fungicide treatments, relative to Elkhart, Bess reduced index by 90%. Using the untreated, susceptible check as reference for comparison, the combination of fungicide and moderate resistance (Proline 3+3 applied to Bess) also reduced index by 90%. For DON, all main and interaction effects were statistically significant. The untreated, susceptible check (Elkhart without fungicide) with mean DON of 18.7 ppm had significantly higher DON contamination that the fungicide-treated resistant cultivars (Roane and Bess + Proline 3+3). The Bess + Fungicide treatment combination led to a 80% reduction in DON relative to the untreated, susceptible check.

For the trial planted into soybean stubble, mean index, incidence, and DON ranged from 0.7 to 18.9%, 5.8 to 32.5%, and 0.6 to 4.7 ppm (Fig. 1), respectively. For both index and DON, all main and interaction effects were statistically significant. Again, the Bess + Proline and Roane + Proline treatment combinations had significantly lower levels of index and DON than the untreated susceptible check (Elkhart without fungicide). The resistance x fungicide treatment combination led to a 85% reduction in DON and a 93% reduction in index relative the untreated susceptible check.

Nebraska. Mean index ranged from 14.7 to 33.4% and mean DON from 8.4 to 15.0 ppm. Only the main effect of cultivar was statistically significant for index, with mean values of 15.6, 32.4 and 24.9% for Harry, Jagalene and Pioneer 21R37, respectively. The difference in index between Harry and Jagalene, 16.8%, represented a 52% reduction in index due to difference in susceptibility. For DON, the main effects of fungicide and cultivar were statistically significant. Pioneer 21R37, with an average DON of 9.2 ppm, had significantly lower DON contamination than Harry and Jagalene, both with a mean DON contamination of 13.6 ppm.

North Dakota. *Two-row Barley.* For the trial planted into canola residue, mean index and DON were low, ranging from 1.4 to 5.2% for index and 0.1 to 1.3 ppm for DON (Fig. 1). However, incidence exceeded 60% in some treatment combinations. Based on index, the effects of cultivar and fungicide, but not the interaction, were statistically significant. Conlon and Rawson and Eslick and Merit had the lowest and highest levels of disease, respectively. A similar trend was observed in the trial planted into the residue of HRSW.

The overall level of disease was also low in the latter trial, with mean index between 1.3 and 5.5% and incidence between 43 and 74%. Mean DON did not exceed 2 ppm.

Six-row Barley. For the trial planted into canola residue, mean index ranged from 6 to 13.3% and mean incidence from 88 to 100%. Only the main effect of cultivar was statistically significant. Averaged across fungicide treatments, Legacy with a mean index of 6.2% and ND20448 with a mean index of 13.2% had the lowest and highest levels of disease, respectively. Similarly, only cultivar had a significant effect on index in the trial planted into residue of HRSW. ND20448 and Tradition with mean index of 15 and 12%, respectively, had the highest levels of disease, whereas Legacy had the lowest, with mean index of 6.5%.

Durum. For both trials (wheat planted after canola and wheat planted after wheat), the effects of fungicide, cultivar, and their interaction on FHB intensity were not statistically significant. This was probably due to the fact that the variability among replicates of the same treatment was very high in both trials.

CONCLUSIONS

Percent control was estimated for a few of the trials to evaluate the efficacy of individual treatments and treatment combinations against index and DON. Trials with nominal levels of disease and DON were not included in this calculation because percent control tends to be highly variable at low index and DON levels. In general, moderately resistant variety x fungicide treatment combination resulted in the highest percent control. Comparing trials with the same treatment combinations, but planted into different types of crop residue, none-host crop + moderately resistant variety + fungicide generally resulted in higher percent control than host crop + susceptible variety + without fungicide.

ACKNOWLEDGEMENT

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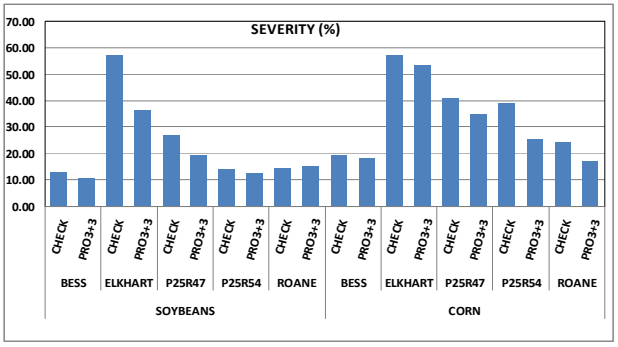

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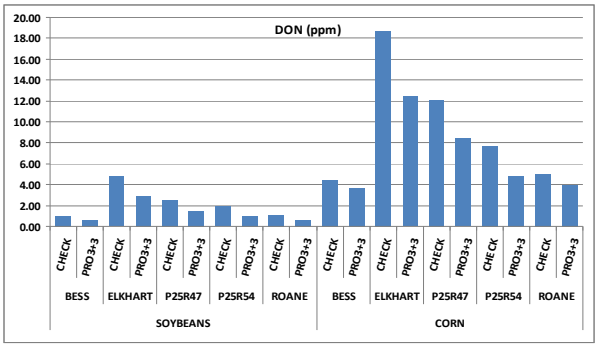

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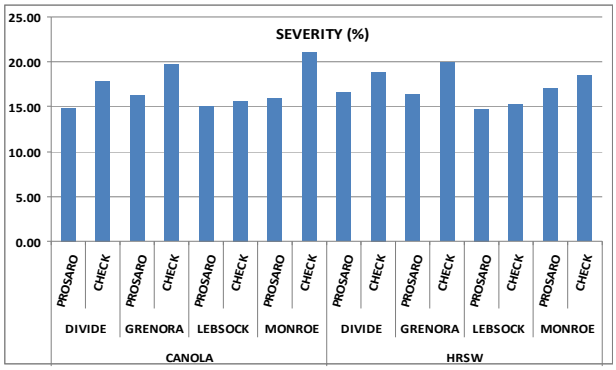
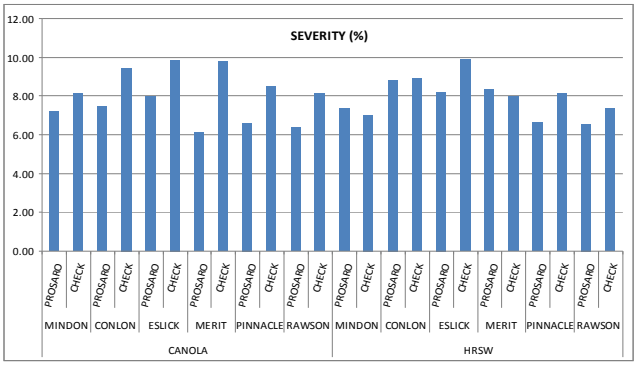


North Dakota – Durum wheat



North Dakota – Two-row barley

Fig.1. Graphs showing side-by-side comparisons of FHB and DON response to fungicide, cultivar and fungicide x cultivar combinations.

INTEGRATING FUNGICIDE AND VARIETY RESISTANCE TO MANAGE FHB/DON IN WHEAT IN DIFFERENT CROPPING SYSTEMS.

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ABSTRACT

Fusarium head blight (FHB) and its associated toxins (especially deoxynivalenol, DON) continue to be a concern in all major wheat-growing regions of the world, causing substantial yield and quality losses. Through years of research funded by the US Wheat and Barley Scab Initiative (USWBSI), several chemical, biological, and cultural management approaches, as well as genetic resistance, have been tested and shown to contribute to FHB and DON reduction. However, when used individually, none of these approaches have been fully effective against FHB and DON. The effects of fungicide, genetic resistance, and residue management through crop rotation and tillage are highly variable and strongly influenced by environment conditions. Beginning in 2007, coordinated studies were initiated to evaluate the integrated effects of multiple management strategies on FHB and DON in all classes of small grain. In particular, studies are being conducted to determine: 1) whether combining fungicide and genetic resistance results in a greater percent reduction of FHB and DON than fungicide or resistance alone and 2) whether the relative magnitude of the reduction varies with wheat class, weather condition, and cropping system. Preliminary results indicate that in general, when FHB and DON levels are moderate to high, combining fungicide and genetic resistance leads to an overall reduction in FHB and DON that is greater than that achieved by either approach used alone. For the studies evaluated thus far, combining the most resistant cultivar with the most effective fungicide results in the lowest levels of FHB and DON in all wheat classes and cropping systems, with percent reduction relative to the untreated, susceptible check as high as 90% for FHB index and 65% for DON, in some cases. However, for a given cultivar and fungicide, the magnitude of the reduction varies from one cropping system to another. Further analyses will be conducted to determine the significance and consistency of the difference in fungicide x resistance interaction effects among cropping systems in all wheat classes.

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2008 UNIFORM FUNGICIDE PERFORMANCE TRIALS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA.

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ABSTRACT

Fusarium head blight (FHB – scab) remains a serious concern for wheat and barley producers in South Dakota. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. One hard red winter wheat cultivar 'Wesley' was planted at two South Dakota locations (Brookings and South Shore/Watertown). Two hard red spring wheat cultivars, 'Briggs' and 'Oxen', were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown) and Robust barley was planted at Brookings. Studies at two of these sites were conducted under ambient conditions. At the Brookings site, both the barley and the spring wheat trials received supplemental mist irrigation. Trial treatments from the Uniform Fungicide Trial treatments list for the suppression of FHB included an untreated check, Folicur (tebuconazole) applied at 4.0 fl oz/A, Proline (prothioconazole) applied at 5 fl oz/A, Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A, Caramba (metconazole) applied at 10 fl oz/A, Caramba (metconazole) applied at 14 fl oz/A and Topguard (flutriafol) applied at 14 fl oz/A. All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. Spring wheat trials were planted in a factorial randomized complete block design with six replications. The winter wheat and the barley trial included four replications. Trial treatments were applied at anthesis (Feekes growth stage 10.51). The spring wheat and barley plots at the Brookings location were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied from 8:00 pm until 8:00 am each day for ten days two weeks following anthesis. Other sites had natural inoculum from corn stalk residue and natural moisture conditions. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity. Samples were collected for Fusarium damaged kernels (FDK), deoxynivalenol (DON), grain yield, and test weight. FHB data in the winter wheat trials were non-significant except for FDK (both locations). Yield differences in the winter wheat trial at Brookings may be related to the highly significant differences in leaf rust control. Spring wheat at all locations had non-significant FHB incidence, however, one location (Groton) showed significant differences in FHB Severity and Disease Index. Yield was also significant in two locations in the spring wheat trial but differences in leaf rust control may have contributed to yield differences as leaf rust developed late in the plots. Significant differences for FHB Incidence and Disease Index occurred in the barley trial; however, FHB Severity and yield were non-significant. Total leaf disease pressure was very significant, as was late season leaf rust pressure.

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2008 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA.

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ABSTRACT

Fusarium head blight (FHB – scab) remains a serious concern for wheat and barley producers in South Dakota. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases under SD conditions. Briggs hard red spring wheat and Robust barley were planted at Brookings, South Dakota. Trial treatments included an untreated check; Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A; TrigoCor 1448 (*Bacillus* sp.) from Cornell University, Ithaca, NY; and TrigoCor 1448 + Prosaro coapplied; Taegro (*Bacillus* sp.) from Novozymes; and Taegro + Prosaro coapplied; 1BA (*Bacillus subtilus*) from South Dakota State University, Brookings, SD; 1BA + Prosaro coapplied. The treatments were applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied from 8:00 pm until 8:00 am each day for ten days following anthesis. Twenty-one days following treatment, 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).

Similar to 2007, the dry weather at flowering in 2008 affected widespread disease development even with an amended environment. In the data analysis, the assessments of FHB Incidence are significant in the barley and spring wheat studies. However, FHB Severity, FHB Disease Index and the yield differences were non-significant.

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COMPARING THE EFFECTS OF MACROCONIDIA AND ASCOSPORES OF *GIBBERELLA ZEAE* ON FUSARIUM HEAD BLIGHT DEVELOPMENT IN WHEAT.

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ABSTRACT

Gibberella zeae (Schwein.) Petch (anamorph: *Fusarium graminearum*) is the primary causal agent of Fusarium Head Blight (FHB) of Wheat in North America. FHB is incited by both sexually produced ascospores and asexually produced macroconidia, with ascospores often considered the epidemiologically important inoculum for FHB development. However, in the field, both ascospores and macroconidia may be equally abundant at the time of crop anthesis and likely contribute equally to FHB development. The objective of this study was to compare the effects of inoculum type and density on FHB development under controlled conditions. During the summer of 2008, SRWW cultivar Cooper (susceptible) was grown under greenhouse conditions at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. Macroconidia and ascospores were harvested from mung bean agar and carrot agar, respectively, and used to inoculate plants at anthesis. Four different inoculum densities (5, 10, 15 and 20 $x10^5$ spores per mL) were prepared for each spore type, and separate groups of approximately 20 plants were spray-inoculated with each spore type x density combination. The experimental design was a split-plot with spore type as whole plot and inoculum density as sub-plot. The experiment was repeated five times, with each time considered a block. After inoculation, plants were incubated in a mist chamber for 24 h and then moved to a greenhouse where FHB development was monitored. Disease incidence was assessed at 24-hour intervals for 21 days after inoculation (DAI) as the proportion of plants with symptoms of FHB. Macroconidia consistently resulted in numerically higher FHB incidence than ascospores at all inoculum densities, at every assessment time. For both spore types, between 5 and 7 DAI, there was a linear increase in incidence with inoculum density, with similar regression slopes for both spore types. However, the intercept (high of the regression line) was greater for macroconidia than ascospore. Depending on the inoculum density, maximum incidence ranged from 93 to 100% for macroconidia and from 80 to 95% for ascospores. Maximum incidence was reached earlier on macroconidia- than ascospore-inoculated plants. At 14 and 21 DAI, incidence was significantly higher on plants inoculated with macroconidia than on those inoculated with ascospore at the two lower inoculum densities, but not at the higher densities. Under the conditions of this study, based on FHB incidence, macroconidia appear to be more efficient at inciting FHB than ascospores. Our results showed that fewer macroconidia than ascospores were required to achieve a similar level of incidence; for a given inoculum density, macroconidia resulted in higher mean incidence than ascospores; and at lower inoculum densities, a given level of incidence was reached earlier for inoculations done with macroconidia than with ascospores.

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EVALUATION OF PROTOTYPE COMMERCIAL MEDIA FOR THE PRODUCTION OF FUSARIUM HEAD BLIGHT ANTAGONIST *CRYPTOCOCCUS FLAVESCENS* OH 182.9.

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OBJECTIVES

1.) Develop a prototype commercial medium for liquid culture production of *Cryptococcus flavescens* OH 182.9 that utilizes inexpensive industrial grade sources of carbon and nitrogen and 2.) Test cells of *C. flavescens* OH 182.9 produced in the selected commercially feasible medium for equivalence in quantity and Fusarium head blight (FHB) biocontrol efficacy to cells produced in the laboratory grade medium SDCL.

INTRODUCTION

While research has shown disease forecasting, resistant varieties, cultural controls, fungicides, and biocontrol agents to be incrementally useful, as individual strategies, in reducing FHB (Dill-Macky and Jones, 2000; Schisler et al., 2002; Beyer et al., 2006; Paul et al., 2007), the integration of multiple control measures offers the best opportunity to substantially and consistently reduce FHB. Using biological control as part of the integrated management of FHB is understudied yet has considerable potential for significantly contributing to the reduction of FHB and DON (Milus et al., 2001). However, the development of a cost effective, commercially feasible medium for producing *C. flavescens* OH 182.9 is a prerequisite step for the inclusion of this agent in the integrated management of FHB.

To date, biomass production of OH 182.9 has been carried out in a laboratory grade medium (SDCL, Slininger et al. 2007) containing a highly purified protein digest product. Using this medium, we have completed crucial studies on optimizing medium carbon loading and C:N ratio to enhance the production and biocontrol efficacy of OH 182.9 biomass. However, the medium is impractical for commercial use due to the high cost of the protein digest component.

MATERIALS AND METHODS

Media with carbon and nitrogen supplied in large part by one of two different cotton seed-derived industrial protein products (Proflo and Pharmamedia, Traders Protein, Memphis, TN) or an industrial source of casein digest (Hy-Case Amino, Kerry Bio-Science, Norwich, NY) were developed using carbon loading and C:N ratios that approximated those of SDCL. Cell production studies were conducted both in shake flasks and benchtop fermentors. For shake flask studies, log-growth phase cells of strain OH 182.9 were transferred to 250 ml Erlenmeyer flasks containing 50 mls of Proflo, Pharmamedia, Hy-Case or SDCL media. Flasks were inoculated to 0.1 OD (A_{620}), incubated at 25°C and 250 rpm with a 2.5 cm stroke for 48 hours, and harvested for determination of CFU/ml and/or use of OH 182.9 biomass in greenhouse studies. In separate replicated studies, cells of OH 182.9 were produced in bench-top fermentors (B Braun Biostat B fermentors, B. Braun Biotech Inc., Allentown, PA) that were charged with 1.5 L of one of the four media types. Antifoam 204 (Sigma, St Louis, MO) was added prior to medium sterilization and cultures were not pH controlled after inoculation at pH 7.0. Log-growth cells of OH 182.9 served as a 5% seed inoculum. Fermentors were operated at 25°C, 1.5L/min aeration and 200 rpm agitation. After dissolved oxygen had recovered to saturation at 48 hours, cells were harvested for determination of CFU/ml and/or use in greenhouse or field trials. Data was analyzed using

one-way ANOVA and Fisher's Protected LSD (Pd"0.05).

Hard red spring wheat (cultivar Norm) was grown in plant growth chambers prior to conducting plant bioassays on greenhouse benches. Conidial inoculum of *Fusarium graminearum* isolate Z-3639 was produced on clarified V8 juice agar under 12 h/day fluorescent light for 7 days at 24°C. At wheat anthesis, one-quarter-strength suspensions of OH 182.9 biomass from the four media were individually misted onto approximately 14 wheat heads per treatment followed immediately by a mist application of a conidial suspension (2×10^4 conidia/ml). Heads treated with water followed by the conidial suspension of *F. graminearum* isolate Z-3639 served as the control. Plants were placed in humidity tents for 3 days, scored for disease severity after 16 days, and data analyzed using one-way ANOVA and Fisher's Protected LSD ($P \leq 0.05$). The reported means are results from pooled replicate experiments.

Field trials were conducted in Peoria, IL and in Wooster, OH in 2008. Biomass of OH 182.9 from the four media was applied to soft red winter wheat cultivar Pioneer Brand 2545 at the beginning of wheat flowering at approximately 85% strength and 20 gal/acre. Corn kernels colonized by *F. graminearum* were scattered through plots (~25-40 kernels/m^2) two to three weeks prior to wheat flowering and mist irrigation was provided periodically for approximately two weeks after treatment application. Heads were scored for disease incidence and severity 20-25 days after treatment using a 0-100% scale. Randomized complete block designs were used in all field trials. Analysis of variance and the Bonferroni mean comparison test ($P \leq 0.05$) were used to compare treatment means.

RESULTS AND DISCUSSION

In shake flask studies, higher cell counts were obtained in SDCL and Hy-Case media than in the Proflo and Pharma media (Table 1). In general, higher cell counts were obtained in the bench top fermentors with the SDCL medium supporting higher counts than the other three media (Table 1). Though OH 182.9 cells tended to reduce FHB severity and incidence when produced in both shake flasks and fermentors, cell production media did not have a significant effect on efficacy in greenhouse trials (Table 2).

Because the Hy-Case medium tended to support OH 182.9 cell production that was nearly equivalent to SDCL (Table 1), these two media were utilized to produce biomass for field trials. Treatment means for DS and DON did not differ significantly from the control in either field location (Table 3). Treatment effects with cells of OH 182.9 produced in the Hy-Case medium were variable with DON being reduced by 41% (NSD) compared to controls in Peoria, IL but having no effect on DON in Wooster, OH (Table 3).

Compared to the SDCL medium, the Hy-Case prototype commercial medium supported the production of nearly equivalent numbers of OH 182.9 cells that were also comparable in biocontrol efficacy to those produced in the SDCL medium. Though the Hy-Case medium was shown to be the most commercially feasible medium of those tested, current work on isolating variants of OH 182.9 with enhanced efficacy may require adjustments of the Hy-Case medium to meet the altered nutritional requirements of improved OH 182.9 strains.

ACKNOWLEDGEMENT

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Table 1. Shake flask and bench-top fermentor production of cells of Fusarium head blight antagonist *Cryptococcus flavescens* OH 182.9 in various prototypes of commercial production media[a,b]

	CFU/ml at 48 h Harvest[c]	
Medium	Shake Flask[d]	Bench-top Fermentor[e]
SDCL	2.80×10^8 A	5.36×10^8 A
Hy-Case	2.38×10^8 A	3.75×10^8 B
Proflo	1.79×10^8 B	3.54×10^8 B
Pharma	1.55×10^8 B	3.14×10^8 B
P value	0.004	0.001

[a] Within a column, values not followed by the same letter are significantly different (Fisher's Protected LSD, $P \leq 0.05$). Average results from two experiments are presented.
[b] See "Materials and Methods" for descriptions of antagonist production media.
[c] CFU/ml= Colony forming units per milliliter.
[d] 250 ml flasks charged with 50 ml growth medium per flask, 48 h cell harvest
[e] Bench-top fermentor vessel capacity of 2 liters charged with 1 liter of medium, 48 h cell harvest.

Table 2. Greenhouse assay of the influence of *Cryptococcus flavescens* OH 182.9 on Fusarium head blight when antagonist cells were produced in differing prototypes of commercially feasible production media[a,b,c]

Treatment	Cell Production Vessel			
	Shake Flask		Bench-top Fermentor	
	DS (%)	INC (%)	DS (%)	INC (%)
OH 182.9 in SDCL	35 A	61 A	60 A	72 A
OH 182.9 Hy-Case	37 A	64 A	68 A	85 A
OH 182.9 Proflo	39 A	62 A	80 A	88 A
OH 182.9 Pharma	56 A	76 A	60 A	69 A
F. graminearum Control	51 A	69 A	75 A	88 A
P value	0.19	0.69	0.09	0.06

[a]Within a column, values not followed by the same letter are significantly different (Fisher's Protected LSD, P≤0.05). Average results from 2 experiments are presented.
[b]See "Materials and Methods" for descriptions of antagonist production media.
[c]DS=Disease severity, INC=Disease incidence

Table 3. Influence of *Cryptococcus flavescens* OH 182.9 on Fusarium head blight and DON in Peoria, IL and Wooster, OH field trials on Pioneer Brand 2545 wheat when antagonist cells were produced in differing prototypes of commercially feasible production media[a,b,c]

Treatment	Field Trial Location			
	Peoria, IL		Wooster, OH	
	DS (%)	DON (ppm)	DS (%)	DON (ppm)
Control	8.0 A	11.8 A	11.9 A	8.1 A
OH 182.9 SDCL	7.6 A	13.6 A	12.6 A	6.8 A
OH 182.9 Hy-Case	5.5 A	6.9 A	11.6 A	8.1 A

[a]Within a column, values not followed by the same letter are significantly different (Bonferroni mean comparison test, P≤0.05). Average results from 2 replicate experiments are presented.
[b]See "Materials and Methods" for descriptions of antagonist production media.
[c]DS=Disease severity, INC=Disease incidence

FUNGICIDES CONTROL OF FUSARIUM HEAD BLIGHT SYMPTOMS CAUSED BY 15-ADON AND 3-ADON *FUSARIUM GRAMINEARUM* ISOLATES IN INOCULATED AND MISTED WHEAT PLOTS IN ONTARIO, CANADA.

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INTRODUCTION

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 may also be produced. A shift in the presence of two *F. graminearum* chemotypes, 15-ADON and 3-ADON was identified in North America (Ward et al. 2008). In Ontario, Canada, *F. graminearum* isolates collected from 2004 to 2007 from winter wheat were mainly of the 15-ADON chemotype (Tamburic-Ilincic et al. 2006, Tamburic-Ilincic et al. 2008). The shift of chemotypes 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, Canada, while PROSARO may be newly registered for commercial application and has active ingredients from both FOLICUR and PROLINE. The objective of this study was to investigate the effect of FOLICUR, PROLINE and PROSARO on FHB symptoms in two spring wheat cultivars after inoculation with 15-ADON and 3-ADON *Fusarium graminearum* isolates in inoculated and misted wheat plots.

MATERIALS AND METHODS

Roblin (FHB highly susceptible-HS) and Alsen (FHB moderately resistant-MR) spring wheat were planted in mid April 2008 in Ridgetown, Ontario. The experiment was designed as a split plot arranged in a randomized complete block design, with blocks replicated three times. Six isolates of *F. graminearum* (three 15-ADON and three 3-ADON) were used as treatments. The plots were fertilized and maintained using provincial recommendations. The fungicide treatments consisted of FOLICUR, PROLINE, PROSARO, and water that were applied at approximately 50% anthesis for each cultivar (Zadoks 65). Two days after the fungicides were applied, plots were spray-inoculated with a suspension of macroconidia of a single *Fusarium graminearum* isolate at 50,000 spores mL^{-1}. The suspension was produced in liquid shake culture using modified Bilay's medium. Plots were misted with approximately 7.5 mm of water daily for 2 weeks after inoculation. Each variety was assessed for visual symptoms at the early dough stage (Zadoks 83) by randomly selecting 20 heads for disease incidence and severity. Disease levels were calculated as Fusarium head blight index (FHBI), which was the product of the percent heads infected and the percent spikelets infected, divided by 100.

RESULTS AND DISCUSSIONS

A significant interaction between fungicides and cultivars was reported for FHB index (Table 1). FHBI (%) was lower in Alsen cultivar (FHB-MR) than in Roblin (FHB-HS) after application of any fungicides or in control (Figure 1). The lowest FHB index in Alsen was recorded after PROSARO application (Figure 1). In the present study, there was no interaction between fungicides and isolates, or between cultivars and isolates, on FHBI (Table 1). A high variation in FHB index was detected among isolates of both 15-ADON chemotype and 3-ADON chemotype (Figure 2), suggesting that isolates ability to produce FHB symptoms might be more important than chemotypes. In the present study, the fungicides reduced FHB index after

inoculation with *F. graminearum* 15-ADON or 3-ADON isolates compared to control, except FOLICUR application after inoculation with 3-ADON isolate 2 (Figure 3). More *F. graminearum* 3-ADON isolates need to be tested to verify this.

ACKNOWLEDGEMENTS

Financially supported by Bayer Crop Science. Technical assistance by Ashley Muckle and Mike Holzworth is gratefully acknowledged.

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Zadoks, J. C., Chang, T. T., and Konznak, C. F. 1974: A decimal code for the growth stages of cereals. Weed Research. 14:415-421.

Table 1. Analysis of variance for the effect of Fungicides, Cultivars, Isolates and their interactions on Fusarium head blight index (FHB %).

SOURCE	DF	MEAN SQUARE	F	Prob (F)
Fungicides	3	420.019	7.89	0.0001
Cultivars	1	9635.057	181.13	0.0001
Fungicides x Cultivars	3	205.880	3.87	0.0117
Isolates	5	534.512	10.04	0.0001
Fungicides x Isolates	15	24.162	0.45	0.957
Cultivars x Isolates	5	97.450	1.83	0.114
Fungicides x Cultivars x Isolates	15	33.651	0.63	0.841
Error	94	53.192		

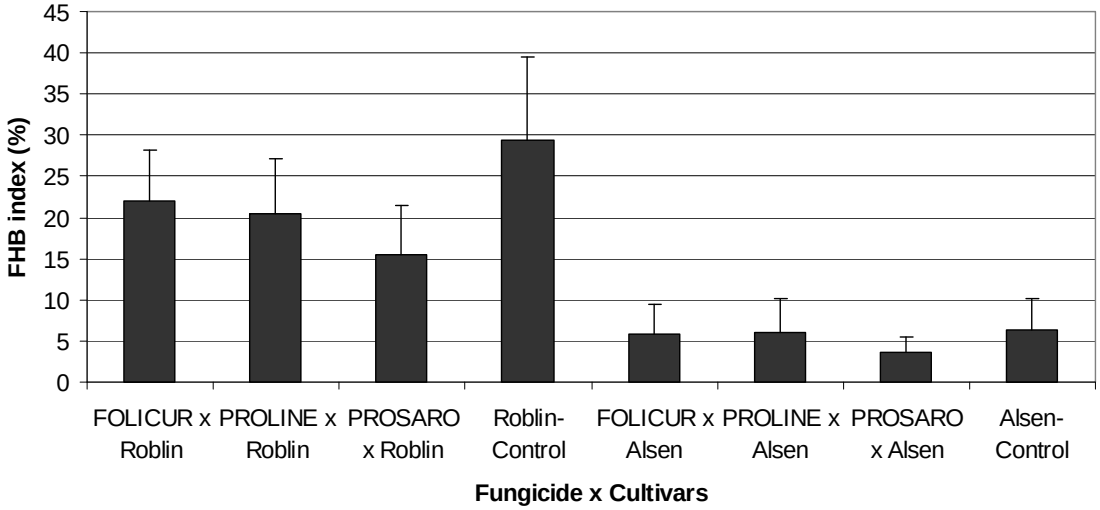


Figure 1. The effect of fungicides x cultivars interaction (+ standard deviation) on Fusarium head blight index (FHB %).

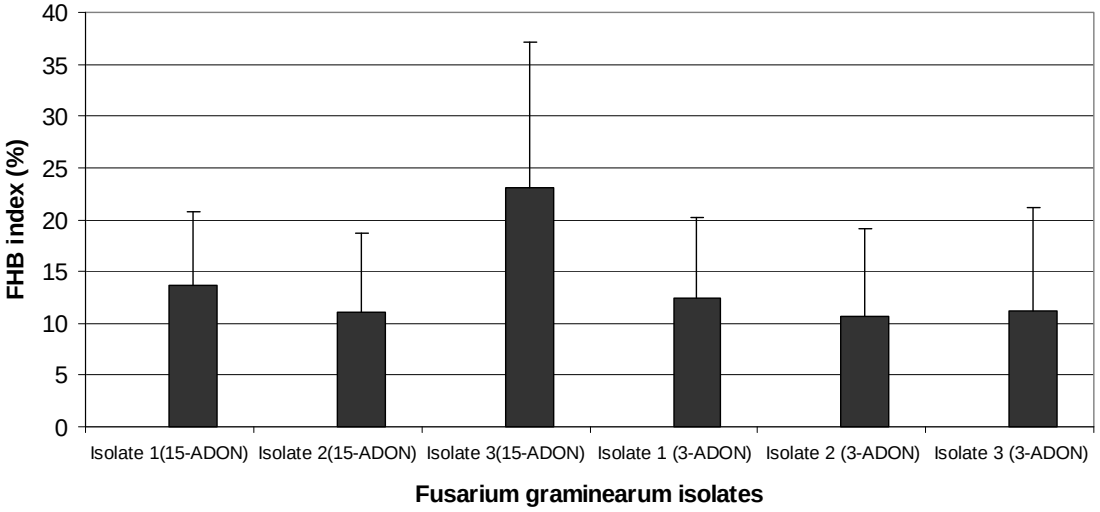
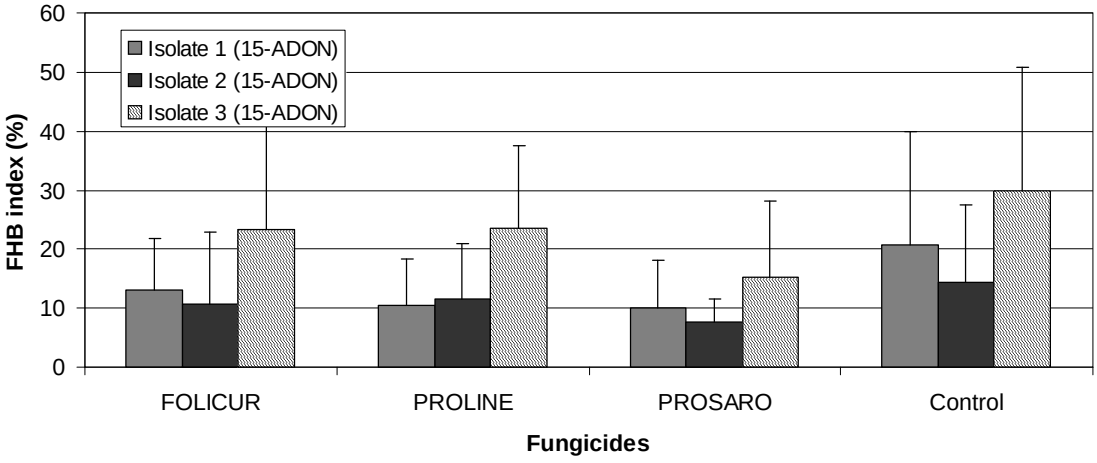
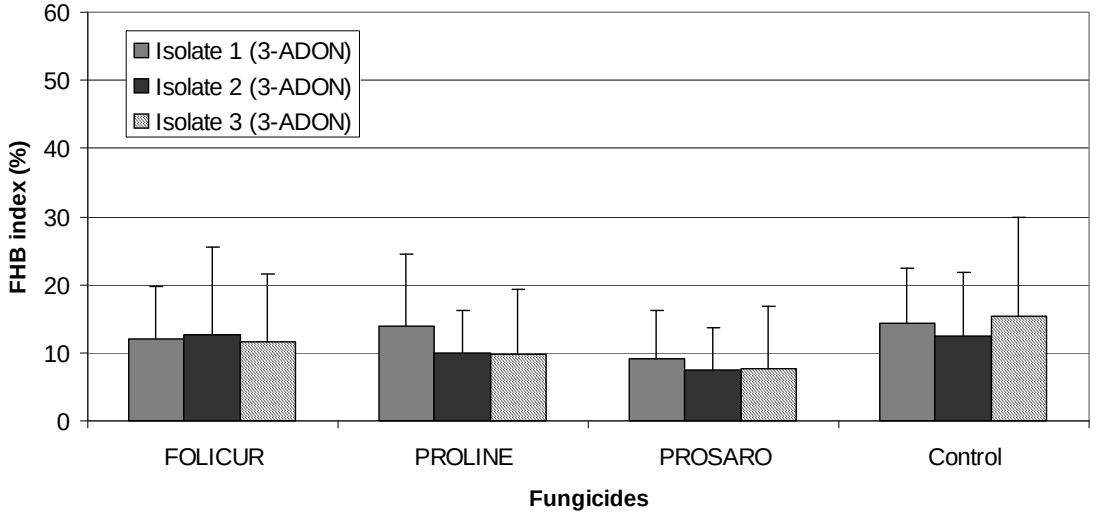


Figure 2. The effect of *Fusarium graminearum* 15-ADON isolates and 3-ADON isolates (+ standard deviation) on Fusarium head blight index (FHB %).



a)



b)

Figure 3. The effect of fungicides on Fusarium head blight (FHB) index (%) in wheat after inoculation with a) *F. graminearum* 15-ADON isolates and b) *F. graminearum* 3-ADON isolates (+ standard deviation).

EVALUATION OF INTEGRATED FHB MANAGEMENT METHODS UNDER LOW DISEASE ENVIRONMENTS IN NEW YORK.

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OBJECTIVE

To evaluate the individual and interactive effects of 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 four 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 multistate, multi-year, coordinated field study. In New York during 2007 and 2008, we conducted a total of four 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 four experimental wheat environments were characterized by the planting of winter wheat 1) following soybean harvest and moldboard plowing in late September 2006; 2) no-till into corn residue in early November 2006; 3) no-till into soybean residue in late September 2007; and 4) no-till into corn residue in early October 2007. 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 10 ft wide commercial grain drills. Sprayed areas in each subplot were 8 ft wide by 20 ft long. Spray treatments applied at Feekes GS10.5.1 were 1) non-sprayed; 2) 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° 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 laboratories of Dr. Dong in 2007 and Dr. Schmale in 2008.

RESULTS AND DISCUSSION

Though varying in yield potential, the four experimental environments in New York had in common very low pressure from FHB and foliar diseases due to dry conditions from pre-flowering through early grain filling periods. FHB incidences at soft dough were at 1% or less, yet detectable levels of DON were recorded for some plots in three of the experiments as a result of infection late during grain development. In the virtual absence of foliar/spike disease, no foliar spray treatment (fungicide, biological control, or combination) alone or in interaction with cultivar had a significant effect on yield, test weight, or DON content of grain. Leaf rust on the flag leaves in the late-planted experiment in 2008 was nearly eliminated by application of Prosaro, yet there was no significant effect of this late rust control on yield. A continuing challenge to the implementation of integrated management strategies is that some of the highest yielding regional cultivars are not resistant to FHB. Such is the case with the soft white winter wheat cultivar 'Caledonia' which

out-yielded the moderately resistant cultivars in three of the four 'low disease' experiments in New York (Table 1). There was a marginally significant effect of cultivar on DON in two experiments though the contrast is between red and white cultivars rather than susceptible vs. moderate resistance to FHB within market class (Table 2). It is hard to extrapolate from these results, as the levels of toxin were quite low.

ACKNOWLEDGEMENT

We thank Drs. Dong and Schmale and staffs for their expert analysis of DON in our grain samples. This material is based upon work supported in part by the U.S. Department of Agriculture under agreement No. 59-070-4-093. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

DISCLAIMER

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

Table 1. Main effect of wheat cultivar on grain yield under four low disease environments at Aurora, NY.

	Adjusted grain yield (bu/A)				
Wheat cultivar:	Expt 1 - 2007	Expt 2 – 2007	Expt 3 – 2008	Expt 4 – 2008	
Planted after >	Tilled soy	No-till corn	No-till soy	No-till corn	Average
Caledonia (Susc., SWW)	70.0	53.4	100.1	85.3	77.2
Freedom (Susc., SRW)	63.4	56.9	82.8	78.6	70.4
Jensen (Mod. Res., SWW)	60.8	50.5	78.7	71.9	65.5
Truman (Mod. Res., SRW)	61.3	50.0	72.1	69.5	63.2
LSD (*P*=0.05)	4.6	NS	3.9	4.6	

Table 2. Main effect of wheat cultivar on deoxynivalenol contamination under four low disease environments at Aurora, NY.

	Contamination of grain by DON (ppm)				
Wheat cultivar:	Expt 1 - 2007	Expt 2 – 2007	Expt 3 – 2008	Expt 4 – 2008	
Planted after >	Tilled soy	No-till corn	No-till soy	No-till corn	Average
Caledonia (Susc., SWW)	0.01	0.26	0.16	0.40	0.21
Freedom (Susc., SRW)	0.00	0.02	0.17	0.16	0.09
Jensen (Mod. Res., SWW)	0.01	0.28	0.20	0.36	0.21
Truman (Mod. Res., SRW)	0.01	0.03	0.20	0.10	0.08
LSD (*P*=0.05)	NS	0.19	NS	0.20	

EFFECTS OF FUNGICIDE TREATMENTS AND CULTIVARS ON FHB AND DON 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 2007 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 2008, corn kernels colonized by *F. graminearum* were applied to the soil surface in the wheat plots one week before flowering at a rate of 50 g/m^2. There also was plenty of natural inoculum. 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 20ft. 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 full heading. Fungicide was applied on June 3. Disease severity and incidence were assessed on 50 randomly selected heads in each plot on June 14, June 20, and June 30 and used to calculate disease index. Severity of Septoria leaf blotch, the predominant foliar disease, was assessed on June 14. 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. Excessively wet weather favored development of severe FHB. Differences in disease index among cultivars were highly significant (P <0.0001) on all three rating dates. Disease index in Harry was lower ($P = 0.05$) than that in either Jagalene or 2137. Jagalene had a higher disease index than 2137, but this difference was mostly non-significant at $P = 0.05$. Fungicide application did not reduce FHB index. This may have been due to high disease pressure. Disease index on June 14 was 14, 11, and 6% for Jagalene, 2137, and Harry, respectively, in the non-sprayed treatment and 13, 10, and 5% for Jagalene, 2137, and Harry, respectively, in the Prosaro treatment. Severity of Septoria leaf blotch on June 14 was 51, 29, and 23% for Jagalene, 2137, and Harry, respectively, in the non-sprayed treatment and 28, 12, and 7% for Jagalene, 2137, and Harry, respectively, in the Prosaro treatment. In the check treatment, yield of Jagalene (14 bu/A) was lower ($P = 0.05$) than that of 2137 (37 bu/A) or Harry (29 bu/A). Yield in the Prosaro treatment was higher (Jagalene, 18 bu/A; 2137, 48 bu/A; Harry, 36 bu/A) than that in the check treatment in all three cultivars, but the difference was not significant at $P = 0.05$. In the check treatment, FDK (47%) and DON (10 ppm) in 2137 were lower ($P = 0.05$) than in Jagalene (61% FDK, 15 ppm DON) and Harry (57% FDK, 14 ppm DON), but did not differ between Jagalene and Harry. In the Prosaro treatment, FDK (34%) and DON (8 ppm) in 2137 were lower ($P = 0.05$) than in Jagalene (51% FDK, 13 ppm DON) and Harry (50% FDK, 14 ppm DON), but did not

differ between Jagalene and Harry. FDK in the Prosaro treatment was lower ($P = 0.05$) than in the check treatment for Jagalene and 2137, but not for Harry. DON concentration in the Prosaro treatment was lower ($P = 0.05$) than in the check treatment for Jagalene, but not for 2137 and Harry. The winter wheat cultivars in this study differed in their reaction to FHB. Although fungicide application did not reduce FHB index, it reduced FDK and DON in some cultivars. Harry, with a moderately resistant reaction to FHB, accumulated more DON than the susceptible 2137. Therefore, some cultivars with a level of resistance to FHB may be susceptible to DON accumulation. Both DON accumulation and reaction to FHB should be considered when selecting cultivars.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-7-080. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

THE 2008 FUSARIUM HEAD BLIGHT EPIDEMIC IN NEBRASKA.

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ABSTRACT

In 2008, Fusarium head blight (FHB) occurred for the second straight year in Nebraska wheat fields. Infection of wheat heads by *Fusarium graminearum* was favored by excessive rainfall before and during flowering. The disease was initially found on June 6 in a grower's field in Lancaster County and in research plots at the University of Nebraska Agricultural Research and Development Center (ARDC) near Mead in Saunders County. The most affected areas were the south central and eastern parts of the state. However, FHB was observed as far west as Imperial in the southwestern part of the state where irrigated fields were more severely affected. Northwest, the Nebraska Panhandle was spared due to dry conditions. A shift towards reduced tillage or no-till to conserve water and soil and inclusion of corn and wheat in crop rotation schemes has led to buildup of FHB inoculum in Nebraska over the last one to two decades. However, because of a variable climate, including drought during some years, FHB has been sporadic in the state. In 2008, as in 2007, above-normal rainfall occurred in south central and eastern Nebraska during the growing season. Heavy rainfall before and during flowering led to outbreaks of severe FHB epidemics. Yields were reduced not only by FHB but by other foliar diseases favored by wet weather. The major foliar diseases were Septoria leaf blotch, powdery mildew, and tan spot. In addition to reducing yield and grain quality, FHB caused accumulation of the mycotoxin deoxynivalenol (DON) in grain. Losses of up to 20% were estimated in the most severely affected areas in the south central and eastern parts of the state. The overall loss statewide in grain yield was estimated at 2.3% or 1.64 million bushels valued at $13.3 million based on an August 28, 2008 wheat price of $8.11/bushel. However the real losses may have been in reduced prices for the infected grain with high levels of DON. In the most severely affected areas, DON concentrations of more than 18 ppm were recorded in the most susceptible cultivars. There were discounts of up to $5/bushel due to DON. In an experiment at the ARDC where wheat heads were grouped into different categories of FHB severity ranging from 0% to 90% in three winter wheat cultivars, DON concentrations ranged from 25 to 57 ppm in the highest severity category. Some growers were reluctant to apply fungicides at flowering because they had already sprayed earlier in the growing season to control foliar diseases and hoped the earlier spray would not require a second fungicide application. However, the fungicides most commonly used to control foliar diseases (Headline, Quilt, and Stratego) do not reduce FHB, hence the damage in fungicide treated fields was also severe. Some growers who wanted to spray with ground equipment were unable to do so because heavy rainfall coincided with flowering. To reduce losses from FHB, growers have been advised to i) plant fungicide treated seed to prevent seedling blights caused by *F. graminearum*, ii) avoid planting wheat after corn or wheat and instead plant wheat after a broadleaf crop such as soybean, iii) select cultivars with good resistance or tolerance to FHB and DON, iv) plant several cultivars that differ in flowering dates to increase the chances that some cultivars will escape infection, v) use the national Fusarium head blight prediction tool to assess the risk of FHB development, and vi) apply an appropriate fungicide at early flowering based on the predicted risk of an FHB outbreak. The severe epidemics of 2007 and 2008 are testimony to the fact that although Fusarium head blight

is sporadic in Nebraska due to a variable climate, it can be devastating when it occurs. Concerted efforts are under way to educate growers, crop consultants, extension personnel, and others involved in the wheat industry about FHB and how to manage it.

EFFECTS OF TEMPERATURE ON DEOXYNIVALENOL TRANSLOCATION AND *F. GRAMINEARUM* INFECTION OF WHEAT HEADS.

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ABSTRACT

The primary causal agent of Fusarium Head Blight in North America is *Fusarium graminearum*. This fungus is known to infect wheat during the flowering and grain-filling stages of development. Shortly after infection, the fungus produces mycotoxins, including deoxynivalenol (DON), which contaminate floral tissue and grain. However, the relationship between mycotoxin levels and fungal infections of wheat heads is not fully understood, in light of the fact that DON can be translocated through the plant. The objective of this research was to study the effects of temperature on fungal biomass and translocation of DON within infected wheat heads. Two spring wheat cultivars were used in this study: Alsen (moderately resistant) and Wheaton (susceptible). A central spikelet was inoculated with macroconidia during mid-anthesis and plants were then incubated at 15 or 22°C. Single spikelets were harvested on days 2, 3, 4, 6, 8, 10 and 12 following inoculation. One floret from each spikelet was placed on Nash agar to establish the presence of *F. graminearum*. DON and ergosterol, a fungal biomass indicator, were extracted from the other floret. DON was extracted using 84:16 acetonitrile-water, while ergosterol was extracted with hexane through a saponification step with methanolic potassium hydroxide. The extracts were combined and a single gas chromatography method was used to detect both compounds. Preliminary results revealed heads of both wheat cultivars were fully colonized with *F. graminearum* by 12 days post-inoculation, regardless of incubation temperature. By 3 days post-inoculation, *F. graminearum* had yet to colonize spikelets beyond the inoculated point in either wheat cultivar. Yet DON translocation to spikelets not colonized by the pathogen was observed at 2 days post-inoculation. DON production also appeared to be stimulated when the fungus was stressed by low temperatures (15°C) or host resistance, possessed by Alsen. Through this research, we have confirmed our methods of DON and ergosterol extraction and detection to be sensitive and effective for point-inoculated wheat heads. We have confirmed the ability of DON to translocate to parts of the wheat head not previously colonized by *F. graminearum*. Results also suggest DON production is a mechanism the fungus uses to adapt to challenging environmental conditions. These findings may, in part, explain the development of asymptomatic grain and help to characterize the complex relationship between DON and disease intensity.

BIOLOGICAL CONTROL OF SCAB: HOW CLOSE ARE WE TO REALITY?

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ABSTRACT

A taxonomically diverse group of microbial agents that includes strains of bacteria and yeasts has been investigated for potential control of Fusarium head blight and deoxynivalenol accumulation in wheat and barley. Biological control has been attributed to antibiotic production, induced resistance and niche exclusion. Each microbe, when applied to cereal heads, consistently controlled the disease in greenhouse experiments and was effective in reducing disease severity or deoxynivalenol levels in separate field experiments. In addition, biocontrol agent-fungicide combinations were shown in some field experiments to enhance disease control over the application of the biological agents or fungicides alone. Since 2004, uniform multistate evaluations of biocontrol agents have been conducted across different environments and cereal crops. These trials also examined the integration of biological control with fungicides. While biological treatments exhibited promise in some trials, consistent field efficacy has been difficult to achieve with any single agent. Significant strides have been made to identify production and formulation methodologies to enhance field efficacy of some biocontrol agents. Research on the population dynamics of biocontrol agents and the expression of biocontrol mechanisms under field conditions is underway. New commercial biocontrol organisms are being made available for evaluations. These collective efforts may lead to biological control becoming an effective and practical strategy for integration with fungicides and host resistance to manage Fusarium head blight and deoxynivalenol.

SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: Anne Desjardins

VIRULENCE OF *GIBBERELLA ZEAE* ON WHEAT FOLLOWING INDEPENDENT DISRUPTIONS OF TRICHOTHECENE BIOSYNTHETIC GENES.

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ABSTRACT

The plant-fungal interaction that occurs when *Fusarium graminearum* invades small grains such as wheat and barley is complicated and involves many interactions between the invading fungus and the plant host. Although trichothecene toxins are not required for the initial infection of wheat, they are involved in the progression of Fusarium Head Blight (FHB) disease. Mutants of *F. graminearum* (*Δtri5*) blocked in the first committed step in the trichothecene biosynthetic pathway do not produce deoxynivalenol (DON) or any other trichothecenes, and are reduced in virulence on wheat. In order to test if biosynthetic precursors of DON are sufficient for disease progression, we disrupted the coding region sequence of 5 genes (*FgTri1, FgTri3, FgTri8, FgTri11*, and *FgTri101*) to produce mutants blocked at various steps in the trichothecene biosynthetic pathway. The mutants were analyzed for production of trichothecenes in liquid media, in a rice solid medium, and *in planta*, and for ability to cause head blight on the FHB-susceptible wheat cultivar Wheaton. Disruption mutants of the esterase *FgTri8* did not show a significant reduction in virulence. However, *ΔFgTri8* mutants accumulated 3,15-diacetylDON in culture, while in the infected seed DON as well as 3,15 diacetylDON was detected. These results suggest that esterases in wheat can contribute to the deacetylation that produces DON following infection by strains that produce 3-acetylDON. Disruptions of four genes, *FgTri1, FgTri3, FgTri11*, and *FgTri101* blocked production of DON and led to the accumulation in culture of early pathway intermediates, such as isotrichodermol and its 3-acetylated derivative isotrichodermin, or calonectrin and its 3 and 15-deacetylated derivatives. Disruption mutants of *FgTri1, FgTri3,* and *FgTri101* were reduced in virulence. However, disruption mutants of the cytochrome P450 monooxygenase *FgTri11* retained wild-type virulence although they accumulated isotrichodermol and isotrichodermin in culture and in infected seed. In a previous study, both isotrichodermol and isotrichodermin were as phytotoxic as DON in an *Arabidopsis thaliana* bioassay. Together, these results suggest that some trichothecene early intermediates are as biologically active as DON, thus the earliest steps of the pathway should be high priority targets for trichothecene control.

METHODS FOR DETECTING CHROMOSOME REARRANGEMENTS IN *GIBBERELLA ZEAE*.

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ABSTRACT

Chromosome rearrangements between fungal strains may reduce fertility in sexual crosses through the production of genetically inviable recombinant progeny. Rearrangements can be important postzygotic reproductive barriers that contribute to the speciation process. The presence of chromosome rearrangements in crosses with *Gibberella zeae* was tested by counting asci with 8, 6, 4, or 2 viable ascospores. Counts were made by observing rosettes of asci extruded from crushed perithecia and by observing unordered ascospore tetrads ejected onto agar slabs from mature perithecia. The two methods gave similar results. Self-fertilized cultures served as controls and produced the normal eight ascospores per ascus in >98% of cases. Crosses with strains known to carry chromosome rearrangements produced significant frequencies of asci with 6, 4, or 2 ascospores, as expected. These results suggest that these methods will be useful to survey populations of *G. zeae* for chromosome rearrangements.

THE ROLE OF TRICHOTHECENE-CHEMOTYPES IN FUSARIUM HEAD BLIGHT DISEASE SPREAD AND TRICHOTHECENE ACCUMULATION IN WHEAT.

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ABSTRACT

Three major strain-specific trichothecene-chemotypes have been identified in *F. graminearum*-infected crops in North America: 3-acetyldeoxynivalenol (3ADON), 15ADON and nivalenol (NIV). The emergence of the 3ADON- and NIV-chemotypes on the continent is a fairly recent phenomenon. In addition, strains with increased 15ADON production have recently been identified on the continent [*Ward et al. 2008. Fungal Genet Biol 45:473; Gale et al. 2007 Phytopathology 97:1434*]. In order to assess the potential impact of these new strains on North American wheat production, we are investigating the role of trichothecene-chemotype variation in FHB-spread and trichothecene-accumulation among susceptible and resistant wheat genotypes. The level of resistance in each of the wheat genotypes used had previously been established by point inoculation with a single isolate of a 15ADON-producer. In the current experiment, we used point inoculation with a composite of strains expressing either 15ADON, high-15ADON, 3ADON, or NIV chemotypes. Stable resistance or susceptibility to disease spread, as well as *Fusarium*-damaged kernel (FDK) scores, were observed in highly-resistant or highly-susceptible wheat genotypes. Chemotype-dependent interactions were observed in moderate or intermediate sources of resistance/susceptibility. Susceptibility to disease spread increased in wheat infected with either of the high DON-producers (3ADON and high-15ADON), and reduced in wheat infected with NIV-producers. Unexpectedly, while 3ADON-producers created as much, if not more, disease in wheat spikes as the high-15ADON-producers, FDK was as low as that caused by NIV-producers. The emergence of 3ADON-producers may imply a greater threat of FHB to North American farmers, although the severity of this impact in terms of grain quality and trichothecene contamination is uncertain. Trichothecene quantification is being performed on the collected kernels to shed some light on these discrepancies and to see if trichothecene accumulation in the grain is reflected in the FDK values. These studies will be followed by spray-inoculation experiments in order to assess the impact of trichothecene-chemotype on establishment of FHB in wheat.

LINKS BETWEEN POPULATION AFFILIATION AND TOXIGENIC POTENTIAL IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

A detailed understanding of the characteristics and dynamics of extant pathogen populations is necessary to evaluate the potential impact of pathogen diversity on specific management strategies, including the deployment of resistant varieties and chemical control strategies. Over the past seven years, several thousand members of the U.S. *Fusarium graminearum* pathogen population were genotyped using molecular markers. The observed variability could be organized into different genetic clusters or populations. Based on this initial molecular characterization and classification of isolates, an assessment of important phenotypic characteristics of the pathogen population could be pursued in a rational manner. We will present outcomes from four studies where we have utilized population affiliation information to inquire into the quantities of trichothecene toxins produced (toxigenic potential) of isolates or populations: 1. Influence of chemotype on the toxigenic potential of *F. graminearum* from the Southern U.S., where we show in greenhouse experiments that nivalenol-producing isolates overall produce much less toxin than DON-producing strains from the same region; 2. Discovery of *F. graminearum* strains that do not produce DON (or nivalenol), based on their multilocus genotypes that are not typical for known U.S. populations of *F. graminearum*; 3. Observation of differential toxigenic potential in greenhouse experiments that is correlated with population membership and that is cultivar-independent on wheat; 4. Observation of differential toxigenic potential in field experiments in wheat that may be indicative of population-synergistic effects, which were not observed in barley.

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COMBINATORIALLY-SELECTED ANTIMICROBIAL PEPTIDES PROVIDE NOVEL MEANS OF RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT.

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ABSTRACT

We are confronting the problem of head blight in wheat by using combinatorial peptide libraries to identify molecules that may contribute to novel forms of disease resistance. Blight-defense peptides are being selected for their ability to inhibit the development of *Fusarium graminearum.* To identify candidate defense peptides, we incubated phage-display libraries that express 12-mer random peptides with *F. graminearum* germlings. Phage clones that bound to the germling surfaces were recovered and amplified. After additional rounds of phage/peptide affinity screenings, we recovered numerous peptides with affinity for the germling surface. By *in vitro* assays, several affinity-selected phage clones have been discovered that inhibit *F. graminearum* germling development. These peptides have been placed into scaffold-display constructs for expression in yeast to assay their inhibitory function in the absence of the phage vector. Upon confirmation of the scaffold-peptide's inhibitory ability, we intend to deliver these constructs into plants. Additionally, the inhibitory peptides, along with peptide-sequence data collected from affinity screenings, will be used to further explore germling surface molecules important for hyphal tip growth.

UNDERSTANDING THE LIFE CYCLE OF *FUSARIUM GRAMINEARUM* AND ITS IMPACT ON DISEASE.

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ABSTRACT

We have been examining the series of life cycle events that take the scab pathogen from infected wheat back to the florets. These events include the production of mycotoxins (DON and zearalenone), the acquisition of carbon sources from the plant, the storage of lipids, the overwintering and production of perithecia, and ultimately dissemination of ascospores and conidia to flowering plants. Gene expression analysis indicates that lipid accumulation is an important step during colonization of wheat tissue. The stored resources are used for development of ascospores under some environmental conditions, or formation of conidia under other conditions. Expression of genes for mycotoxin biosynthesis can be predicted by the stage of infection and the nutritional status of the fungus. In this presentation we will frame our findings in the context of the disease cycle on wheat. Implications of these findings for forecasting and for disease control will be discussed.

THE POWER OF OMICS: ANALYZING GLOBAL EXPRESSION PROFILES TO REVEAL INFECTION MECHANISMS.

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ABSTRACT

Genomics and proteomics technology has revolutionized the study of how fungal pathogens attack their hosts. Identifying the infection mechanisms of a pathogen permits the development of targeted resistance strategies. We have used gene, protein, and metabolite expression profiling to examine the early infection process of *Fusarium graminearum* and its cereal hosts. We are especially interested in the secondary metabolites *F. graminearum* produces during infection. Cellular targets of these metabolites are under investigation using the extensive yeast genomic tools such as the deletion mutant collection. Our gene expression libraries have been mined to identify novel mycotoxin biosynthetic genes. The disruption of known and putative regulatory factors of these genes is underway to study the regulation of mycotoxin production. A shotgun proteomics approach was used to monitor protein expression changes under conditions conducive to mycotoxin production, revealing 130 *F. graminearum* proteins that exhibited significant changes in expression. Seventy-two proteins were significantly up-regulated relative to their level at the initial phase of the time course and this group included predicted secreted proteins, cellular transport proteins, homologs of other fungal virulence proteins, and many conserved hypothetical proteins. We are currently disrupting several genes encoding proteins identified in this study to explore function and contribute to our search for mechanisms of host invasion and novel antifungal targets.

USING NATURAL VARIATION TO CHARACTERIZE VIRULENCE: THE TRI13 STORY.

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ABSTRACT

The *Fusarium graminearum* population in Nepal was characterized for population structure, toxin production and virulence on both wheat and maize. AFLP analyses divided the population into four distinct groups. All strains in AFLP 1 produced 4-deoxynivalenol (DON), while nivalenol (NIV) was produced by all strains in AFLP 2. The remaining two groups, AFLP 3 & 4, were variable with some strains producing NIV and others DON. The pattern suggests that: 1. *F. graminearum* can be highly variable within a small geographic area, and 2. different AFLP lineages can coexist within an area and remain distinct. Trichothecene levels were lower generally for NIV producers compared to DON producers. There was a trend for DON producers to be more virulent than NIV producers. This pattern was evident even for strains in AFLP 3. Within this group, pairs of strains that were genetically similar but differed in toxin type were compared. On average, DON producers caused 20% more disease than NIV producers. The pattern of toxin accumulation suggests different patterns of toxin accumulation and virulence in NIV versus DON producers. NIV producers caused more disease per unit of toxin accumulation, while DON producers accumulated more toxin and ultimately caused more disease.

PHYLOGENETIC RELATIONSHIPS OF FUSARIUM HEAD BLIGHT PATHOGENS FROM DIFFERENT SOURCES BASED ON *TRI101* GENE SEQUENCING DATA.

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ABSTRACT

A molecular data set from nucleotide sequences of trichothecene 3-*O*-acetyltransferase (*Tri101*) gene (1336 bp) was constructed for 58 isolates of Fusarium head blight (FHB) pathogen from Canada, Mexico, and Iran along with sequences of 7 representative isolates of *Fusarium graminearum* (*Fg*) clade sequenced in this study and 11 representatives of *Fg* clade species plus 1 *F. pseudograminearum* isolate as the outgroup available at NCBI. Cultures were grown and DNA extracted and sequenced according to published procedures. DNA sequences were processed and assembled using SOOMOS 0.6 and sequence multiple alignments were conducted using MEGA 4. Phylogenetic analysis was conducted using PAUP* 4.0 to characterize the genetic diversity and evolutionary relationships of the isolates. Maximum parsimony searches were conducted using 100 random sequence addition replicates and the tree bisection-reconnection method of branch swapping. Maximum parsimony analyses of the sequences identified 11 *Fg* clade species. All Canadian and Iranian isolates clustered with lineage 7 (=*F. graminearum*) of *Fg* clade (BP=91%). Mexican isolates fell into two different groups: 7 isolates clustered with lineage 3 (=*F. boothii*) (BP=100%), and 8 isolates formed a new monophyletic group (BP=100%) which is different from any of the 11 known lineages (species) in the *Fg* clade. Sequencing data from the present study and from NCBI supported isolates of *F. asiaticum*, *F. acasiae-mearnsii*, and *F. cortaderiae* as being single species (BP=99%, 99%, and 98%, respectively) but there was insufficient support for *F. meridionale*, *F. austroamericanum*, *F. brasilicum*, *F. mesoamericanum*, *F. gerlachii*, and *F. vorosii* isolates. Based in part on the results of this study, two isolates from each of Canada, Mexico, and Iran, with different characteristics, will be selected and used in the future in a host-pathogen interaction study.

COMPARATIVE GENE EXPRESSION ANALYSIS OF *FUSARIUM GRAMINEARUM* IN *TRITICUM AESTIVUM* AND *ORYZA SATIVA* SPP. JAPONICA.

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ABSTRACT

Negligible amounts of Type B trichothecenes accumulate in *Oryza sativa* spp. japonica infected with *Fusarium graminearum* relative to *Triticum aestivum* inoculated with an identical strain of the fungus. To identify differential fungal gene expression patterns that could be responsible for differences in toxin accumulation in these plants, analyses of expression were conducted during infection of *O. sativa* or *T. aestivum* using *F. graminearum* Affymetrix GeneChips. Gene expression profiles were generated for time points 48, 96, and 192 hours after inoculation (hai) of plants. Profile analyses revealed a subset of genes (236) expressed only in *T. aestivum*. Classification of these genes using MIPs FunCat categories showed 110 of these genes fell into the Unclassified category. Five of these genes encode InterPRO predicted zinc-finger transcription factors and are being targeted for functional analysis via gene knock-out mutagenesis. Sixty-three genes fell into the Metabolism category, the next highest representation among the remaining genes. An *in silico* search of non-coding upstream regions for regulatory sequences in all 236 genes revealed an enrichment of two nucleotide sequences: ACGTCA and CCCCGC. Differences in temporal patterns of global fungal gene expression were observed during infections of the different hosts. In *T. aestivum*, expression levels of all genes increased from time point to successive time point, whereas expression levels of genes in *O. sativa* remained relatively constant. These results were well correlated with symptoms observed on both plants. Onset of symptoms first occurred on *O. sativa* at 48 hai and slowly increased in severity over time. Symptoms were first observed on *T. aestivum* 72 hai and intensified continuously and more quickly than those observed on *O. sativa*.

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THE TRANSCRIPTIONAL REGULATOR *TRI*6 PLAYS A MULTIFUNCTIONAL ROLE ASSOCIATED WITH VIRULENCE IN *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Tri6 which encodes for a C_2H_2 zinc finger protein positively regulates the trichothecene pathway genes in *F. sporotrichioides* (Proctor *et al.* 1995). RNA-mediated silencing of *Tri6* suppressed mycotoxin production in *F. graminearum* (McDonald *et al.* 2005). Here we report the phenotypic, exo-proteomic and transcriptomic characterization of *Tri6* mutant in *Fusarium graminearum*. Targeted disruption of *Tri6* failed to *in vitro* synthesize 15-aceytyldeoxynivalenol, a derivative of the mycotoxin deoxynivalenol (DON). Further, infection on a *Fusarium* susceptible variety of wheat was restricted to the inoculated site. Exo-proteomic evaluation of *ΔTri6* and wild-type strains revealed that disruption of the *Tri6* gene is associated with effects on secretion. The secreted proteins that are affected have been previously implicated in pathogen virulence. One such protein *Tri*8, located within the trichothecene cluster, is a member of a lip5 class of secreted lipases, and is associated with virulence in *Candida albicans*. Targeted disruption of *Tri*8 in *F. graminearum* resulted in drastic reduction in the virulence of the pathogen. Finally, whole gene expression profiling by Illumina- Solexa technology confirmed that *Tri6*, in addition to regulating secretion also affects transcription of genes involved in sequestering nutrition for pathogen growth.

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THE *CID1* CYCLIN C-LIKE GENE IS IMPORTANT FOR PLANT INFECTION AND DON PRODUCTION.

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ABSTRACT

Fusarium head blight is an extremely destructive disease on wheat and barley. Losses are due to reduction in yield and contamination of infected grain with mycotoxins. Although structural genes for deoxynivalenol (DON) biosynthesis have been well characterized, fungal regulatory genes and plant factors controlling trichothecene synthesis in infested wheat kernels are not well understood. In this study, we functionally characterized a cyclin C-like gene *CID1* in *F. graminearum*. *CID1* is homologous to *SSN8* of *Saccharomyces cerevisiae* and the *FCC1* gene of *F. verticilloides*, which is required for regulating fumonisin production on infected corn kernels. Complementing all defect phenotype in fcc1 mutant of *F.verticilloids* with *CID1* gene showed this gene really conserved in filamentous fungi. In *F. graminearum*, the *cid1* mutant was enhanced in the production of a reddish pigment on V8 agar plates and liquid cultures. In infection assays with flowering wheat heads and corn stalks, the *cid1* deletion mutant was significantly reduced in virulence. Only a very low amount of DON and 15ADON was detected in wheat kernels colonized by the mutant. The expression level of the trichodiene synthase gene *TRI5* was reduced in the *cid1* mutant. Re-introduction of the wild-type *CID1* allele into the *cid1* mutant complemented all its defects. These data suggest that *CID1* may function as a regulatory factor in DON synthesis.

SESSION 3:

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Co-Chairpersons: David Kendra and David Schmale

A USER-FRIENDLY LAB-ON-A-CHIP CARTRIDGE FOR QUANTITATIVE DETERMINATION OF MULTIPLE MYCOTOXINS.

James Bloomberg[1*], Randy Myers[1], Jens Burmeister[2], Ingmar Dorn[2], Karin Wieczorek[3], Jerry Outram[3] and Friedrich Kerz-Möhlendick[3]

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ABSTRACT

Since mycotoxins entering the food chain bear a significant risk to human health, legislators in Europe have set maximum levels for a number of mycotoxins in grain and grain products, which in turn impacts global trade flows. However, to date no suitable test is available that allows quantification of multiple mycotoxins directly at the point-of-interest, e.g. at grain collection points. Bayer Technology Services and Bayer CropScience have jointly developed a mycotoxin test kit based on the proprietary biochip platform planar waveguide technology (PWG) with plans for its market introduction from 2009. The system is based on fluorescently labelled antibodies and consists of a reader and an easy-to-use lab-on-a-chip cartridge that allows quantification of multiple mycotoxins in one analysis, within 20-30 min. including grinding and extraction. The kit can work in outside the laboratory environment in humid and dusty conditions associated with harvest time. First field tests in three sites in Germany have shown the suitability of the test to fit into the process at grain collection points. Analytical performance characterization demonstrated agreement of the method with EU guideline 401/2006. Performance comparison demonstrated superior accuracy of the newly developed test kit with commercially available ELISA-kits and performance closer to chromatographic analysis. The unique features of the PWG are its multiplexing capability and the ease of use in combination with quantitative results in a wide range of individual mycotoxin concentrations.

REDUCING THE COST OF DEOXYNIVALENOL TESTING SERVICES IN WHEAT AND BARLEY: MOVING TOWARD A SMALLER GRAIN SAMPLE.

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ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is a common contaminant of small grains in the U.S. Current DON testing labs supported by the United States Wheat and Barley Scab Initiative (USWBSI) operate sensitive and specific Gas Chromatography/ Mass Spectrometry (GC/MS) machines to detect and quantify DON with detection thresholds $\leq$ 0.05 ppm. Though DON assessments via GC/MS are extremely accurate, the machines and associated maintenance contracts are expensive. Standard extraction protocols for DON require large samples of homogenized grain (5.0g) and a substantial volume of extraction solvent (40mL) for each sample. Large grain samples require bulky extraction vessels, sizeable quantities of expensive solvents, and a considerable amount of space for processing. We hypothesized that wheat and barley samples of varying weights (0.5g, 1.0g, 2.5g, and 5.0g) taken from single 100g ground grain lots would yield similar concentrations of DON. The specific objective of this study was to evaluate the efficiency, accuracy, and repeatability of small grain samples (< 5.0 g) for DON testing. Grain was ground from 30 unique 100g grain lots (10 winter wheat, 10 hulled barley, and 10 hulless barley lots) from Virginia FHB field trials in 2007. Each ground 100g sample was divided into four sample weights (0.5g, 1.0g, 2.5g, and 5.0g), with each sample weight replicated at least twice. DON was extracted from grain samples with acetonitrile/water; 4 mL of the solvent was added for every 0.5g of sample. Extraction, clean-up, and quantification of DON were conducted following standard protocols. An analysis of variance showed that there was no difference in mean DON concentrations across sample weights ($P = 0.255$). Mean DON concentrations were significantly correlated across all of the sample weights ($P < 0.001$), with r ranging from 0.88 (0.5g and 5.0g) to 0.97 (1.0g and 2.5g). Our results indicate that small samples from 100g lots of wheat and barley (particularly those $\geq$ 1.0g) provide a reliable measure of DON contamination from FHB field trials. New front-end methods (before the sample is processed on the GC/MS) during sample preparation and DON extraction may help offset high operating costs associated with the GC/MS and improve the efficiency and timeliness of DON testing services. USWBSI DON-testing labs use an estimated 3,000L of acetonitrile every year (~75,000 samples, 40mL per sample), with an estimated cost of more than $100K (based on 19L containers purchased at $662 each). A 10-fold reduction in acetonitrile use (based on 0.5g samples) could save USWBSI DON-testing labs nearly $90K annually.

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FY08 DEOXYNIVALENOL (DON) TESTING SERVICES AT VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY.

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ABSTRACT

Concerns about the mycotoxin deoxynivalenol (DON) continue to mount, and there is a growing need to develop and expand USWBSI diagnostic laboratories for mycotoxins throughout the United States. DON testing services are vital to the development of new varieties of wheat and barley with reduced mycotoxin potential and are necessary to identify and/or exclude appropriate strategies for managing FHB. In FY08, the Schmale Laboratory at Virginia Polytechnic Institute and State University launched a new regional diagnostic laboratory for mycotoxins in the eastern United States. Approximately 6,000 samples of wheat and barley are slated to be tested from USWBSI investigators (Bergstrom, Cowger, Griffey, and Grybauskas) in four states (New York, North Carolina, Virginia and Maryland). Most of the samples received for testing in FY08 were 100g kernel lots from FHB field trials, but some were ground 5-25g samples from greenhouse experiments. Extraction, clean-up, and quantification of DON were conducted following standard protocols. DON testing services are currently managed by two talented scientists (Patricia Gundrum and Diane Reaver) and four dedicated undergraduates (D'Lourdes Cuadra, Shannon Grosse, Tamara Fetters, and Will Russell). The ultimate goals of this work are to provide analytical services necessary to develop new cultivars of wheat and barley with reduced potential for DON contamination and to facilitate DON testing that will improve chemical and cultural practices necessary to reduce DON contamination in wheat and barley. The availability of these new testing services will continue to expedite the acquisition and delivery of data from DON analyses and will ensure increased uniformity, quality, and sample capacity for stakeholders in the eastern United States.

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This material is based upon the work supported by the USDA under Agreement Number 59-0790-7-078, and the Virginia Small Grains Board. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed are those of the authors and do not necessarily reflect the views of the USDA or the Virginia Small Grains Board.

DEALING WITH DON CONTAMINATED WHEAT – A MILLER'S PERSPECTIVE.

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ABSTRACT

The Mennel Milling Company is a regional flour miller dating back to 1886. In 1996, there was a Fusarium epidemic in the Ohio soft wheat crop. This resulted in major financial losses to farmers and millers, raised the awareness of the dangers of FHB and DON, and changed the way we do business from purchasing to operations to sales. Each crop year we diligently monitor the development of the wheat. We scout fields and obtain samples prior to harvest. We no longer sell wheat ahead of harvest when the crop is at risk for FHB. We test each inbound load of wheat at harvest and segregate as necessary. We have improved our cleaning houses and thus, have taken defensive measures which have raised our costs of doing business, while also inconveniencing our suppliers and restricting the ability of our customers to buy forward flour when they may want to do so. FHB and DON in wheat continue to be major problems for the wheat flour milling industry.

RAPID DON TESTING AND METHOD PERFORMANCE EVALUATION AT THE USDA.

Tim D. Norden*

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ABSTRACT

The USDA Grain Inspection, Packers and Stockyards Administration (GIPSA) provides official testing services throughout the U.S. for domestic and export grain lots for aflatoxins, deoxynivalenol (DON), fumonisins, zearalenone, and ochratoxin A. Rapid, simple, inexpensive, and accurate test methods are required for the effective facilitation of grain marketing. GIPSA evaluates and certifies the performance of both qualitative and quantitative rapid mycotoxin test methods according to specific criteria. Only GIPSA-certified rapid test methods can be used for official mycotoxin testing. Reference methods are developed and / or validated as needed to provide the benchmark criteria for evaluating the accuracy of potential rapid test methods. Current rapid DON testing technology and GIPSA method performance criteria for evaluation of this technology will be presented.

EVALUATION OF VISUAL AND OPTICAL SORTING OF *FUSARIUM*-DAMAGED KERNELS IN WINTER WHEAT.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a destructive disease of wheat. FHB causes premature whitening or bleaching of infected spikelets. Bleached spikelets are either sterile or contain shriveled and/or discolored kernels, which are referred to as *Fusarium*-damaged kernels (FDK). *Fusarium*-damaged kernels lower yield, test weight, and quality of grain, and often contain the toxin deoxynivalenol (DON). Therefore, FDK is a major grain grading factor and is routinely measured for purposes of quality assurance. Measurement of FDK usually is done visually. Visual sorting of grain for FDK can be laborious especially if many samples need to be sorted. Furthermore, if several raters sort grain for FDK, as is often necessary, inconsistency can result from variability in intra-rater repeatability and/or inter-rater reliability. The objective of this study was to assess the ability of a single-kernel near-infrared system to detect FDK. Twenty one wheat grain samples were obtained from fields, a grain inspection facility, and elevators in south central and eastern Nebraska, USA, an area where severe epidemics of FHB occurred in 2007. Four 100-kernel subsamples from each of the 21 samples were sorted by an automated single-kernel near-infrared (SKNIR, Perten Instruments, Stockholm, Sweden) system. The system feeds single kernels into a near-infrared spectrometer, and then sorts each kernel into either a healthy or FDK portion using a partial least squares regression calibration model. The same subsamples sorted by the SKNIR system were visually sorted by an experienced rater and a recently trained rater. Agreement in FDK sorting between the SKNIR system and the two raters (inter-rater reliability) was strong. Correlation coefficients between the SKNIR system and the raters were $r = 0.91$ (rater #1) and $r = 0.89$ (rater #2). Agreement between the two raters (inter-rater reliability) also was strong ($r = 0.91$). Agreement between replicate runs in sorting FDK (intra-rater repeatability) was strongest for the SKNIR system ($0.91 \leq r \leq 0.96$, $P < 0.0001$) followed by rater #1 ($0.68 \leq r \leq 0.80$, $P \leq 0.0007$) and rater #2 ($0.49 \leq r \leq 0.66$, $P \leq 0.0236$). The mean FDK in each of the 21 samples ranged from 1 to 71% for the SKNIR system, 7 to 51% for rater #1, and 4 to 44% for rater #2. Compared to the SKNIR system, the raters generally overestimated low FDK and underestimated high FDK. Plots of standard deviations of FDK means showed that the SKNIR system was more consistent in sorting FDK than the two raters. In conclusion, visual sorting was strongly correlated with sorting by the SKNIR system; the SKNIR system had a wider range of FDK detection than visual raters; visual raters overestimated low FDK and underestimated high FDK; and the SKNIR system was more consistent than visual raters.

DEOXYNIVALENOL ALTERED CIRCULATING AND SPLENIC LEUKOCYTES AND CELL MIGRATION MARKERS: TIME COURSE AND DOSE RESPONSE IN YOUNG AND OLD BALB/C MICE.

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ABSTRACT

It was hypothesized that deoxynivalenol (DON) changed leukocyte subset numbers and their migration potential in peripheral blood with interaction of age and sex. These leukocyte markers could be functional biomarkers of DON exposure in humans. In BALB/c mice fed DON at 0, 1.0 and 2.0 ppm, age, sex and feeding interval altered immune response of peripheral blood (PB) and splenic leukocytes, as measured by flow cytometry using cell surface markers. In 2-3 month old female mice, after feeding DON for 14 d, PB granulocytes were increased at 1 and 2 ppm DON, but not after 28 d. Also, a decreased percentage of PB $CD4^+$ cells and a decreased percentage of $CD11b^+$ (macrophage) splenic leukocytes at 2.0 ppm DON were seen after 14 d only. An increased percentage of $CD19^+$ cells occurred at 2.0 ppm DON after 14 and 28 d. In old females, decreased PB $CXCR5^+$ B cells were noted after feeding 2.0 ppm DON for 14 d, and 1.0 ppm DON decreased splenic $CD11b^+$ cell %, but no dose-response in these cells was observed. In old male mice, 1.0 and 2.0 ppm DON increased granulocytes and $CD29^+CD11a^+$ neutrophils after 14 d and 2.0 ppm DON increased $CCR9^+$ T cytotoxic cells after 28 d suggested that DON stimulated digestive system inflammation in old male mice. DON caused no changes to immune cell markers in young male mice. As a conclusion, low-dose DON changed leukocyte balance in peripheral blood and spleen, and interrupted B cell, neutrophil, and T cell migration in interaction with sex and age.

SESSION 4:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Michael Lawton

A GENOMICS APPROACH TO CHARACTERIZE TRICHOTHECENE MODE OF ACTION REVEALS A CELLULAR WIDE RESPONSE IN YEAST.

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ABSTRACT

Trichothecenes constitute a large family of low-molecular-weight sesquiterpenoid mycotoxins produced by various species of *Fusarium, Trichoderma*, *Cephalsporium*, and other fungi. Identified by their characteristic trichothecene ring, these toxins include deoxynivalenol (DON), diacetoxyscierpenol (DAS), T-2, and trichothecin (T-cin). Each toxin varies with regard to toxicity and as a group is known to have a wide array of effects in plants, animals, and humans. The plant pathogen *Fusarium graminearum* causes Fusarium head blight (FHB) in both wheat and barley resulting in reduced plant yield and contamination of cereal grains with trichothecenes, in particular DON. DON is an inhibitor of translation. However, the inhibitory effects of trichothecenes are often not limited to translation and information on other downstream targets of trichothecenes is lacking. To obtain a comprehensive picture of the pathways involved in trichothecene metabolism and resistance, we have used the yeast, *Saccharomyces cerevesiae*, as a model organism to study the impact of trichothecences on eukaryotic cells. We screened the yeast knockout (YKO) collection, composed of 4700 strains, to identify mutants that exhibit hypersensitivity to T-cin. We selected T-cin over DON to screen the library since yeast is sensitive to micromolar levels of T-cin compared to mM levels of DON. Bioinformatic analyses of the select sensitive mutants have revealed components of pathways that play a role in trichothecene resistance, such as MAP kinases, components of protein synthesis, vacuolar protein sorting, and ribotoxic stress pathways, suggesting a cellular-wide response. These genes represent new candidates for engineering resistance to DON and FHB in cereals. Further characterization of these genes will provide important new insights into the trichothecene metabolism.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-6-069. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

2008 FHB ANALYSIS OF TRANSGENIC BARLEY LINES.

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ABSTRACT

Transgenic lines have been developed with the goal of reducing FHB and DON in barley. Replicated field trials for FHB reaction of 48 Conlon transgenic lines were conducted in 2008 in Langdon, ND and Rosemount, MN. The Langdon trials consisted of three replicates in hill plots in an inoculated misted nursery and three replicates in the adjacent un-inoculated un-misted nursery. The misted nursery was inoculated with a mixture of five *F. graminearum* isolates three times at two weekly intervals beginning 2 weeks prior to heading. Rosemount plots included 4 replicates of 8 foot rows which were spray-inoculated at heading and misted after inoculation. FHB severity (% infected kernels) and FHB incidence (% infected spikes) were measured at both locations. Disease severity was highest in the Langdon inoculated plots (31-55%) and lowest in the plots at Rosemount (2-10%). FHB incidence at Langdon was essentially 100%, with almost all spikes showing some FHB except the resistant checks. Data were ranked from low to high for each measurement and Spearman rank correlations were not significant between the locations, i.e. lines with the lowest measurements in either Langdon nursery showed moderate to high FHB at Rosemount. Lines did show significant correlations between measurements within a location. Two lines showed significant reductions in FHB (compared to Conlon) in the Langdon un-inoculated nursery and two different lines showed significant reductions in incidence and severity at Rosemount. All four lines contained rice genes for a chitinase and a thaumatin-like protein. All 48 lines will be tested again in 2009 to validate the reductions in FHB.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2008 FIELD NURSERY REPORT.

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ABSTRACT

The 2008 field screening nursery, with 64 wheat and 208 barley plots was located at UMore Park, Rosemount MN. Trial entries were submitted by Rutgers University (5 wheat), University of North Texas (2 wheat) and USDA (48 barley). In addition to the submitted transgenic entries, untransformed controls were also submitted from each program. Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks used were the moderately resistant Alsen and the susceptible cultivars Wheaton, Norm and Roblin while the barley checks were the moderately resistant line M122 and the susceptible cultivars Robust and Stander. The experimental design was a randomized block with four replicates. Plots were 2.4 m long single rows. The trial was planted on May 8, 2008. All plots, except a non-inoculated Wheaton check, were inoculated twice. The first inoculation was applied at anthesis for wheat and at head emergence for barley. The second inoculation was applied three days after the initial inoculation (d.a.i.) for each plot. The inoculum was a composite of 41 *F. graminearum* isolates at a concentration of 100,000 macroconidia.ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml.L^{-1} as a wetting agent. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10ml.sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from June 26, two days prior to the first inoculation, till July 22 to facilitate FHB development. FHB incidence and severity were assessed visually 20-24 d.a.i. for wheat and 17-21 d.a.i. for barley on 20 arbitrarily selected spikes per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 spikes observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets observed in these 20 spikes. Plots were harvested at maturity on August 11. The harvested seed from each plot was split using a Boerner Divider to obtain a 50 g sub-sample, which was then cleaned by hand. These sub-samples were used to estimate the percentage of visually scabby kernels (VSK) for wheat and then all samples (wheat and barley) were analyzed for deoxynivalenol (DON). The data indicated that resistance was expressed in some of the transformed lines.

ACKNOWLEDGEMENT AND DISCLAIMER

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DIFFERENTIAL TRANSCRIPTOMICS AND PROTEOMICS OF *FUSARIUM GRAMINEARUM*- AND TRICHOTHECENE-CHALLENGED WHEAT GENOTYPES.

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ABSTRACT

While a few fusarium head blight (FHB) host transcript- and protein-accumulation studies have emerged in the past few years, no global comparisons have been made between resistant and susceptible wheat genotypes, or between trichothecene- and *Fusarium*-induced defense responses. The current study explores the differential transcriptome and proteome of three wheat genotypes ('Superb', DH1 and DH2) in their response to isolated components of the FHB-wheat interaction (fungus, deoxynivalenol, and aggressive-factors) within the first 24h of interaction. 'Superb' is a FHB-susceptible Canadian wheat cultivar that shares 75% genetic identity with each of the double haploid lines, DH1 (CIMMYT11-derived type I resistance) and DH2 ('Sumai 3'-derived type II resistance). Uninfected spikelets of point-inoculated heads were harvested in order to identify changes in transcript and protein accumulation associated with induced systemic resistance. Differential transcription is elicited as early as 3hai (and up to 24hai) by the different components of the FHB-wheat interaction in the uninvaded tissues of all three wheat genotypes. Such an early induced response in distal spikelets suggests that a mobile alarm signal is produced in the infected tissue and transmitted to the uninvaded tissues, preparing the tissue for *Fusarium* invasion. On the other hand, few differences are elicited in the proteome of the aforementioned interactions at 3dai. It is possible that either (a) a change in the proteome of uninvaded tissues occurs later than 3dai, or (b) a change in proteome is induced at 3dai, but the assay method used is not sensitive enough to detect these changes. Fewest differences were observed in the uninvaded tissues of the type II resistant line, suggesting that resistance to disease spread is regulated at/near the site of infection.

DEOXYNIVALENOL-INDUCED GENE EXPRESSION IN BARLEY.

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ABSTRACT

Trichothecenes are a major group of mycotoxins that are produced by many types of phytopathogenic fungi including *Fusarium, Myrotherium, and Stachybotrys*. Trichothecenes inhibit protein synthesis in eukaryotic cells and function as phytotoxins that promote fungal disease. Therefore, the role of plant host response to trichothecene accumulation is an important aspect of plant defense to fungal disease overall. In FHB, the yield and quality of infected grain is severely reduced because of blighted kernels and the presence of the trichothecene deoxynivalenol (DON). Our objective was to examine the barley host response after DON application. A susceptible barley genotype (cv. *Morex*) inoculated with DON was shown to convert DON to DON-3-glucoside. In a subsequent experiment, a susceptible barley genotype (cv. *Morex*) was inoculated with the equivalent of 2.0 µg DON per floret or mock-inoculated with water. Microarray analysis was conducted with the Barley1GeneChip® to examine gene expression at 1, 12, 24, 48 hours after inoculation. A total of 255 transcripts exhibited increased accumulation, with fold changes of ≥ 2.0 in DON treated versus water treated plants. Eleven transcripts exhibited decreased accumulation (fold change ≤ 0.5 between DON and water treatment). Comparative analysis with previous barley-*Fusarium* studies shows that 135 of these genes may be trichothecene-specific. These genes comprised many functional classes; those groups that were of particular interest included putative trichothecene detoxification and transport, regulatory, signal transduction, and ubiquitination. We validated the expression of a subset of these genes in a near-isogenic line pair respectively containing resistant and susceptible alleles at an FHB resistance QTL on barley chromosome 2H-Bin8. Real-time PCR results validate the DON-specificity of the transcripts as observed from the microarray data. In addition, we found that some transcripts were differentially expressed between DON-treated resistant and susceptible near-isogenic lines, indicating that the transcripts encode genes that may be contributing to either resistance or susceptibility to FHB.

VIRUS-INDUCED GENE SILENCING IDENTIFIES A PUTATIVE ROLE FOR ETHYLENE SIGNALING IN TYPE II RESISTANCE TO *FUSARIUM GRAMINEARUM* IN WHEAT.

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ABSTRACT

Ethylene (ET) and Jasmonic Acid (JA) have been shown to be important for resistance to necrotrophic pathogens in Arabidopsis. While it remains unclear as to whether *Fusarium graminearum* is a hemibiotroph or a necrotroph, its necrotrophic mode of growth is most damaging. Thus, ET and JA are potential candidates for disease resistance signaling. We have used a Virus-Induced Gene Silencing (VIGS) system to silence genes in both the ethylene biosynthesis pathway and the ethylene signaling pathway. Preliminary results indicate that a number of these genes may indeed be important for defense signaling against *Fusarium graminearum*. The genes were silenced in the resistant variety 'Ning' 7840. Upon application of the virus, containing a portion of a wheat gene, the plants were screened for conversion from resistance to susceptibility. The genes involved in ET signaling screened thus far include SAMs, ACS, ETO, CTR, EIN2, and an ERF.

FUSARIUM HEAD BLIGHT RESISTANT TRANSGENIC WHEAT EXPRESSING ANTIFUNGAL PLANT DEFENSIN FROM *MEDICAGO TRUNCATULA* (MTDEF4).

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ABSTRACT

Defensins belonging to a superfamily of similarly folded antimicrobial peptides comprise representatives in vertebrates, invertebrates and plants. Plant defensins are small cysteine-rich peptides with a net positive charge which have been isolated from both mono- and dicotyledonous plants. We have identified and isolated a plant defensin MtDef4 from *Medicago truncatula* which is a potent inhibitor of *Fusarium graminearum* (IC_{50} = 0.75-1.5 μM) during *in vitr*o antifungal activity assay. Transgenic wheat lines expressing monocot intron optimized MtDef4 were generated using *Agrobacterium tumefaciens*-mediated transformation of spring wheat cultivar Bobwhite (BW) and a Chinese cultivar Xin Chun 9 (XC9). A total of six and one events were generated in BW and XC9 background, respectively. Based on the segregation analysis of these 7 events, 3 in BW and single one in XC9 background segregated for single copy of the *MtDef4* gene. Homozygous plants from all four single-copy events were identified in the T2 generation, all of which were expressing MtDef4 protein based on ELISA. Type II resistance to FHB was evaluated in three homozygous lines using single floret inoculation method in the greenhouse. Of the three lines (independent events) tested, one transgenic line 431-1-3-1 showed improved resistance when compared to non-transformed Bobwhite both in the T3 and T4 generation, thereby showing the heritability of FHB resistance. Moreover, the level of resistance in this line was similar to that of FHB resistant cultivar Alsen. The results of this study show the potential of plant defensin *MtDef4* in conferring heritable resistance to FHB.

BIOPROSPECTING FOR *TRI101* IN *FUSARIUM*: SEARCHING FOR A BETTER ENZYME TO DETOXIFY DEOXYNIVALENOL (DON).

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ABSTRACT

The mycotoxin deoxynivalenol (DON) is a common contaminant of wheat and barley in the United States. New strategies to mitigate the threat of DON need to be developed and implemented. Previous research has shown the value of an enzyme (TRI101) to modify DON and reduce its toxicity. Recent work by Garvey et al. (2008) highlighted differences in the activity of TRI101 from two different species of *Fusarium* (*F. graminearum* and *F. sporotrichioides*), but little is known about the relative activity of TRI101 enzymes produced by other species of *Fusarium*. We cloned *TRI101* from four different species of *Fusarium*: *F. sporotrichioides*, *F. graminearum*, *F. oxysporum*, and *F. fujikuroi*. Pairwise comparisons of genetic identity between *TRI101* sequences ranged from 65% (*FgTRI101* and *FjTRI101*) to 85% (*FoTRI101* and *FjTRI101*). To increase the transfer of mycotoxin in and out of the yeast cells for our expression studies, we also cloned *TRI12* (a trichothecene efflux pump) from *F. sporotrichioides*. Both genes were cloned into the yeast expression vectors pYes2.1 (*TRI101*) and pESC-LEU (*TRI12*), and the resulting vectors were co-transformed into the yeast strain RW2802. Transformed strains of RW2802 expressing *TRI101* and/or *TRI12* were fed DON at a concentration of 10ppm for 4 days at 28C. Fungal secondary metabolites were extracted, and DON and 3-acetyl-deoxynivalenol (3-ADON) were quantified using GC/MS. All of the *TRI101* genes tested were able to acetylate DON *in vitro*, and the ratio of [3-ADON]/[DON] ranged from 0.77 (FoTRI101) to 10.44 (FsTRI101). Our results suggest that other species of *Fusarium* (even those that do not produce DON) may contain functional *TRI101* genes, some with the potential to 'outperform' those evaluated in the present study. We are currently developing an *Agrobacterium* transformation vector to move these *TRI101* genes into hulless barley lines. We plan to monitor potential decreases in DON in both raw grain and dried distiller's grains with solubles (a byproduct of ethanol fermentation and a significant source of feed for domestic animals) following ethanol production using our genetically-engineered lines. TRI101 has tremendous potential to enhance food safety in the United States in the near future.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported in part by the USDA under Agreement Number 59-0790-7-078, and the Virginia Small Grains Board. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed are those of the authors and do not necessarily reflect the views of the USDA or the Virginia Small Grains Board.

HR-LIKE LESION MIMIC CONTRIBUTES TO IMPROVED RESISTANCE TO *FUSARIUM GRAMINEARUM* IN WHEAT.

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ABSTRACT

Lesion mimics (LM) that resemble plant disease symptoms have been reported to confer a broad spectrum resistance to diverse pathogens and to be involved in plant defense responses. A Chinese scab-resistant wheat line Ning7840 starts expression of LM around heading. To investigate whether LM in Ning7840 contributes to type II resistance to *Fusarium graminearum,* a recombinant inbred population from cross between Ning 7840 and Chokwang, a Korean moderately scab-resistant cultivars, was evaluated for LM and scab resistance. The gene responsible for LM in Ning 7840, designated as *lm,* also associated with type II resistance to scab. Lines with LM phenotype showed a significantly higher level of scab resistance than non-LM lines ($P < 0.05$). *lm* reduced the percentage of scabbed spikelets with or without presence of *Fhb1*, the major QTL responsible for type II resistance to scab on 3BS chromosome. The interaction between the two QTLs was not detected. Composite interval mapping consistently detected a minor QTL, *Qfhb.pser.1BL,* for type II scab resistance on 1BL across two experiments. *Qfhb.pser.1BL* was flanked by *lm* and SSR marker *Xbarc181*, and explained 5.0-8.0 % of phenotypic variation for scab resistance. *Qfhb.pser.1BL* is a new QTL that has not been reported before and may be due to a pleiotropic effect of *lm.*

TOWARD POSITIONAL CLONING OF *FHB1*, A MAJOR QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT.

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ABSTRACT

DNA markers near *Fhb1*, a major QTL for Fusarium head blight resistance on chromosome 3BS in wheat, were used to screen the Chinese Spring chromosome 3B BAC library, and a BAC contig spanning the *Fhb1* region was constructed. The genic regions of two overlapping BAC clones were sequenced. Based on new DNA markers developed from the BAC sequences, *Fhb1* was narrowed down to a 261 kb region with seven putative genes. The expression of the candidate genes was examined by RT-PCR. Four out of the seven genes are expressed in wheat spikes. But, there is no clear expression difference between water-inoculated and Fusarium-inoculated wheat spikes. Five cosmid clones containing all seven candidate genes were isolated from a cosmid library of Sumai 3. The cosmid clones were used to transform FHB-susceptible cultivar Bobwhite and transgenic plants were obtained for all cosmid clones. Transgenic plants for four out the five cosmid clones have been tested for Type II resistance to FHB, and none of them are resistant. FHB evaluation of the transgenic plants of the fifth cosmid clone is in progress. A highly diagnostic, codominant marker, UMN10, was developed and used for MAS for gene *Fhb1*.

A GENOME-WIDE SCREEN IN YEAST TO IDENTIFY POTENTIAL TARGETS OF TRICHOTHECENE MYCOTOXINS.

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ABSTRACT

Fusarium graminearum infection contaminates wheat and barley grain with the potent trichothecene mycotoxin deoxynivalenol (DON). Trichothecene mycotoxins are known to target cytosolic ribosomes and can cause cell death by permanently disrupting translation. In addition to the inhibition of protein synthesis, these toxins have been reported to influence a diverse set of biochemical processes in the eukaryotic cell. However, the molecular mechanisms that control sensitivity of wheat and barley to trichothecenes are not well understood and cellular factors that influence how the toxins are taken up, processed, and transported before inhibiting translation have not been identified. The goal of our research is to develop a better understanding of the genetic basis of eukaryotic cell susceptibility to trichothecene mycotoxins. Yeast, *Saccharomyces cerevisiae*, is sensitive to a wide variety of trichothecene mycotoxins and thus provides an ideal model organism to identify the cellular targets of these toxins. The availability of several complete sets of deletion libraries provides a powerful approach to identify genes critical for conferring sensitivity to trichothecenes on a genome-wide scale. We have carried out a genome-wide screen of the non-essential yeast knockout library (YKO) to identify the genes that confer resistance to trichothecenes when deleted. We screened 4720 homozygous diploid YKO strains and identified 122 strains that showed resistance to 4 μM trichothecin (T-cin), 27 of these strains also showed resistance to 6 μM T-cin and 14 strains showed resistance to 8 μM T-cin. The broad categories of genes identified in the resistance screen include genes that influence translation, trafficking, signal transduction, protein folding/degradation, biosynthesis/metabolism, the cell cycle, membranes, gene regulation, and mitochondria. The majority of identified genes were associated with mitochondria, implicating mitochondria in the toxin mechanism of action. These genes represent potential targets for engineering resistance to FHB and for developing effective approaches to prevent mycotoxin contamination of cereals.

IDENTIFYING PLANT GENES AND MECHANISMS THAT CONTRIBUTE TO DEFENSE AND SUSCEPTIBILITY TO *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium head blight (FHB)/scab caused by the fungus *Fusarium graminearum* is a destructive disease of wheat and barley. Previously, we had demonstrated that ectopic expression of the *Arabidopsis thaliana* At*NPR1* gene, which is a key regulator of the salicylic acid-dependent systemic acquired resistance (SAR) and the jasmonic acid-dependent induced systemic resistance (ISR) mechanisms, enhanced FHB resistance in the hexaploid wheat cv. Bobwhite and in Arabidopsis (Makandar *et al*. 2006). Subsequent studies have indicated that both SA and JA contribute to wheat and Arabidopsis resistance to *F. graminearum* during different stages of the infection. The Arabidopsis-*F. graminearum* host-pathogen system has been utilized to identify additional plant genes and mechanisms that modulate host defense or susceptibility against *F. graminearum*. For example, mutations in the *PAD4* and *WRKY18* genes enhanced Arabidopsis susceptibility to *F. graminearum*. In contrast, overexpression of *PAD4* and *WRKY18* enhanced disease resistance. PAD4 is an important modulator of camalexin and salicylic acid synthesis and is also required for phloem-based defenses against sap sucking insects. WRKY18 on the other hand is a transcription factor that regulates expression of defense associated genes. Transgenic wheat containing a *Ubi*:At*PAD4* chimera, in which *PAD4* expression is driven from the maize *Ubi* gene promoter have been generated and efforts are underway to transform a *Ubi*:At*WRKY18* chimera into the wheat cv Bobwhite. Other mechanisms that contribute to controlling *F. graminearum* growth in Arabidopsis include a flagellin-inducible non-host defense mechanism that enhances FHB resistance and a 9-lipoxygenase-dependent mechanism that enhances susceptibility to the fungus. Efforts are underway to determine if these processes also impact FHB severity in wheat.

ACKNOWLEDGEMENT AND DISCLAIMER

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RAPID GENE ASSAY IN *PHYSCOMITRELLA PATENS* REVEALS MULTIPLE MECHANISMS AND APPROACHES FOR CONTROLLING FUSARIUM HEAD BLIGHT.

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ABSTRACT

There is a pressing need for novel sources of resistance against *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) and mycotoxin contamination in wheat and barley. Our ability to perform functional screens for novel genes that can confer FHB resistance has been limited by the relatively inefficiency of transformation in wheat and barley. We have developed the recombinogenic plant *Physcomitrella patens* as a rapid assay system for genes that confer resistance to FHB and Fusarium-derived mycotoxins such as deoxynivalenol (DON).

We have used this system to define a collection of genes (conserved between *Physcomitrella* and wheat) that condition FHB resistance and DON sensitivity. Our studies have revealed that a number of distinct mechanisms can contribute to resistance and susceptibility to FHB. These include: (i) the suppression of host programmed cell death (PCD) through the overexpression of anti-PCD genes; (ii) the suppression of host PCD through the disruption of genes required for PCD; (iii) the overexpression of mutant versions of natural genes that are known targets of *Fusarium* mycotoxins; (iv) the disruption of genes that affect recognition at the plant cell surface: (v) the alteration of cell wall properties; (vi) the overexpression of genes involved in induced immunity.

These reverse genetic approaches define useful genes whose efficacy in crops can be evaluated through the use of VIGS and transgenic plants. In addition, we have also found *Physcomitrella* to be a useful system for chemical genetic studies to define compounds that phenocopy specific mutants and protect plants against infection with *Fusarium*.

USING VIRUS-INDUCED GENE SILENCING (VIGS) TO IDENTIFY GENES MAKING ESSENTIAL CONTRIBUTIONS TO FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT.

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ABSTRACT

This presentation will describe a new method we have developed to rapidly identify genes that function in the Fusarium head blight (FHB) resistance mechanism of wheat. In this method, called virus-induced gene silencing (VIGS), genes thought to function in FHB resistance are switched-off, or silenced, and their role in FHB resistance is inferred if silencing results in resistant wheat plants becoming susceptible to FHB. This method utilizes the RNA virus, Barley stripe mosaic virus (BSMV), to activate RNA-mediated gene silencing in wheat. RNA-mediated gene silencing is an evolutionarily conserved defense mechanism in plants and animals that targets viral RNAs for sequence-specific degradation. In VIGS, the plant's RNA-based defense response is exploited to cause plant genes selected by the experimenter to be silenced by inserting a piece of the chosen plant gene into the viral RNA. In this way, the messenger RNA from the chosen plant gene is targeted for degradation, thus silencing the expression of the gene, as the plant defense mechanism works to degrade all the viral RNA. This approach has several important advantages: 1) As it is homology-dependent, it can simultaneously silence multiple copies of genes, which are almost always present in hexaploid wheat. Without this capability, the expression of any closely related genes would prevent observation of the effects of silencing. 2) It is rapid; an experiment can be accomplished in as little as 2 months from identification of a candidate gene to observing the effect of its silencing. We are using VIGS to test if candidate genes make essential contributions to FHB resistance by silencing the target gene in an FHB resistant wheat genotype and assessing whether or not the silenced plant remains resistant to FHB.

GENES AND MECHANISMS ASSOCIATED WITH PLANT INTERACTION WITH *F. GRAMINEARUM*.

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ABSTRACT

Genes and signaling mechanisms associated with plant interaction with *F. graminearum* were identified in *Arabidopsis thaliana* and their involvement in wheat interaction with *F. graminearum* validated. These studies in wheat and Arabidopsis have indicated an important role for salicylic acid (SA) and jasmonic acid (JA) signaling in plant defense against *F. graminearum*. SA-dependent defenses were most effective during the early stages of infection, inhibiting germination of fungal spores. In contrast, JA-dependent defenses were most effective during later stages of infection. However, during the early stages of infection, JA attenuated the activation of SA signaling, thereby contributing to susceptibility. The induction of SA signaling can be expedited in susceptible hexaploid wheat and durum cultivars by constitutive overexpression of Arabidopsis *NPR1*, resulting in enhanced FHB resistance. Similarly, overexpression of two other genes associated with SA signaling, enhanced resistance to *F. graminearum* in Arabidopsis. Resistance could also be enhanced by knockdown of lipoxygenase expression in Arabidopsis, suggesting that plant oxylipins function as susceptibility factors. Experiments are underway to test the role of these and additional genes/mechanisms in wheat interaction with *F. graminearum* and to determine if their manipulation provides a viable strategy for enhancing FHB resistance in wheat.

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RAPIDLY IDENTIFY AND TEST SCAB RESISTANCE GENES.

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ABSTRACT

Our overall goal is to identify genes that play role in resistance to Fusarium Head Blight (FHB) and to develop and test transgenic wheat carrying these genes. Previously, we conducted a large array of RNA profiling experiments during *Fusarium graminearum* infection of barley. We identified a set of regulatory genes that respond to trichothecene accumulation and a set of genes encoding UDP-glucosyltransferases that may detoxify trichothecenes. The regulatory genes encode a WRKY transcription factor, a Myb transcription factor, a Spl7 cell death regulatory protein, a Cys2/His2 zinc-finger protein, an F-box domain containing protein, and a NF-X1 zinc finger protein. We used a virus-induced gene silencing (VIGS) assay to functionally test the regulatory genes for their role in FHB resistance/susceptibility in wheat. The NF-X1 gene functions as a negative regulator of trichothecene-induced defense response in Arabidopsis. Wheaton and Bobwhite inoculated with VIGS-NF-X1 constructs exhibited statistically significant reduction in disease severity during the early stages of disease development compared to the empty vector VIGS control lines ($P<0.05$). In addition, we have established collaboration with Dr. Gerhard Adam (Universität für Bodenkultur Wien, Austria) and to study barley UDP-glucosyltransferases in yeast.

ARABIDOPSIS THALIANA AS A MODEL PLANT TO TEST ANTIFUNGAL GENES FOR RESISTANCE TO *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Recent studies have shown that *Arabidopsis thaliana*, a model host plant is susceptible to *F. graminearum*. Taking advantage of the foliar *Fusarium-Arabidopsis* pathosystem, we tested antifungal defensins, MsDef1 and MtDef4, from *Medicago* spp., for their ability to confer resistance to this pathogen. We generated chimeric defensin gene constructs that resulted in overexpression of MsDef1 or MtDef4 either extracellularly or intracellularly (*i. e.,* vacuole or endoplasmic reticulum) in transgenic *A. thaliana* ecotype Columbia). Here, we demonstrate that constitutive overexpression of MsDef1 and MtDef4 confers strong resistance to *F. graminearum*. Transgenic *A. thaliana* lines overexpressing MsDef1 or MtDef4 either extracellularly or intracellularly showed up to 68% reduction in disease severity (DS) index as compared to that of the wild type plants (100%) and supported significantly less fungal growth as evaluated by trypan blue staining. Transgenic inoculated plants also bolted normally like the mock inoculated wild-type plants, whereas the inoculated wild-type plants showed much delayed bolting. Our results indicate that *A. thaliana* is a useful model plant to test antifungal genes for their ability to confer resistance to *F. graminearum.*

SESSION 5:

VARIETY DEVELOPMENT AND HOST RESISTANCE

Co-Chairpersons: Steve Harrison and Brian Steffenson

VALIDATION OF QTL ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN THE SOFT RED WINTER WHEAT, 'ERNIE'.

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OBJECTIVES

To validate, QTL on 2B, 3BSc, 4BL and 5AS associated with type II resistance (FHB severity; FHBS) and low deoxynivalenol (DON) in the soft red winter wheat 'Ernie'.

INTRODUCTION

Fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)] reduces yield and quality of wheat (*Triticum aestivum* L.) when persistent rainfall occurs during heading. Although a significant amount of genetic variation exists for FHB resistance in winter wheat, breeding has been hindered by the complexity of resistance, the difficulty associated with screening large numbers of wheat genotypes at heading and the need for multiple screening environments to both identify and confirm resistance. Marker-assisted selection (MAS) applied at the seedling stage, should enable breeders to discard large numbers of susceptible lines earlier in the breeding stream thereby accelerating the development of FHB resistant genotypes. Provided markers linked to FHB resistance genes are available and validated, MAS should also facilitate the rapid introgression of multiple FHB resistance genes into individual lines and the quick recovery of recurrent parent genetic backgrounds thus avoiding the linkage drag that commonly plagues the use of exotic resistance sources. In the past decade more than 100 QTL associated with different types of FHB resistance have been reported, distributed on 20 out of the 21 wheat chromosomes with multiple QTL often being located in same genomic regions (Buerstmayr et al., 2008). The only exception is chromosome 7D. To date, however, only a limited number of QTL have been validated and thus most are not being used for MAS. The U.S. cultivar, Ernie, is a widely-used source of FHB resistance in an adapted soft red winter wheat background. In Ernie, four QTL have been identified on chromosomes 2B, 3BSc, 4BL and 5AS that are associated with FHBS (Liu et al., 2007), low deoxynivalenol (DON), and kernel quality retention (Abate et al., 2008), however, none has been validated in genetically related populations or breeding lines. This study was designed to validate these four QTL using advanced breeding lines derived from crosses involving Ernie and other susceptible parents. Marker-trait associations were used to confirm the effect of each QTL on both FHBS and DON content.

MATERIALS AND METHODS

Thirty-one F_4-derived F_7 and F_8 lines from three crosses made between Ernie as the resistant parent and AgriPro Hickory, Pioneer ® Variety 2510, and IL 87-1917-1 as susceptible parents were used for this validation study. Lines within crosses were selected based on their FHBS reaction. Across all three crosses 17 resistant lines and 14 susceptible lines were included. Ten plants per line arranged in a randomized complete block design with three replications were evaluated in the greenhouse for type II resistance. Lines within each cross were classified as resistant, moderately resistant, moderately susceptible, and susceptible based on the respective $LSD_{(0.05)}$. Seed from each plant was bulked within line and replication and evaluated for DON content at Michigan State University in East Lansing, MI. Deoxynivalenol content was quantified in ¼g g^{-1} using the mycotoxin extraction kit Veratoxin for DON 5/5 (Veratox®, Lansing, MI). Thirteen *Xgwm* (Röder et al., 1998), *Xbarc* (Song et al., 2005), or *Xwmc* (Somers et al., 2004) SSR markers flanking QTL on chromosomes 2B,

3BSc, 4BL and 5AS were used to genotype the Ernie-derived lines and validate previously identified QTL. Markers spanned < 5, ~ 17, ~12 and ~50 cM on chromosomes 2B, 3BSc, 4BL and 5AS, respectively. Genotyping followed procedures described by Liu et al. (2007). Alleles were scored as derived from Ernie (E), non-Ernie (N) or as heterozygous (H). Dummy variables were defined for markers that amplified two (E or N) and three (E, N, and H) alleles according to the following formula where, X_is are marker codes and a, b, c represent variables for lines carrying the E, N and H alleles, respectively.

$$X_{ia} = \begin{cases} 1 & \text{If Ernie} \\ 0 & \text{Otherwise} \end{cases} \quad X_{ib} = \begin{cases} 1 & \text{If not Ernie} \\ 0 & \text{Otherwise} \end{cases} \quad X_{ic} = \begin{cases} 1 & \text{If heterozygous} \\ 0 & \text{Otherwise} \end{cases}$$

Further statistical analyses were carried out using these dummy variables. Marker variables were selected using stepwise regression analysis with PROC REG (SAS Institute 2007). Markers included in the model for the two resistance traits were determined based on 5 % significance level, adjusted R^2, and C_p criterion, where C_p measures the predictive ability of a fitted model. Multiple regression analysis was carried out using the selected markers and the variation explained by each marker was obtained from the partial R^2 in the sequential analysis option of PROC REG (SAS Institute 2007). Regression models were used to estimate the predicted values of lines carrying combinations of markers. The predicted values for the two resistance traits were compared with the respective observed values of lines carrying similar combinations of alleles to determine the usefulness of markers in selection programs. Useful markers linked to each QTL associated with FHBS and DON were considered important if lines carrying favorable QTL alleles had significantly lower FHBS and/or DON accumulation. Final marker-trait association was determined based on genotypic and phenotypic association in resistant and susceptible lines from each cross.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) conducted on combined data across the three crosses showed significant differences for the both FHBS and DON content among lines derived from Ernie crosses. Within crosses, lines also differed significantly for both traits. Single factor ANOVA on combined data across the three crosses indicated a significant marker-trait association for most of the tested markers. Of the six 5AS markers, however, only one marker (*Xbarc165*) was significant across combined data reflecting the lower marker density on that chromosome arm. Sample data for 4 markers associated with QTL peaks for FHBS and DON (Liu et al., 2007; Abate et al., 2008) are given in Table 1. Within crosses, marker-trait associations were less consistent. Most markers on 2B, 3BSc were significant in Ernie/AgriPro Hickory and Ernie/IL87-11917-1 while those on 4BL were significant in Ernie/AgriPro Hickory and Ernie/Pioneer ® variety 2510. Markers on 5AS were significant only in Ernie/AgriPro Hickory, probably due again to the lack of marker density on 5AS and the lack of tight linkage of markers to the QTL on that chromosome arm. Reduction in FHBS associated with *Xgwm319* on 2B was 33% in Ernie/AgriPro Hickory lines and 46% in lines derived from the Ernie/IL87-1917-1 cross (Table 1) whereas the average reduction associated with *Xgwm285* on 3BSc was 34 % and 53 % in lines derived from the two crosses, respectively. The Ernie allele on 4BL was associated with reduced FHBS in Ernie/AgriPro Hickory and Pioneer ® Variety 2510/Ernie lines, but was not significant in lines derived from Ernie/IL 87-1917-1. Similar trends were observed for DON.

Multiple regression analysis was used to identify combinations of markers significantly associated with reduced FHBS and DON accumulation. Across crosses, results suggested that the critical markers were *Xgwm319* on 2B, *Xgwm285* on 3BSc, and *Xgwm495* on 4BL which together were predicted to reduce FHBS by 67% and DON by 69%. Where marker combinations were actually observed in the data set, predicted and observed reductions in both FHBS (R^2=0.97) and DON (R^2=0.93) were highly correlated.

Table 1. Mean Fusarium head blight severity (FHBS) and deoxynivalenol (DON) content of thirty-one F_7 or F_8 lines derived from Ernie crosses. Lines were greenhouse inoculation with *Fusarium graminearum*, genotyped with SSR markers linked to 4 QTL associated with FHBS and DON content in Ernie, and classified according to whether or not they carried alleles from Ernie (E), non-Ernie (N), or were heterozygous (H).

		Markers linked to QTL for FHBS and DON							
		2B - *Xgwm 319*		3BSc – *Xgwm 285*		4BL – *Xgwm 495*		5AS – *Xbarc56*	
Cross	Allele	FHBS	DON	FHBS	DON	FHBS	DON	FHBS	DON
Combined	E	24±4	9±3	27±4	10±2	24±4	8±2	28±4	12±2
	N	36±4	17±3	76±7	56±5	50±5	28±3	36±5	13±4
	H	-	-	-	-	-	-	29±5	10±4
Significance		**	**	***	**	***	***	NS	NS
Ernie/AgriPro Hickory	E	19±3	6±3	23±2	9±3	19±3	7±3	22±3	9±3
	N	52±5	30±3	61±10	37±7	53±5	30±4	49±7	27±5
Significance		***	***	**	**	***	***	***	**
Ernie/IL87-1917-1	E	21±7	8±8	31±6	10±3	35±10	10±9	35±10	41±9
	N	67±7	44±8	84±9	74±5	53±10	42±9	53±10	10±9
	H	-	-	-	-	-	-	-	-
Significance		**	*	**	***	NS	NS	NS	NS
Pioneer 2510/Ernie	E	-	-	27±4	9±3	24±4	7±3	27±6	9±4
	N	27±4	9±3	-	-	44±6	19±4	24±6	6±4
	H	-	-	-	-	-	10±5	29±5	10±3
Significance		NA	NA	NA	NA	***	***	NS	NS

Understanding the genotypic difference with respect to markers present in resistant and susceptible lines is central to validating QTL and eventually recommending markers for marker-assisted-selection programs. Phenotypic and genotypic information for resistant and susceptible lines are given in Table 2. For all crosses, most resistant lines inherited all three alleles from the resistant parent, Ernie, while the majority of susceptible lines carried two or three non-Ernie (N) alleles.

Across crosses, lines classified as moderately resistant or moderately susceptible were less consistent. These results were not unexpected in that breeders frequently have difficulty classifying lines that have intermediate levels of resistance.

In summary, results suggest that QTL on 2B, 3BSc and 4BL have significant effects on both FHBS and DON accumulation in lines derived from Ernie. As such, they should be useful in combination with phenotypic selection for identifying resistant lines and eliminating susceptible lines from breeding streams. These markers were less useful in differentiating among moderately resistant and moderately susceptible lines. Markers on 5AS may also be useful; however, more tightly linked markers must be identified prior to using this QTL for MAS.

ACKNOWLEDGEMENT

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DISCLAIMER

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

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Table 2. Phenotypic and genotypic differences among resistant and susceptible lines derived from crosses with the resistant parent Ernie and four susceptible soft red winter wheat lines. Lines were phenotyped by point-inoculation with *Fusarium graminearum* in the greenhouse at the University of Missouri and genotyped with significant markers at each of four QTL on chromosomes 2B, 3BSc, 4BL and 5AS.

Cross	Line no.	Resistance level†	FHBS (%)‡	DON µg g^{-1}	2B *Xgwm319*	3BSc *Xgwm285*	4BL *Xgwm495*
Ernie/AgriPro Hickory	F_8-11	R	9.0 a	4.8 a	E	E	E
	F_8-21	R	11.6 a	2.7 a	E	E	E
	F_8-14	R	12.2 a	2.8 a	E	E	E
	F_8-23	R	12.6 a	5.4 a	E	E	E
	F_8-15	R	15.0 a	2.6 a	E	E	E
	F_8-22	R	15.3 a	2.4 a	E	E	E
	F_8-20	R	16.0 a	4.1 a	E	E	E
	F_8-12	MR	19.3 b	4.5 a	E	E	E
	F_8-16	MR	27.1 b	6.7 a	E	E	E
	F_8-13	MS	28.7 c	9.3 a	E	E	E
	F_8-19	MS	31.9 c	15.8 a	E	E	E
	F_8-24	MS	33.1 c	16.5 a	E	E	E
	F_8-17	MS	37.6 c	20.6 b	N	E	N
	F_8-18	S	60.4 d	33.3 b	N	E	N
	F_8-10	S	60.7 d	37.0 c	N	N	N
LSD (0.05)			8.9	7.4			
Ernie/IL 87-1817-1	F_7-35	MR	20.9 a	13.7 a	N	E	E
	F_7-37	MR	21.8 a	7.2 a	E	E	E
	F_7-36	MS	49.8 b	8.2 a	E	E	N
	F_7-38	S	84.6 c	7.2 a	N	N	N
LSD (0.05)			10.4	9.0			
Pioneer 2510/Ernie	F_8-101	R	13.9 a	4.2 a	N	E	E
	F_8-115	R	15.2 a	2.1 a	N	E	E
	F_8-100	R	17.1 a	4.3 a	N	E	E
	F_8-107	R	17.9 a	4.3 a	N	E	E
	F_8-111	R	19.3 a	3.8 a	N	E	E
	F_8-106	R	20.6 a	8.5 b	N	E	E
	F_8-96	R	20.8 a	4.9 a	N	E	E
	F_8-94	MS	34.2 b	9.8 b	N	E	E
	F_8-98	MS	37.5 b	14.2 c	N	E	E
	F_8-105	MS	40.2 b	14.5 c	N	E	E
	F_8-113	S	42.6 c	21.5 d	N	E	N
	F_8-99	S	44.7 c	17.3 c	N	E	N
LSD (0.05)			7.4	4.1			

† Resistance level based on resistance in Ernie (R=≤ 20%) and the respective LSD for the cross.
‡ FHBS = Fusarium head blight severity determined as the proportion of infected spikelets on the inoculated head following greenhouse point inoculation with *Fusarium graminearum*.

GENOTYPIC AND PHENOTYPIC SELECTION FOR HEAD SCAB RESISTANCE IN WHEAT.

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OBJECTIVE

To compare phenotypic selection with genotypic selection for FHB resistance in early generations.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a highly destructive disease that affects wheat (*Triticum aestivum* L.) throughout the world (Mc Mullen et al., 1997). Breeding for FHB resistance is arguably the best way to combat this disease. Historically, the selection process for resistance to scab has been based on phenotypic evaluation of disease incidence and severity in the field, and then estimation of percentage of fusarium diseased kernels (FDK) and deoxynivalenol (DON) content after harvest (Bai and Shaner 1994). However, phenotypic evaluation is time consuming, costly and often inaccurate. Moreover, the inheritance of resistance to FHB is complex and the phenotypic expression is greatly affected by weather (Bai and Shaner, 2004). Given these facts, molecular markers are potentially very useful in breeding for FHB resistance (Van Sanford et al., 2001; Bai and Shaner, 2004). Still, optimizing the balance between phenotypic and genotypic selection remains a significant challenge in the way of improving FHB breeding programs.

MATERIALS AND METHODS

Plant Material - An $F_{2:3}$ population derived from a cross between FHB-susceptible KY93C-1238-17-2 and FHB-resistant VA01W- 476 was divided into two subpopulations (Figure 1): one was aimed to be subjected to phenotypic selection (SPp) and the other to genotypic selection (SPg). The first subpopulation (SPp) comprised 48 $F_{2:3}$ lines planted in headrows in October 2006 in a scab nursery in Lexington, KY. This material was subjected to phenotypic screening: field ratings, incidence and severity were measured in the field; FDK and DON were measured in the seed. For the second subpopulation (SPg), 10 seeds from each of a second group of 48 $F_{2:3}$ lines were planted in pots in the greenhouse in December 2006. Each plant was evaluated for the presence of QTL associated with FHB resistance: the major FHB resistance QTL on chromosome 3BS *Fhb1* (markers used: *Xbarc147-3B, Xgwm533-3B* and *Xsts3B-256*) and the resistance QTL on chromosome 2DL (marker used: *Xcfd233*). Plants homozygous resistant and susceptible for each QTL were selected to be planted in the field in the fall of 2007 (Fig.1).

In October 2007, seed from the greenhouse and field was planted in headrows in scab nurseries located at Lexington, KY (LEX) and Princeton, KY (PRN). The experimental design at each location was a RCB with two replications. Field ratings were recorded. After harvest in June 2008, FDK and DON were measured in kernels harvested from headrows.

Scab Nurseries – the Lexington nursery had an overhead mist irrigation system on an automatic self timer while Princenton nursery was not irrigated. Scabby-corn inoculum (30 g m^{-2}) was spread at both locations three weeks before anthesis. At Princeton, plants were additionally treated with conidial suspensions (100,000 spores ml^{-1}) at anthesis at a rate of 30 ml per m of row.

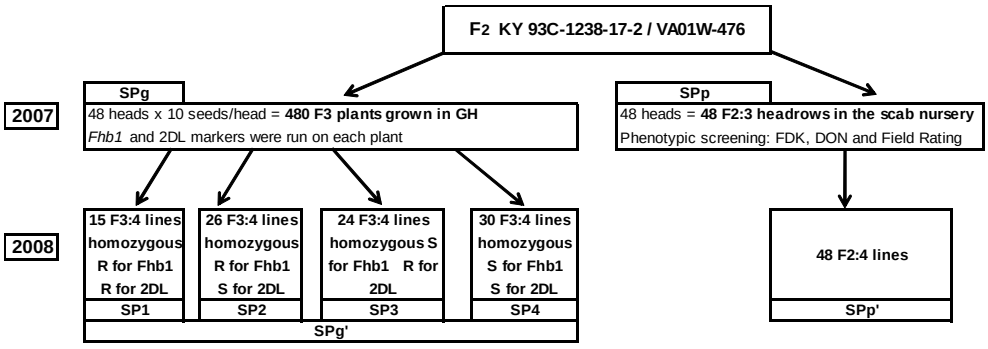

Figure 1. Schematic of the derivation of subpopulations (SP) subjected to genotypic (SPg) and phenotypic (SPp) selection in 2007-2008.

Phenotyping - In 2007, incidence was based on 20 spikes and severity was recorded as an average of 10 spikes (21 days after anthesis). For both 2007 and 2008, field ratings were an estimation of FHB incidence and severity using 1 to 3 scale (1<10%, 2=10% - 90%, 3>90%). FDK was measured using an air separation machine developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from asymptomatic ones. DON was determined by GC-MS.

Genotyping - Dried leaf tissue samples from 10 seedlings of 48 F2:3 families were submitted to the USDA/ ARS Regional Small Grains Genotyping Lab (RSGGL) at Raleigh, NC in 2006. DNA was extracted and markers amplified by the RSGGL. PCR products were sized using an ABI 3130XL DNA Analyzer and analyzed using GeneMarker (SoftGenetics, LLC).

Data Analysis - For the comparison between genotypic and phenotypic selection, we graphed p-value as a function of the percentage of the population phenotypically selected (Fig.2). The p-value indicates the likelihood that both phenotypically and genotypically selected populations are equal. Thus, when we applied a high phenotypic selection intensity (low % of the population selected), the phenotypically selected population had significantly lower FHB than the genotypically selected one ($p<0.05$). At low selection intensities (high % of the population selected), it was the other way round. At intermediate selection intensities, both phenotypically and genotypically selected populations were not significantly different ($p>0.05$).

RESULTS AND DISCUSSION

Phenotypically Selected Subpopulation - The 2007 field experiment represents a typical breeding program selection scheme in which unreplicated headrows are phenotypically selected at one location. Out of the 10 top lines for FDK in 2007, only 4 were among the top 10 in 2008. Out of the 10 top lines for DON in 2007, only 6 were among the top 10 in 2008. This reinforces the general idea that FHB selection should be based on more than one observation.

Despite the fact that in 2007 there was a higher FHB incidence (Mundell, personal comunication), in our study DON was higher in 2008. This may have been due to the fact that the 2007 seed came from the scab nursery. Mean FDK was similar across years and locations. Standard deviations and ranges for both FDK and DON were higher in 2008 (Table 1).

Genotypically Selected Subpopulation - The subpopulation having both resistance QTL (SP1) showed significantly lower FDK and DON than subpopulations having any single resistant QTL (Table 2, 3). The presence of either resistance QTL (*Fhb1* or 2DL) significantly reduced FDK and DON (Table 2, 3). When averaged over both locations, FDK reduction was similar for both resistance QTL but the 2DL QTL showed a significantly higher reduction in DON (mean value, Table 3). Additionally, the 2DL QTL showed a significant ($p<0.01$) interaction with the environment, while *Fhb1* was stable across environments (data not shown). The relative effectiveness of the 2DL QTL in

Table 1. Means, Standard Deviations (ST DEV) and ranges for a set of $F_{2:3}$ lines at Lexington in 2007 and their $F_{2:4}$ progeny at Lexington and Princenton in 2008.

Year	2007		2008		2008	
Location	LEX		LEX		PRN	
Parameter	FDK	DON	FDK	DON	FDK	DON
Mean	20.65	18.47	19.71	25.37	20.10	25.46
S.D.	7.14	10.94	11.91	17.42	10.59	14.25
Range	9.4 - 41.2	4 - 41.4	3.3 - 58.6	2.4 - 68.7	4 - 42.6	2.3 -61.8

Table 2. Means and Standard Deviations (SD) of parents and subpopulations. Subpopulations reflect the presence of resistance alleles at zero, one or both QTL. Different letters indicate significant differences at $p<0.05$.

		FDK				DON			
	N	LEX		PRN		LEX		PRN	
Parents		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
VA01W-476	4	3.05 a	1.21	4.05 a	1.47	3.30 a	1.39	2.10 a	0.57
KY93C-1238	4	33.97 b	7.69	32.85 b	8.58	30.15 a	16.17	44.82 b	10.11
Subpopulation									
Fhb1 R + 2DL R (SP1)	30	6.56 a	4.16	7.38 a	4.34	7.49 a	5.07	6.37 a	4.92
Fhb1 S + 2DL R (SP2)	48	12.21 b	3.97	10.36 a	6.75	12.19 b	7.32	8.27 a	5.74
Fhb1 R + 2DL S (SP3)	52	10.07 b	8.11	15.45 b	7.28	14.84 b	7.46	17.71 b	9.99
Fhb1 S + 2DL S (SP4)	60	16.15 c	9.55	22.06 c	11.69	19.88 c	10.06	25.97 c	14.93

this study was surprising. However, results from this one year, one population study must be viewed with caution, although the same trends have been seen in other studies (Jiang et al., 2007; Agostinelli, unpublished).

Genotypic vs. Phenotypic Selection – In contrast with the high level of FHB inoculum of SPp' seed (coming from 2007 scab nursery), SPg' seed came from the greenhouse where it was not exposed to FHB. The different level of inoculum in seed between SPg' and SPp' hindered us from drawing conclusions by comparing both populations. Thus, for making the comparison between phenotypic and genotypic selection we simulated a phenotypic selection using SPg'. To simulate phenotypic selection, one location was treated as the selection environment and the other as the validation environment. For example, entry means ranking from LEX were used to select entries at PRN and vice versa (Fig. 2).

When the percentage of the phenotypically selected population was between 10 and 55 %, the phenotypically selected population did not differ significantly from the subpopulation having both resistance QTL (SP1) for either FDK or DON in either environment. When <10% of the population was selected, the phenotypically selected population was more resistant than SP1. When > 55% of the population was selected, SP1 was more resistant than the phenotypically selected population (Fig. 2I, 2II and Table 3). The results from the comparison between phenotypically selected populations and subpopulations having one resistance QTL varied with parameter measured (FDK or DON) and location (Fig. 2III, 2IV, 2V, 2VI and Table 3).

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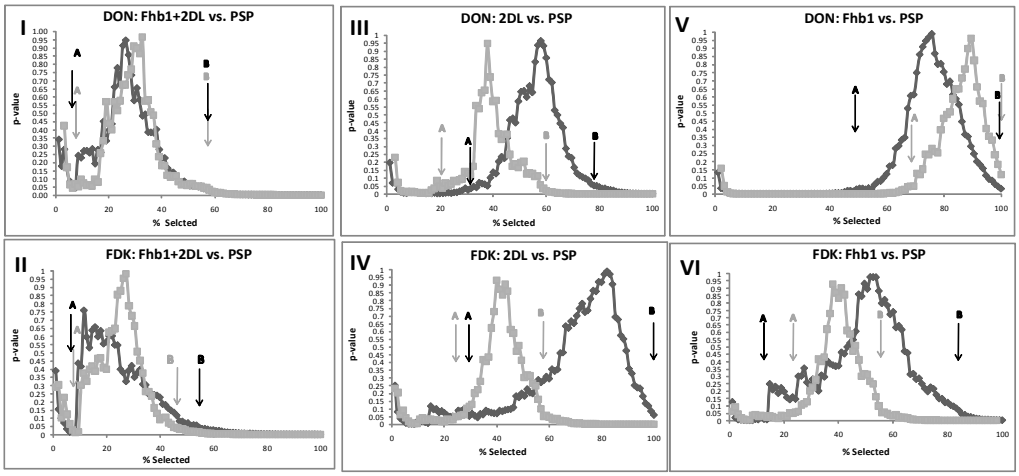

Figure 2. P-value associated with Student's T-test and percentage of the phenotypically selected population (PSP). Higher p-values indicate higher likelihood that PSP is equal to subpopulations with both QTL (I and II), 2DL (III and IV) and *Fhb1* (V and VI) for DON (I, III and IV) and FDK (II, IV and VI) in LEX (dark grey) and PRN (light grey). Between arrows A and B, phenotypic and genotypic selection are not significantly different ($p<0.05$). To the left of the A arrow phenotypic selection is significantly more resistant and to the right of the B arrow, genotypic selection is more resistant.

Table 3. Means of subpopulations at Lexington (LEX) and Princeton (PRN). Different letters indicate significant differences at $p<0.05$. *QTL effect was calculated by subtracting the mean of the subpopulation containing the resistance alleles from the mean of subpopulation containing the susceptible alleles and dividing it by the mean of the subpopulation with susceptible alleles.

							EQUIVALENT RANGE**	
		N	LEX	PRN	Mean Value	QTL Effect*	LEX	PRN
FDK	***Fhb1* R + 2DL R (SP1)**	30	6.56 a	7.38 a	6.97 a	63.5%	8% - 55 %	9% - 45%
	2DL R (SP1+SP2)	78	10.01 b	9.24 a	9.62 b	40.4%	33% - 100%	25% - 60%
	Fhb1 R (SP1+SP3)	82	8.78 ab	12.56 b	10.67 b	31.7%	14% - 85%	23% - 58%
	MEAN (SP1+SP2+SP3+SP4)	190	11.99 c	15.02 c	13.50 c	-	-	-
DON	***Fhb1* R + 2DL R (SP1)**	30	7.49 a	6.37 a	6.93 a	69.8%	7% - 56%	8% - 56%
	2DL R (SP1+SP2)	78	10.35 ab	7.55 a	8.95 a	54.9%	35% - 79%	21% - 59%
	Fhb1 R (SP1+SP3)	82	12.12 b	13.65 b	12.88 b	25.4%	55% - 99%	71% - 100%
	MEAN (SP1+SP2+SP3+SP4)	190	14.61 c	16.20 b	15.40 c	-	-	-

**Equivalent range: range of selection intensities between arrows A and B in Fig 2.

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PERCENTAGE OF FUSARIUM DAMAGED KERNELS MEASURED BY AIR SEPARATION.

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ABSTRACT

One of the greatest problems in breeding for Fusarium head blight (FHB) resistance lies in the difficulty of assessing the disease. Air separation methods have long been used in the seed industry for seed conditioning purposes and in seed labs to measure the proportion of different components of seed samples. An air separation machine was specifically developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from healthy ones. Once a sample is loaded into the machine, air-driven elevation of the lighter portion of wheat (i.e. scabby seeds) occurs until it reaches the top of the column where is collected in a receptacle. The heavier portion of wheat (i.e. asymptomatic seeds) is suspended midair and does not reach the top of the column. Once the air is turned off, the asymptomatic seeds fall and are collected in the bottom of the column. Finally, both portions of the sample are weighed separately and FDK is calculated. Time per sample is about a minute.

A population of 128 F3:4 and 48 F2:4 lines derived from a cross between FHB-susceptible KY93C-1238-17-2 and FHB-resistant VA01W- 476 were grown in headrows in October 2007 in scab nurseries located at Lexington and Princeton, KY. In 2008, scab ratings were recorded in the field; percentage of Fusarium damaged kernels (FDK) and deoxynivalenol (DON) concentration was measured in kernels harvested from headrows. FDK was measured using the air separation machine and DON was determined by GC-MS. The correlation between FDK and DON using all data points was 0.852, indicating that FDK measured by air separation can be a highly useful way to assess FHB in scab breeding programs.

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CHARACTERIZING BARLEY NEAR-ISOGENIC LINES FOR A DON QTL ON CHROMOSOME 3H.

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OBJECTIVES

Investigate ergosterol and DON content in barley spikelets following point inoculation in lines isogenic for a DON accumulation QTL on chromosome 3H.

INTRODUCTION

Previously, we evaluated a FHB mapping population using a point inoculation assay to assess accumulation of DON in barley spikelets 72 hours after inoculation (Smith et al., 2004). The mapping population segregated for DON concentration. Interestingly, the parent with resistance to FHB, Frederickson, accumulated higher levels of DON compared to the susceptible parent Stander. In this mapping study, we identified a QTL for DON accumulation on chromosome 3H. This QTL for DON accumulation was not associated with field resistance to FHB, and thus suggested independent mechanisms for host resistance to infection and DON accumulation. Possible explanations for a host genetic effect on DON accumulation include a host effect on: fungal growth, fungal production of DON, and degradation of DON. In this study, we use a pair of near-isogenic lines for this 3H QTL region to confirm the 3H QTL effect on DON accumulation and assay ergosterol to determine if the host effects growth of the fungal pathogen.

MATERIALS AND METHODS

DON and Ergosterol assay of spikelets. Inbred lines FEG8-14-083-F(29) and FEG8-14-083-S(1) are isogenic for the 3H DON QTL region. In addition, the parents of the mapping population, Frederickson and Stander, were used as checks (Mesfin et al., 2003). Two seeds of each line were planted in each of five pots in two separate greenhouse experiments as previously described (Smith et al., 2004). Plants were inoculated when at least one head on the plant reached full head emergence or anthesis. A single isolate of *Fusarium graminearum* (Butte86ADA-11) was used to produce macroconidial inoculum (spore suspension of 100,000 conidia/mL). Two central spikelets of a single barley spike of each plant were injected with 10µL of inoculum and immediately transferred to the dew chamber for 72 hours with conditions as described in Smith et al. (2004). If only one plant in the pot had reached heading, the other plant was not used. At 72 hours, pots were removed from the dew chamber and inoculated spikelets were collected, a visual score was taken on each for the percent necrosis and chlorosis of each spikelet and then stored at -20°C until analyzed. One of the two spiklets harvested from each plant was used for DON analysis (Smith et al., 2004) and the other was used for ergosterol analysis (Dong et al., 2006) using methods described previously.

Data Analysis. Analysis of variance was performed using SAS Proc GLM (SAS, Institute, Inc. 2003). Each pot was treated as the experimental unit, so in the case where both plants were inoculated, the data were averaged. The two greenhouse experiments were analyzed both separately and together. Mean separation was performed using a protected LSD (P=0.05) for heading date, percent necrosis, percent chlorosis, seed weight, DON ppm, 15ADON ppm, and ergosterol ppm.

RESULTS AND DISCUSSION

Comparison of near isogenic lines: As expected, Frederickson headed later than Stander and the NILs. However, there was no significant difference in heading date between the NILs (data not shown).

- The NILs did not differ in seed weight, percent necrosis, or percent chlorosis (data not shown).

- As previously observed, the NIL carrying the Stander allele at the 3H QTL had significantly lower DON concentration compared to the NIL carrying the Frederickson allele (Figure 1).

- The NILs did not differ in ergosterol (Figure 2) but were significantly higher than both Frederickson and Stander.

- The NILs did not differ for 15ADON concentration (data not shown)

In our previous study, we showed that the QTL on 3H was significantly associated with accumulation of DON in point inoculated spikelets 72 h after inoculation. Among a set of NILs we observed that on average lines carrying the Frederickson allele had 2.5 fold higher levels of DON compared to lines carrying the Stander allele (Smith et al., 2004). In this experiment using one selected pair of NILs, we saw a 4-fold difference in DON accumulation. However, we did not observe any difference in ergosterol concentration between the NILs. This suggests that the host effect on accumulation of DON in infected spikelets is not related to growth of the pathogen. Future experiments will investigate the role of the host on fungal production of DON and host degradation of DON.

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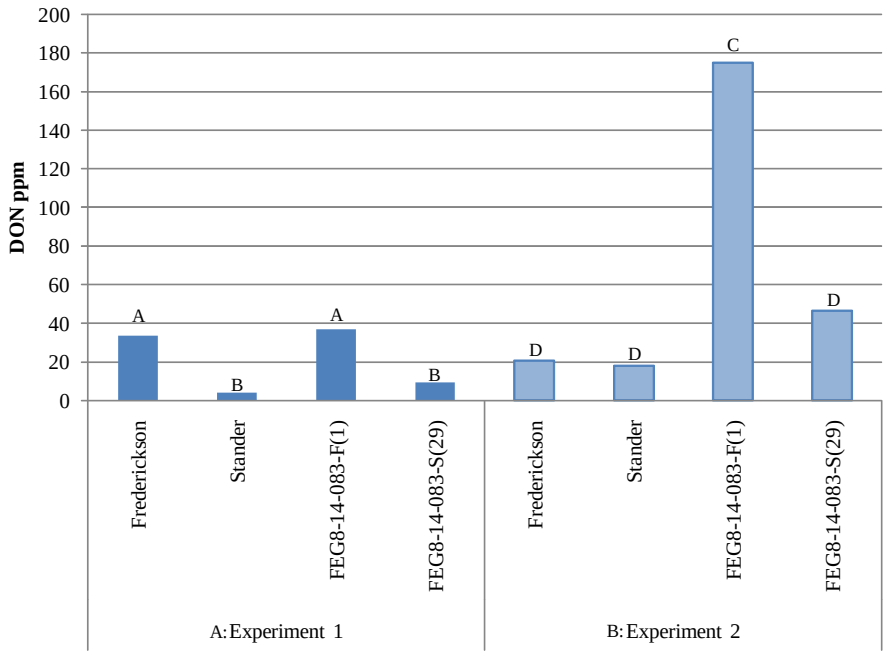

Figure 1. DON concentration for Frederickson, Stander, and the NILs in single spikelets harvested 72 h after inoculation. A: experiment 1; B: experiment 2. Bars labeled with different letters are significantly different (LSD, P=0.05).

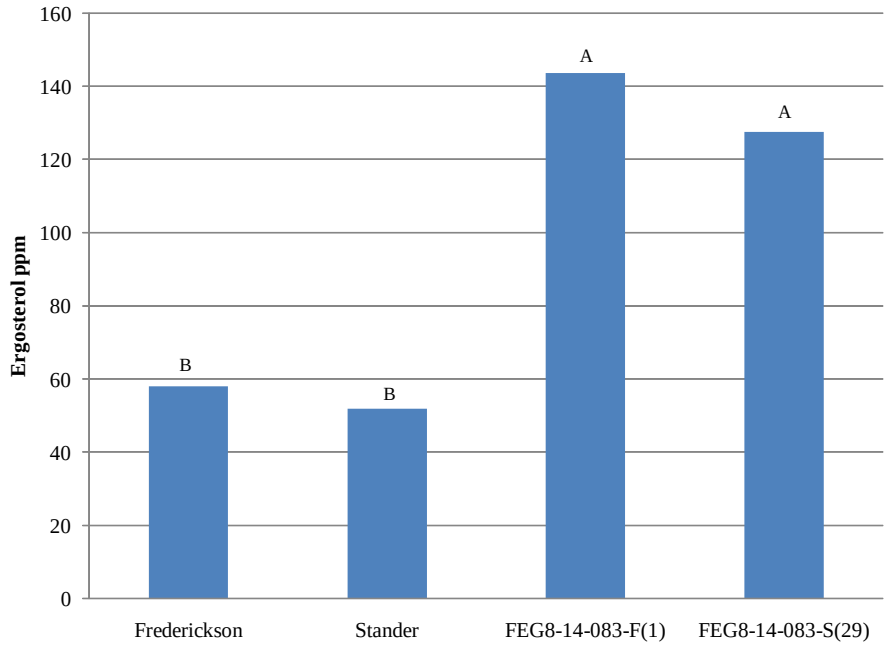

Figure 2. Ergosterol concentration for Frederickson, Stander, and the NILs in single spikelets harvested 72 h after inoculation. Combined analysis of experiments 1 and 2. Bars labeled with different letters are significantly different (LSD, P=0.05).

INVESTIGATING HOST VARIATION FOR DON ACCUMULATION IN WILD BARLEY.

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OBJECTIVE

Assess variation for toxin accumulation and ergosterol concentration in twenty *Hordeum vulgare* ssp *spontaneum* accessions.

INTRODUCTION

Previously, we showed that there was host genetic variation for accumulation of DON in point inoculated barley spikelets (Smith et al., 2004). Interestingly, the FHB resistant parent, Frederickson, accumulated more DON in this assay than the FHB susceptible variety Stander. While we were able to identify host genetic variation within cultivated barley in this mapping study, we were curious as to the extent to which variation for this trait was present in wild barley. In this study, we analyzed a set of 20 ecogeologically diverse accessions from the Wild Barley Diversity Collection (WBDC, Steffenson et al., 2007).

MATERIALS AND METHODS

Wild Barley Diversity Collection. Three hundred and eighteen wild barley (*Hordeum vulgare* ssp. *spontaneum*) accessions were genotyped on a DArT platform (Steffenson et al., 2007). These data were used to calculate the pairwise genetic distance among the 318 accessions in PAUP v 4.0 (Swofford 2003) and were used to construct a radial dendrogram in Dendroscope (Huson et al., 2007). Twenty lines were selected to best represent the diversity of the entire collection. Resistant and susceptible barley cultivars, Frederickson and Stander, respectively, were used as checks. Two seeds of each line were planted in each of five pots in two separate greenhouse experiments as previously described (Smith et al., 2004). Wild barley lines were vernalized on moistened filter paper in sealed petri plates for 28 days at 4°C before being transferred to the greenhouse for transplanting.

DON and Ergosterol assay of spikelets. See the report "Characterizing barley near-isogenic lines for a DON QTL on chromosome 3H" by Beaubien et al. in these proceedings.

Spread of FHB within the spike. Due to preliminary evidence of variation for spread of FHB in the head, FHB severity was assessed as a measure of spread for all lines in experiment 2. After pots were removed from the dew chamber and inoculated kernels were collected, pots were returned to the greenhouse bench. Two weeks after being returned to the greenhouse bench, the number of visually symptomatic kernels and the total number of kernels from each inoculated spike were counted and used to calculate FHB severity.

Data Analysis. For analysis of variance, only the wild barley accessions were considered; the checks were not included. The pot was treated as the experimental unit, so in the case where both plants were inoculated, the data were averaged. The two greenhouse experiments were analyzed both separately and together. Proc GLM (SAS, 2003) was used to assess variation for heading date (including 28 days vernalization for wild barley accessions), percent FHB severity (experiment 2 only), percent necrosis and percent chlorosis for the inoculated spikelets. The two spikelets harvested for DON and ergosterol analysis were averaged for percent necrosis and chlorosis. We also analyzed seed weight, DON ppm, 15ADON ppm, and ergosterol ppm.

RESULTS AND DISCUSSION

Phenotypic variation in wild barley:

- We observed significant (P<0.0001) variation for percent necrosis on inoculated spikelets (Figure 1)
- There was significant (P=0.0102) variation for FHB severity among wild barley accessions. In general, severity was greater in the wild barley accessions than in Frederickson and Stander (Figure 2).
- There was significant variation (Exp1, P=0.0028; Exp2, P=0.0113) for ergosterol accumulation among wild barley accessions. However, some lines were inconsistent from experiment 1 to experiment 2 (Figure 3).
- Significant variation among wild barley accessions for both DON and 15ADON was observed in experiment 1 only (Figures 4 and 5 respectively).
- Wild barley accessions also differed significantly for heading date and seed weight (data not shown).

While we observed some phenotypic variation for DON accumulation among the wild barley accessions, the extent of variation was generally within what we observed for Frederickson and Stander. This suggests that additional variation for this trait may not be available in wild barley. We did observe significantly more variation for FHB severity (spread in the head) in wild barley compared to Frederickson and Stander. It is important to note that the spread that we observed was likely due to surface growth of mycelia rather than growth in the rachis as is typically observed in wheat. In cultivated barley, there is very limited spread through the rachis compared to wheat. While this variation may not be the same as type II resistance in wheat, our results suggest that further investigation in wild barley may be warranted.

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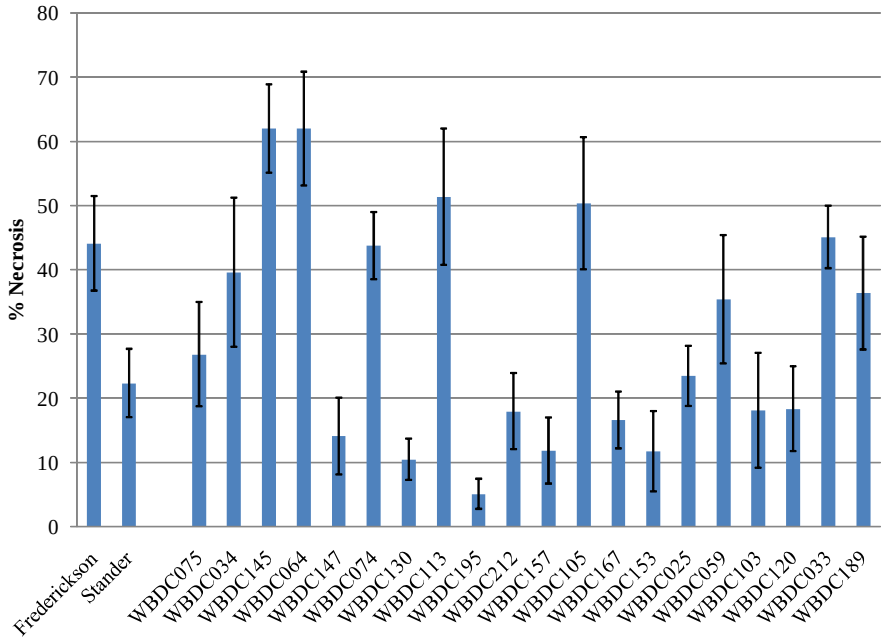

Figure 1. Percent necrosis 72 hours after inoculation of single spikelets. Data is combined for two experiments.

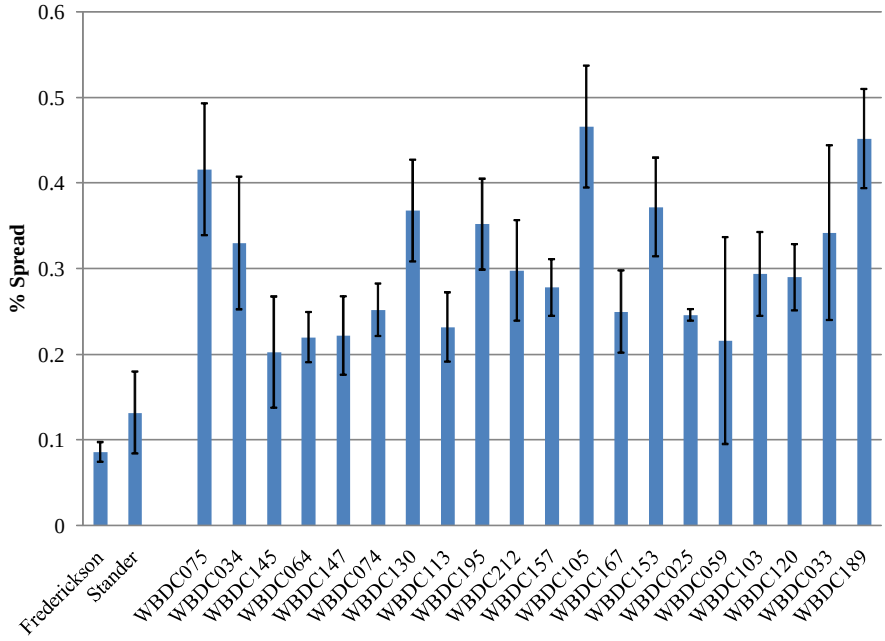

Figure 2. Percent FHB severity 72 hours after inoculation of single spikelets. Data was collected only in experiment 2.

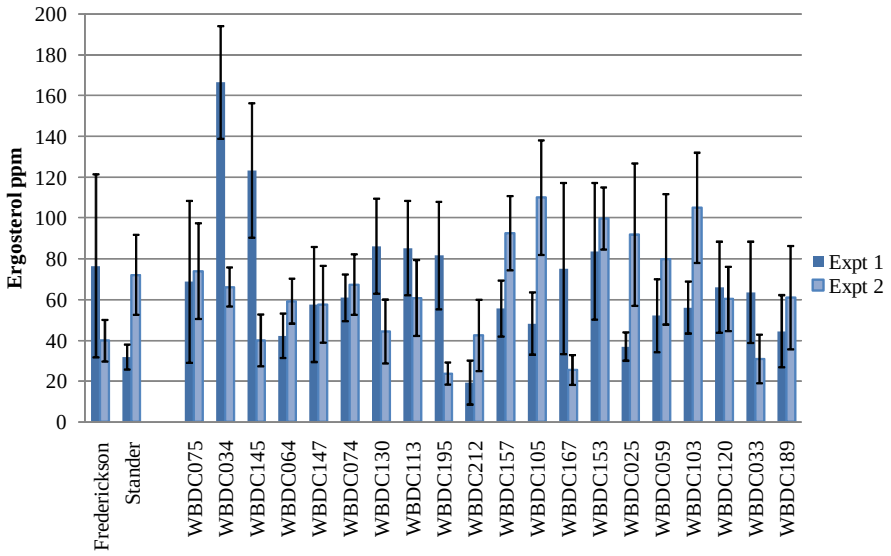

Figure 3. Ergosterol ppm 72 hours after inoculation of single spikelets.

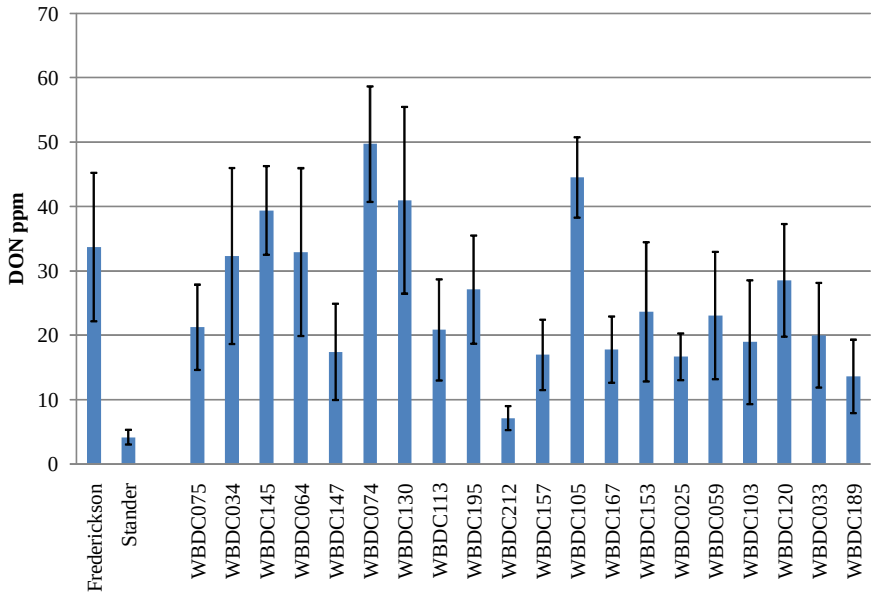

Figure 4. DON ppm 72 hours after inoculation of single spikelets for experiment 1. Variation among accessions was not significant in experiment 2.

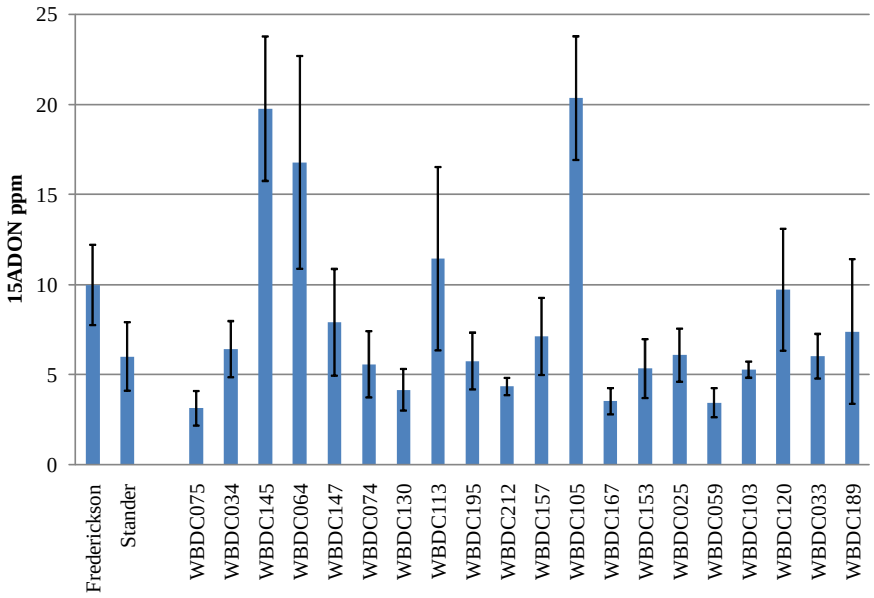

Figure 5. 15ADON ppm 72 hours after inoculation of single spikelets for experiment 1. Variation among accessions was not significant in experiment 2.

DISCOVERY AND MAPPING OF SINGLE FEATURE POLYMORPHISMS IN WHEAT USING AFFYMETRIX ARRAYS.

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ABSTRACT

Affymetrix arrays have been used to discover single feature polymorphisms (SFPs) in several crop species. To demonstrate the utility of the Affymetrix GeneChip® Wheat Genome Arrays in SFP discovery and mapping in wheat (*Triticum aestivum* L.), complimentary RNAs synthesized from mRNA isolated from seedlings of 71 F_{8-12} recombinant inbred lines (RILs) from the cross of Ning 7840/Clark were hybridized to the Affymetrix array. SFP prediction on the array data was done following the method of Kirst et al. (2006). A total of 955 SFPs were selected and combined with simple sequence repeats (SSR) data for mapping. A high-density genetic map consisting of 923 SFPs and 269 SSR markers and covering 1,944 cM genetic distance was constructed with 877 SFPs assigned to 21 chromosomes. The SFPs were randomly distributed within a chromosome and effectively filled gaps between SSRs, but were unevenly distributed among different genomes. B genome had the most SFPs, and D genome had the least. Map positions of a selected set of SFPs were validated by SNaPshot analysis and comparison with previous EST physical mapping data. Results indicate that Affymetrix array is a cost-effective platform for SFP discovery and mapping using RILs. The new map will be an important source of markers for quantitative trait loci (QTL) detection and high resolution mapping.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

SINGLE NUCLEOTIDE POLYMORPHISM MARKERS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT.

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease in humid and semi-humid wheat growing regions of the world. The quantitative trait locus (QTL) on 3BS (Fhb1) of Sumai 3 and Ning 7840 has been identified to have the largest effect on FHB resistance. Simple sequence repeat (SSR) markers flanking the Fhb1 are identified. These SSR markers have been widely used for marker-assisted screening of Fhb1. However, the SSR markers flank a relatively large chromosome region of the QTL and more closely linked markers to the QTL may improve selection efficiency. The rich sources of wheat expressed sequence tags (ESTs) and abundance of single nucleotide polymorphism (SNP) markers makes SNP ideal markers for fine mapping. We developed SNP markers based on wheat ESTs that mapped to the 3BS QTL region. A total of 15 SNPs were identified between Ning 7840 and Clark (FHB-susceptible) based on sequence analysis of three different ESTs. SNP primers were designed and the single base extension method was used to analyze the SNPs in 125 Ning 7840 /Clark recombinant inbred lines. Three SNP markers mapped between *Xgwm533* and *Xgwm493*. Two of them, *Xsnp-21-1* and *Xsnp–20-1a*, have higher coefficient of determination (R^2) than *Xgwm533* and should be good markers for marker-assisted selection of Fhb1 QTL in breeding programs.

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TOWARDS RAPID CANDIDATE GENE DISCOVERY IN THE BARLEY CHROMOSOME 2(2H) BIN 10 FUSARIUM HEAD BLIGHT RESISTANCE QTL.

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INTRODUCTION

The U.S. Wheat and Barley SCAB Initiative has now been funding research for over ten years and the genes controlling resistance to Fusarium Head Blight (FHB) in barley are still unknown. Many sources of resistance have been reported, but the highest, most stable source of resistance known is located on chromosome 2(2H) (Dahleen et al., 2003; de la Pena et al., 1999; Hori et al., 2005; Hori et al., 2006; Horsley et al., 2006; Kolb et al., 2001; Ma et al., 2000; Mesfin et al., 2003; Zhu et al., 1999). Our chromosome 2(2H) map has been delineated by QTL mapping (Horsley et al. 2006) and by phenotyping of our recombinant lines to include only bin 10 (MWG699-MWG503). The importance of this region has been challenged by the recent findings of Sato et al. (2008) who failed to find a FHB resistance QTL in the *Vrs1* region but did find a major QTL in the *cly1/Cly2* region on chromosome 2(2H) at approximately bin 14. Sato studies were done with crosses between 2-rowed lines using a single resistance source and a cut spike FHB assay. Mesfin et al. (2003) also reported a major FHB resistance QTL in the *cly1/Cly2* region, detectable only in greenhouse studies. These results suggest that environmental conditions and the source of resistance may play a major role in the detection of the *cly1/Cly2* QTL. The *vrs1* mutant we have isolated in CIho4196 (see below) should help resolve this controversy.

Though the bin 10 map is quite saturated, genetic mapping continues—a process that has gained momentum with the development of PCR-based markers allowing a cleaved amplified polymorphic sequence (CAPS) marker to be mapped in a single day. Many of our random fragment length polymorphism (RFLP) markers have been converted to CAPS markers, making genotyping faster and safer. Our physical map of the region is also reasonably saturated and with adequate funding we are prepared to sequence 24 bacterial artificial chromosomes (BACs) with the purpose of rapid gene discovery.

Mutagenesis of CIho4196 with subsequent analysis and phenotyping has resulted in several mutants of agronomic interest including 6-row, early, and semi-dwarf, which retain CIho4196-like levels of FHB resistance. The *vrs1* mutant giving a 6-rowed phenotype contains a nine base pair deletion in the homeobox domain, a mutation unlike any previously reported 6-rowed varieties or mutants (Komatsuda et al., 2007).

RESULTS AND DISCUSSION

Phenotyping of quantitative disease resistance is difficult, at best, and FHB phenotyping is no exception. The recombinant lines previously reported (Boyd et al., 2007) were tested for FHB levels in a China and a North Dakota nursery in 2008. There are 22 lines derived from recombinants 07-76 and 07-84 indicating that the FHB resistance QTL resides at marker BG365406 (co-segregates with Uni4780 and BF254012). However, this is not confirmed by recombinants 07-85-1 and 07-97, which appear to have this chromosome region and yet are susceptible (Fig. 1). Recombinants derived from 07-91 indicate that the region at marker ctg15632 (co-segregates with MWG503) may be important, which is supported by the QTL mapping of Zhu et al. (1999). However, recombinants derived from line 07-87 have this marker, lack the marker BG365406, and are susceptible. The

simplest explanation may be that both regions contribute. Line 07-90-8 appears resistant in the first trial and susceptible in the next, underscoring the difficulties of phenotyping QTL-controlled resistance and the importance of finding closely linked genetic markers.

Due to the above difficulties in further refining the location of the FHB resistance QTL, we chose to saturate the genetic and physical map for the entire bin 10 region from *Vrs1* to MWG503 (Fig. 2). There are 10 loci in this region represented by 26 markers and 152 BAC clones. The region just below *Vrs1* was fine-mapped by Pourkheirandish et al. (2007), providing further markers for BAC identification (markers identified in gray, Fig. 2). We recently mapped the rRN5S1 gene to our target region. Its tandem repeats may account for the lack of genes in the area (Kanazin et al., 1993). We continue to identify and map additional markers and select BAC clones for this region, currently focusing on the recently released *Brachypodium distachyon* sequence as a possible source (brachypodium.org).

Using the contigs identified in the barley physical map database from the Tim Close lab and hybridization of *Hind*III-digested BAC clone filters, we eliminated redundant BAC clones and those from loci that do not map to this region. The minimum tiling path of 24 BAC clones makes up 14 contigs covering more than 2 Mb of the genomic region (Fig. 2). For rapid marker and candidate gene detection, the 24 total BAC clones must be sequenced.

Gamma irradiation of CIho4196 seed has proved quite successful in developing lines with better agronomic qualities that maintain FHB resistance. The 6-row line, designated g07-014, contains a unique mutation in the *vrs1* gene and is available for use in breeding programs, as are the early and semi-dwarf lines (Boyd et al., 2008).

ACKNOWLEDGEMENTS

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DISCLAIMER

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

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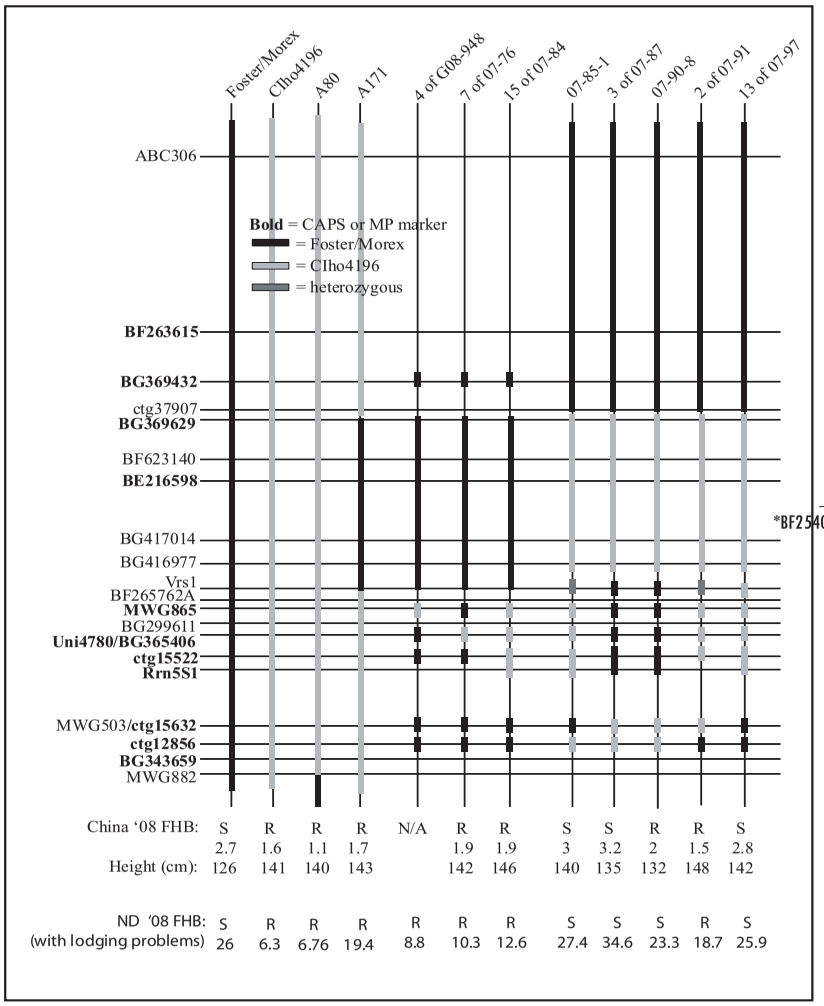

Fig. 1 Genotype of recombinants lines and phenotyping data from one year in China and one year in North Dakota. The recombinants were isolated from crosses of A171 or A80 with Morex.

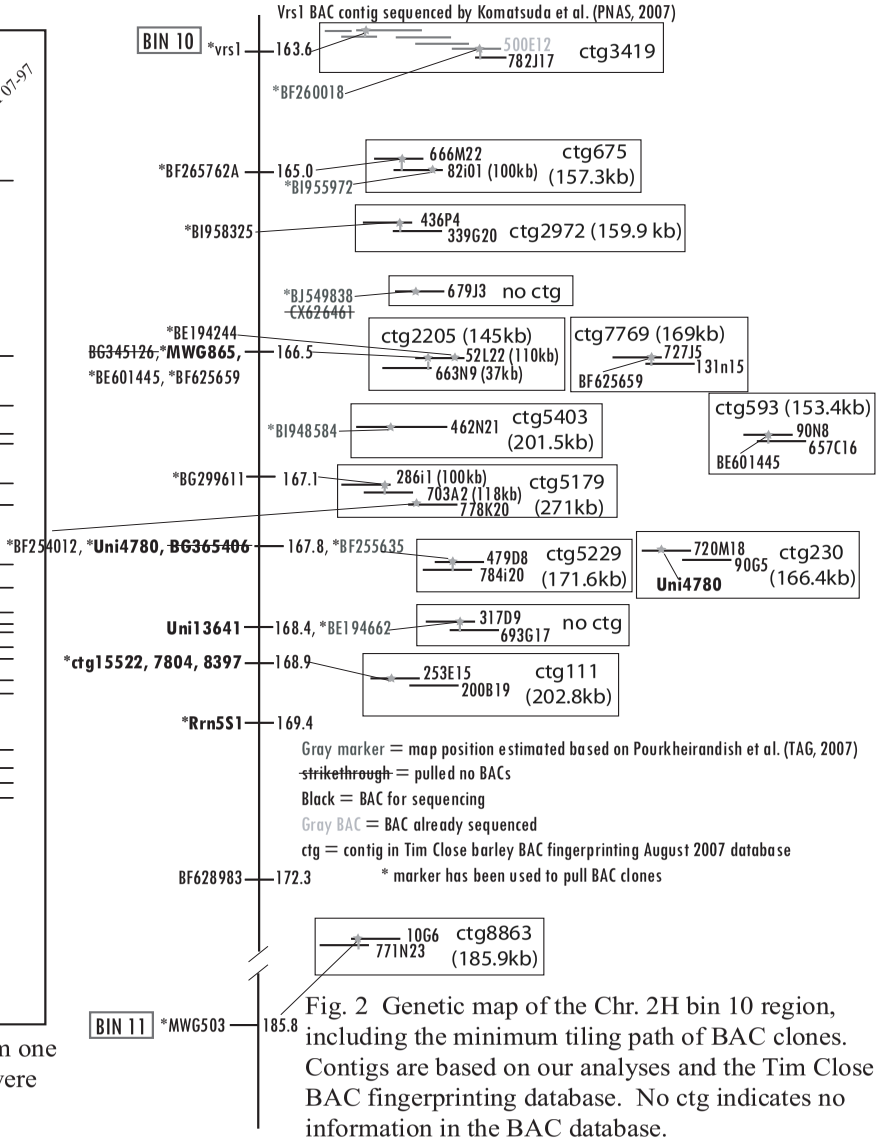

Fig. 2 Genetic map of the Chr. 2H bin 10 region, including the minimum tiling path of BAC clones. Contigs are based on our analyses and the Tim Close BAC fingerprinting database. No ctg indicates no information in the BAC database.

MARKER-ASSISTED SELECTION FOR FHB AT THE EASTERN REGIONAL SMALL GRAINS GENOTYPING LAB.

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ABSTRACT

Head scab of wheat, caused by *Fusarium graminearum*, is a disease that affects wheat production in the Eastern soft wheat growing region of the U.S.A.. Genotyping and marker-assisted selection (MAS) are being applied in the Eastern wheat growing region to develop resistant wheat varieties and to characterize germplasm. Since 2005, the Eastern Regional Small Grains Genotyping Lab has worked with breeders in eastern region to conduct MAS for FHB resistance. During 2008, samples for screening with markers linked to FHB resistance QTL were received from 13 soft wheat breeding programs. The *Fhb1* resistance gene is by far the most frequent gene being deployed by MAS. However, marker-assisted selection has also been conducted for resistance QTL on chromosomes 5A, 2D, 4B, 6B, 2B, and 3BS near the centromere. In addition to MAS, genotyping with markers linked to FHB resistance is also done on collaborative regional nurseries. The 2007 and 2008 Uniform Southern Fusarium Head Blight Nursery and Northern and Preliminary Northern Uniform Winter Wheat Scab Nurseries, were screened for SSR markers linked FHB resistance QTL on chromosome 3BS (*Fhb1*), 5AS, and 2DL using SSR markers. The 2008 nurseries were also screened with markers linked to the 3BS centromere QTL mapped in Ernie, as well as markers associated with genes for resistance to leaf, stripe and stem rust, the Hessian fly, and barley yellow dwarf virus. Markers for the *Bx7^{oe}* allele for gluten strength, reduced height genes *Rht-B1* and *Rht-D1* and the short arm of rye chromosome 1R were also evaluated. No lines were found in any nursery evaluation to contain all the scab resistance QTL evaluated. In 2007, seven lines evaluated had at least one scab resistance QTL in the NUWWSN, while the USFHBN had six lines with at least one QTL. In 2008, the number of lines postulated to have a mapped resistance QTL increased to 12 lines and 11 lines in the NUWWSN and USFHBN, respectively.

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COMPARISON OF TWO FUSARIUM HEAD BLIGHT INOCULATION METHODS IN WHEAT.

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ABSTRACT

Fusarium head blight (FHB), or head scab, is a widespread and destructive disease of wheat and barley. Identifying breeding lines with host plant resistance to FHB is an important breeding objective. Many inoculation and evaluation methods are used to identify breeding lines with resistance to FHB. Often, the phenotypic data collected using different inoculation and evaluation methods are poorly correlated. Our objectives in this study were to determine if FHB resistance ratings from two different inoculation methods were highly correlated, and if the same breeding lines with the highest resistance were selected using the two inoculation methods. The two inoculation methods used were a spray-and-bag method using a macroconidial suspension and an infected grain spawn with mist irrigation system to enhance natural infection. If data from the two methods agree, the spray and bag method would provide a way to evaluate breeding lines in multiple environments and locations without establishing misted, inoculated FHB evaluation nurseries at all sites. Both methods were slightly modified from a similar experiment in 2005. These methods were tested in 2008 on 87 lines in three separate experiments. Scab incidence and severity data were collected, and FHB index was calculated. Data from the two methods were combined and analyzed using the PROC CORR procedure of SAS with a significance threshold of $\alpha = 0.05$. Disease pressure was high in 2008 as indicated by the resistant check Ernie. Scab incidence ($r = 0.41$), severity ($r = 0.83$), and FHB index ($r = 0.76$) were significantly correlated between the two methods. Based on these preliminary results it is possible to obtain highly correlated FHB resistance ratings between two different inoculation methods. Analyzing the data for a subset of the lines with a FHB index in the top 20% and bottom 20% based on the grain spawn infection method increased the correlations between the two methods slightly for incidence and FHB index, but not for scab severity. More than half of the breeding lines with the most resistance under the grain spawn/mist infection agreed with the most resistant lines under spray-and-bag infection. Some of the lines with the highest resistance using one method were not selected using the second method. Nevertheless, the high linear relationships between methods indicate that either method is useful for selecting breeding lines; however, additional data collected in multiple environments are required to validate these results. It appears that the spray and bag method may have potential to supplement data from misted, inoculated FHB evaluation nurseries, but should not be used in place of data collected in these nurseries.

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

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ABSTRACT

The use of resistant wheat cultivars is an effective means of reducing losses due to Fusarium head blight (FHB), caused by *Fusarium graminearum*, and deoxynivalenol (DON) accumulation. Breeding efforts have produced FHB-resistant wheat lines, but many of these lines yield less than FHB-susceptible wheat cultivars when FHB pressure is low. Farmers frequently plant FHB-susceptible wheat cultivars with the intent of spraying fungicides when necessary. Recent fungicide technology has greatly improved control of FHB in wheat and barley, but fungicides do not provide complete control of FHB. Most fungicide efficacy studies report a significant yield increase in treated plots compared to the untreated check. Fungicides with triazole chemistry are the most effective in reducing FHB and DON. Our objectives were to evaluate the effectiveness of host plant resistance and fungicide treatment for suppression of FHB and DON accumulation and the effect of FHB on yield and test weight. Using an inoculated and irrigated disease nursery we tested two triazole fungicides, tebuconazole (Folicur®) and tebuconazole + prothioconazole (Prosaro®), and twelve wheat cultivars ranging from FHB susceptible to FHB resistant. The experiment was a split-plot design with fungicide treatment as the main plot and variety as the sub-plot with four replications. Data were collected on scab incidence, severity, FHB index, Fusarium damaged kernels (FDK), incidence/severity/kernel quality index (ISK index), DON, yield, and test weight were all measured. Both fungicide and cultivar had a significant effect on all variables. Significant interactions between fungicide and cultivar were detected for FHB incidence, FDK, ISK index, DON, yield, and test weight. In individual untreated plots, FHB incidence ranged from 10% to 100% thereby confirming high disease pressure and varying cultivar FHB resistance levels. Yield varied greatly (110.0 – 67.5 bu/A) and test weights were moderate to low (58.3 – 48.7 lbs/bu). Averaged over all cultivars both Folicur and Prosaro significantly increased yield and test weight, and lowered ISK index and DON. Folicur increased yield by an average of 9.5 bu/A and decreased DON by an average of 44%, while Prosaro increased yield by 13.8 bu/A and decreased DON by 67%. Prosaro treated plots significantly outperformed Folicur treated plots in yield, test weight, incidence, FHB index, FDK, and ISK index. The three cultivars with the greatest host resistance in the untreated plots realized the lowest yield increase from the addition of fungicides, whereas, except for one cultivar, the most susceptible cultivars realized a greater than 20% increase in yield with Prosaro fungicide. Notably, in the untreated plots, the most resistant cultivar, IL02-18228, had the lowest DON level (0.7 ppm) and the highest yield and test weight. IL02-18828 in the untreated plots yielded more than all but one of the susceptible cultivars even when these six cultivars were treated with Prosaro, the most effective fungicide. This is preliminary data from one year, but our data indicate that under severe FHB pressure, wheat producers can produce high yields of sound grain, with DON below the FDA's guideline of 1 ppm, by use of cultivars with good FHB resistance in combination with either Folicur or Prosaro.

CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN ALSEN-FRONTANA-DERIVED RECOMBINANT INBRED LINES.

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OBJECTIVES

Evaluate the recombinant inbred lines (RILs) of wheat for resistance to FHB and DON accumulation, and characterize the RILs with known SSR markers.

INTRODUCTION

Fusarium head blight of wheat (FHB), caused primarily by *Gibberella zeae* is a destructive disease of wheat, and other small grains worldwide (McMullen et al., 1997). In the United States, the fungus causes millions of dollars in losses and poses serious socio-economic problems (Nanje et al., 2004). The fungus produces several mycotoxins that cause serious health problems to both humans and livestock (McMullen et al., 1997).

Mesterházy et al. (1995) reported five different types of resistance to FHB in wheat. Among them, type II resistance has been studied extensively and reported to be more genetically stable than other types of resistance (Bai and Shaner, 2004; Mesterházy, 1995). Chinese wheat cultivar 'Sumai 3' (PI481542) and its derivatives, which exhibit the type II resistance, are widely used in wheat breeding programs worldwide (Bai and Shaner, 2004). Brazilian cultivar 'Frontana' (PI500147) is believed to exhibit type I resistance for FHB and has also been used in breeding programs of wheat (Singh et al., 1995; Van Ginkel et al., 1996).

North Dakota State University (NDSU) has released the FHB-resistant cultivar 'Alsen' (PI615543) (Frohberg et al., 2006) developed from Sumai 3, which is widely grown in the Midwest of the United States (USDA, 2007). To combine the type I resistance in Sumai 3 background, spring wheat cultivar Alsen was crossed with Frontana and W9207 (Chinese wheat line); consequently, 135 F_9 recombinant inbred lines (RILs) were developed. The long-term goal of this study was to develop wheat germplasms for durable resistance to FHB by combining both type I and II resistance.

MATERIALS AND METHODS

Inoculation and Disease Assessment: One hundred thirty-five F_9 RILs developed from crosses between Frontana/W9207//2*Alsen, were evaluated for FHB reactions and DON content during fall 2006 and spring 2007. Nine spikes of each RIL were spray-inoculated at mid anthesis (Zadok's scale 65) in the greenhouse. The FHB severity was assessed 7, 14, and 21 days after inoculation (DAI) as described previously (Stack and McMullen, 1998). The area under disease progress curve (AUPDC) was calculated from FHB severity values taken 7, 14, and 21 DAI (Campbell and Madden, 2006). FHB severity values estimated at 7 DAI were used to measure the resistance to initial infection (type I), while FHB severity values estimated 21 DAI and AUDPC were used to measure the resistance to fungal spread (type II resistance). The terms 'initial disease severity' (IDS) and 'final disease severity' (FDS) referred to the FHB severity 7 DAI and 21 DAI respectively. The inoculated spikes were harvested and threshed manually. The Fusarium damaged kernels (FDK) per spike were counted for parents and each RIL. Deoxynivalenol (DON), 3-ADON (3-Acetyldeoxynivalenol), 15-ADON (15-Acetyldeoxynivalenol), and NIV (Nivalenol) were estimated from these grain samples using gas chromatography (Tacke and Casper, 1996)

at the Veterinary Diagnostic Laboratory, NDSU, Fargo, ND. Analysis of variance (ANOVA) was performed for each greenhouse experiment using SAS (version 9.1, Statistical Analysis System; SAS Institute, Cary, NC).

Molecular Marker Analysis: Genomic DNA was extracted as described previously (Burlakoti et al., 2007) with some modifications. Forty two resistant RILs were characterized with seven SSR markers known to be linked to FHB resistance (GWM493, GWM533, BARC133 and BARC147 on the 3BS, WMC397 and WMC398 on the 6BS, BARC197 on the 5AS). The PCR amplification was performed as described in Roder et al. (1998) in a PTC-100 Thermal Cycler (MJ Research, Watertown, MA).

RESULTS AND DISCUSSION

Data analysis showed that the variances of experiment, RIL, and RIL × experiment were highly significant ($P < 0.001$) for IDS, FDS, AUDPC, and FDK (Table 1). Among the three parents, Alsen had lowest FDS (28.16%), AUDPC (319.49) and DON (7.90 µg/g), and Frontana had the lowest IDS (13.47%) (Table 2). The RIL population showed larger variation for all these three FHB parameters and FDK; however, their means did not deviate significantly from the parental means (Table 2). The average value of DON content for the RIL population was lower (10.11 µg/g) than the parental mean value (14.22 µg/g) (Table 2). Among the RILs, 22 lines had less than 10% IDS and 5 µg/g DON content, and 20 lines had 10-30% FDS. Approximately 11% of the RILs showed higher levels of resistance to initial infection (type I), FHB spread (type II), and DON accumulation (type v) than the resistant parents.

In molecular marker analysis, 78.57%, 80.95%, 95.23% and 88.09% of the resistant RILs showed the Alsen type allele for GWM493, GWM533, BARC133, and BARC147 (3BS), respectively. Similarly, 78.57% and 73.80% of resistant RILs showed Alsen type allele for WMC397 and WMC398 (6BS), respectively. On the other hand, 59.53% of resistant RILs showed Frontana type allele for BARC197 (5A). Among the 24 resistant RILs exhibiting overall resistance to IDS, FDS and DON, 83% RILs showed Alsen type alleles for markers from 3BS and 6BS, and Frontana type allele for marker BARC197, indicating that these resistant RILS had markers linked to both type I and II resistance. This result suggests that that the combination of the two sources (type I and type II) of resistance may provide high levels of resistance to initial infection, FHB spreads, and DON accumulation, and these RILs may be useful for FHB resistance breeding programs of wheat.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA.

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Table 1. Mean square of 113 recombinant inbred lines (RILs) and three parents for initial disease severity (IDS) (%), final disease severity (FDS) (%), area under disease progress (AUDPC), and Fusarium damaged kernel (FDK) per spike.

Source of Variation	Df	IDS (%)	FDS (%)	AUDPC	FDK
Experiment†	1	7449.12***‡	20427.12***	1564753.79***	0.14**
Rep (Exp)	4	272.99**	1063.07**	160399.90***	183.97***
Treatment	115	277.35***	1116.09***	107736.85***	43.32***
Treatment × Exp	115	141.29***	640.01***	60749.27***	25.05***
Error	460	70.34	257.07	25333.81	13.77
CV (%)		51.68	38.32	38.33	54.07

†After homogeneity test, data from both experiments conducted in greenhouse were combined and analyzed using SAS.
‡ ** and ***, Indicates significant at $P < 0.01$ and $P < 0.001$, respectively.

Table 2. Mean values of 113 recombinant inbred lines (RILs) and three parents averaged for two greenhouse experiments for initial disease severity (IDS) (%), final disease severity (FDS) (%), area under disease progress (AUDPC), Fusarium damaged kernel (FDK) per spike, and deoxynivalenol (DON) content.

Parameter	IDS (%)	FDS (%)	AUDPC	FDK	DON (µg/g)
RILs mean	16.22	41.83	408.28	6.85	10.11
W9207	21.50	64.59	573.49	8.94	16.60
Frontana	13.47	32.93	331.49	7.46	18.15
Alsen	14.97	28.16	319.49	3.65	7.90
Mean parental value	16.65	41.89	408.11	6.68	14.22
LSD ($P < 0.05$)	9.52	18.19	180.60	4.18	-

THE ICARDA PROGRAM FOR BREEDING FHB RESISTANCE IN BARLEY.

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ABSTRACT

ICARDA, in cooperation with CIMMYT, has been producing barley with enhanced resistance to Fusarium head blight (FHB) since the early 1980s. ICARDA's germplasm bank in Syria offers a diverse reservoir of genes that are being explored as new sources of resistance for this devastating disease. The crop's wild relatives represent even richer reservoirs of genes for stress tolerance and adaptation, as their history in the Central and West Asia and North Africa (CWANA) region is very long and includes periods with very harsh climate in the Pleistocene Era. The ICARDA barley breeding program started research on FHB resistance in response to the needs of the Andes countries. In 1986, a total of 5,000 barley accessions were screened in Mexico; of these, 23 were found with some level of resistance, and were subsequently intensively introgressed into the main program. Resistance sources were shared with programs worldwide, especially after the FHB outbreaks of the 1990s. Collaboration and cooperative efforts with advanced research institutions, such as the Busch Agricultural Resources Inc. (BARI) and the US Wheat and Barley Scab Initiative (USWBSI), allow the project to make germplasm sources with enhanced levels of resistance widely available. Environmental conditions at the CIMMYT's Toluca Experiment Station in Mexico at the early years and El Batán since 2006 are ideal for FHB development and evaluation. In addition, the project obtains data through collaboration with programs in the USA, Canada, China, Ecuador, Brazil and Uruguay. A recent initiative to comprehensively screen ICARDA's gene bank for unique and undiscovered sources of resistance has identified some potentially promising barley sources. A BARI/ICARDA collaboration line (ADV BARI 57) continues to show lowered levels of DON and is now being used in crossing blocks with superior malting parents. Several other ones have been found as highlights in the nursery network carried out in cooperation. Overall, this collaboration has illustrated the need for multi-year data collection and the usefulness of FHB disease nurseries for barley breeding.

MOLECULAR MARKER-ASSISTED EVALUATION AND CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT GENOTYPES GROWN IN THE PACIFIC NORTHWEST.

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ABSTRACT

This study was to deploy molecular marker-assisted selection and to evaluate and characterize Fusarium head blight (FHB) resistance in wheat genotypes grown in the Pacific Northwest (PNW). A total of 276 wheat genotypes from all classes except soft red winter wheat were evaluated and characterized with 13 markers flanking the six known FHB QTL (2DL, 3AS, 3BS, 5AS, and 6B) previously identified. Seventy-eight genotypes were released and adapted cultivars from California, Utah, Colorado, Washington, Oregon, and Montana. The rest of 198 genotypes were University of Idaho released cultivars and advanced lines. These genotypes have no Sumai3 related backgrounds. By comparing haplotypes of 276 genotypes with four known resistance sources Sumai 3, W14, Renwood3260, and Ernie, we found that the six known QTL existed in the 276 genotypes. Especially, the known UMN10 marker allele on 3BS was present in 66 lines out of the 276 genotypes studied. Among the 66 genotypes, twenty have combined three QTL of 3BS, 2DL, and 6BS. Eight of the twenty have additional 3AS QTL combined; while five of the twenty have additional 5AS QTL combined. The WMC 152 marker allele on 6BS was common and present in 108 lines; while the wmc264 marker allele on the 3AS QTL was rare and only present in 10 genotypes. The Gwm120 marker allele on the 2BS QTL was present in twenty-three genotypes, the gwm261 marker allele on the 2DL QTL was present in thirty-eight genotypes, the known Barc117 marker allele on 5AS was present in 68 lines. These identified cultivars/lines having good field FHB resistance and/or known FHB resistance QTL can then be grown in PNW region and be used as adapted resistance sources in the PNW and Great Plains breeding programs.

HAPLOTYPE ANALYSIS OF GENES FOR FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID WHEAT GERMPLASM.

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ABSTRACT

Haplotype analysis at the molecular marker loci associated with the known Fusarium head blight (FHB) resistance QTL in wheat can be used to identify resistance genes in the resistant germplasm, and thus provides practical information of pyramiding different sources of resistance for the development of resistant germplasm or cultivars. In this research, we analyzed haplotypes of 132 tetraploid wheat accessions with various levels of FHB resistance at 19 molecular marker loci associated with the known FHB resistance QTL on the chromosomes 2B (*Triticum carthlicum* 'Blackbird'), 3A (*T. aestivium* 'Frontana' and *T. dicoccoides* 'Israel A'), 3B (*T. aestivium* 'Sumai 3' and 'Wanshuibai'), 5A ('Sumai 3' and 'Frontana'), 6B ('Blackbird' and 'Wangshuibai'), and 7A (*T. dicoccoides* PI 478742). Among the tetraploid wheat accessions included 40 accessions of *T. carthlicum*, 81 accessions of *T. dicoccum*, and 9 accessions of *T. turgidum*. We found 43 accessions, including two accessions of *T. carthlicum*, one accession of *T. turgidum*, and 39 accessions of *T. dicoccum*, showed different haplotypes at all the marker loci investigated, suggesting that these accessions may carry FHB resistance genes different from those in the known resistance sources. The novel FHB resistance genes carried by the tetraploid wheat accessions identified in this research could be utilized to enhance FHB resistance of durum wheat as well as bread wheat.

INTROGRESSION OF EXOTIC QTL INTO SOFT RED WINTER WHEAT USING MARKER-ASSISTED SELECTION AND EVALUATION OF NEAR-ISOGENIC LINES FOR SCAB RESISTANCE.

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ABSTRACT

Scab of wheat, caused by *Fusarium graminearum*, is a disease that periodically strikes the U.S. mid-Atlantic region. Breeding for resistant wheat varieties is an effective method of disease control. The objective of this study was to develop scab resistant soft red winter wheat germplasm adapted to the US mid-Atlantic region using marker-assisted selection. McCormick, a genotype adapted to the Mid-Atlantic region, was used in a backcross program with the Chinese variety Ning7840. An accelerated backcross scheme was developed to incorporate scab resistance QTL found on chromosomes 3BS, 5A and 2DL in Ning7840. Two rounds of backcrossing were completed using McCormick as the female parent. Progenies from the first round of backcrossing were selected for the presence of the Ning7840 scab resistance alleles at 3BS, 5A, and 2DL and for a high background of McCormick alleles. Two backcross progenies had over 60% McCormick background. Using these two selected BC_1F_1s, 400 BC_2F_1s were produced in a second round of backcrossing. Additionally, the two selected BC_1F_1s were crossed with a wheat line with leaf and stripe rust resistance (Southern States 8641). 800 BC_2F_1 seeds were screened with molecular markers to identify those with Ning7840 alleles and a predominance of McCormick background. A single BC_2F_2 population derived from a selected BC_2F_1 plant was screened with markers to select those homozygous for the resistant alleles. Additionally, we derived eight near-isogenic lines (NILs) from this BC_2F_2 population. Seven BC_2F_3 NILs segregated into both awned and awnless types. A field study conducted in Salisbury, MD in 2007/2008, showed that the combination of scab-resistant QTLs in 3BS and 2DL conferred the lowest deoxynivalenol (DON) content: 1.7 ppm and 1.1 ppm for awned and awnless lines, respectively. We plan to further characterize the BC_2F_4s derived in field and greenhouse studies in 2008/2009 at Maryland and Kentucky. In the fall of 2008, F_4 seed of McCormick and SS8641 derivatives with scab QTLs were distributed to seven breeding programs (AR, GA, KY, LA, NC, VA, and Westbred) for crossing and further evaluation.

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DEOXYNIVALENOL (DON) ACCUMULATION IN EIGHT WHEAT LINES WITH VARIOUS FUSARIUM HEAD BLIGHT RESISTANCE GENES.

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ABSTRACT

Breeding new cultivars, particularly with fusarium head blight (FHB) resistance genes from different sources combined is considered to be the most effective and durable approach to reduce crop production losses (Mesterhazy, 1995) and to minimize DON accumulation in the grain. The objective of this study was to compare DON concentration at four dates, at approximately weekly intervals, after grain physiological maturity (during June – early July) in grain of eight wheat cultivars that differ significantly in FHB resistance, in 2007 and 2008 at Lafayette, IN. DON accumulation was significantly greater in FHB susceptible wheat lines than in wheat lines with various resistance genes, both in 2007 (with limited rainfall in June) and 2008 (with more and also more frequent rainfall).

LINKAGE DISEQUILIBRIUM ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN TUNISIAN DURUM WHEAT.

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ABSTRACT

To expand the number of genes for FHB resistance in gene pyramiding programs, it is necessary to find genetically varied sources of resistance. In this study we used 184 BC1F6 and 189 BC1F7 lines derived from crossing of Tun7, Tun18, Tun34, Tun36 (all lines identified as sources of resistance from Tunisia) with durum cultivars 'Ben', 'Maier', 'Lebsock' and 'Mountrail' for association studies. As the pedigree of Tunisian lines show no relation to the popular Chinese sources of resistance, they could potentially carry different genes or alleles for resistance to FHB. We checked the parents and RILs in the greenhouse in two seasons for type II resistance to FHB by single floret injection inoculation method. The data showed that the Tunisian lines have different amount of resistance varying from 23% to 11% infection rate through the spikes as compared with D8750 (susceptible control) and Sumai3 (resistant control) with 41% and 9% of infection rate respectively.

We conducted the Diversity array (DArT) marker analysis to have a good coverage of the whole genome. DArT analysis used 2300 markers which showed 25% polymorphism between the parents. About 8% of the polymorphic markers were present in all the Tunisian lines but not the susceptible cultivars. The cluster analysis of the polymorphic markers revealed three distinct groups. Tun7 was in a separate group far from the other two and all the other Tunisian lines fell in a separate group from susceptible cultivars. As both Tun7 and Tun18 are more resistant to FHB than others and have different genetic backgrounds, they may be considered as potential candidates for new sources of resistance.

Linkage disequilibrium analysis on DArT markers revealed seven QTL associated to FHB resistance in Tun34 pedigree were located on Chromosomes 3B, 6B, 2A, 5B, 1B, 7A and 7B. Tun18 carries three QTL on 7B, 7A and 1B for FHB resistance. The 1B QTL were exactly in the same location as the one from Tun34 population. We have found three QTL on 3AS, 1BL and 2AL associated to FHB resistance in the Tun7 pedigree but the 2AL region associated with increased resistance was from the susceptible source parent. With this study we revealed some QTL in the same chromosome as had been reported in hexaploid wheat, which may change the belief of lacking the genes for FHB resistance in durum wheat as compared to hexaploid wheat. This may lead to future analysis identifying loci that may act as suppressors of resistance in the durum wheat making FHB resistance genes less effective compared to their action in hexaploid genetic background. Although we found several new potential QTL regions for FHB resistance in durum wheat, two regions located on 5B and 7B have not reported in the hexaploid wheat and may be valuable new sources for pyramiding once transferred into cultivated bread wheat background. This study also shows the power of pedigree based association mapping to find the minor QTL although we had problem with the pedigrees with less than 100 entries especially when there are selections in favor of the other agronomic traits beside FHB resistance.

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DEVELOPMENT OF FHB RESISTANT SPRING WHEAT IN THE NORTHERN GREAT PLAINS.

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ABSTRACT

Released in 2000, 'Alsen' was the first Hard Red Spring Wheat (HRSW) cultivar made available to growers in the northern Great Plains known to carry the major Fusarium Head Blight (FHB) resistance QTL, *Fhb1 (Qfhs.ndsu-3BS)*. With the progression of time, cultivars possessing *Fhb1* released by the North Dakota State University (NDSU), South Dakota State University (SDSU), and University of Minnesota (UMN) HRSW breeding programs have become more prevalent. Resistance sources derived from *Triticum dicoccoides*, as an example, have also been utilized. The cultivar 'Steele-ND' would be placed within this category. Although *Fhb1* and other resistance genes are presently found within most releases from these programs, their resistance is incomplete, and therefore, losses caused by FHB can still be significant. Continual germplasm screening efforts combined with marker-assisted selection are a requirement if further advances in resistance levels are to be realized. In an attempt to achieve this goal, the NDSU, SDSU, and UMN HRSW breeding programs each operate significant FHB resistance screening programs. Within each program, thousands of early-generation, preliminary, and advanced breeding lines are either screened at multiple field locations or in both field and greenhouse environments each year. Though not to the same extent, phenotypic observations are collected for disease incidence, severity, and disease index values as well as the frequency of Fusarium damaged kernels and deoxynivalenol concentrations. Significant procedural differences exist among the programs, although each is successful in identifying lines with progressively elevated FHB resistance. Classical phenotypic selection techniques coupled with an increased usage of molecular markers should allow resistance levels to gradually increase, although it is anticipated that progress will be more tempered after *Fhb1* becomes widely utilized.

VALIDATION OF A FAMILY-BASED QUANTITATIVE TRAIT LOCUS MAPPING APPROACH FOR SELECTION OF FUSARIUM HEAD BLIGHT RESISTANT SPRING WHEAT BREEDING LINES.

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ABSTRACT

Traditional Quantitative Trait Loci (QTL) mapping approaches are based on analysis of bi-parental populations. Mapping populations, however, are not widely known for the creation of new cultivars. In addition, markers linked to QTLs of interest are often not immediately available for use in breeding, and may never be useful within some genetic backgrounds. Use of multiple segregating populations for simultaneous QTL mapping, marker validation, marker-assisted selection (MAS) and prospective cultivar development has recently caught the attention of plant breeders because weaknesses of traditional mapping approaches can potentially be circumvented. Using a non-traditional family-pedigree based mapping approach, we previously localized the well-characterized Fusarium Head Blight (FHB) resistance QTL, *Fhb1 (Qfhs.ndsu-3BS)* within 82 segregating populations. The objective of this study was to demonstrate advantages of the family-pedigree based approach in the context of generating breeding lines for potential cultivar release. In the mapping portion of the study, ten Simple Sequence Repeat (SSR) markers on chromosome 3B, with *Gwm389*, *Gwm533*, and *Gwm493* being of most interest, were used to genotype F_1 plants. A single spike from heterozygous plants in each population was threshed individually and grown as a head row during winter 2007-2008. Each row was harvested in bulk and grown as a yield trial plot in 2008. Thirty F_3 spikes from each of 18 desirable yield trial plots were selected prior to harvest and a sample of F_4 seed from each spike was sown as a hill for FHB screening in the greenhouse during fall 2008. Four plants from each hill were also genotyped using the SSR marker *Gwm533*. Results from FHB disease screening and marker genotyping will be presented. Although the location of Fhb1 was known prior to initiation of this study, it was chosen to illustrate the speed with which 1.) this approach can localize QTL using widely applicable molecular markers and 2.) several FHB resistant breeding lines can be selected from within many agronomically acceptable populations.

CHARACTERIZATION AND DEVELOPMENT OF FHB RESISTANT SOFT WINTER WHEAT CULTIVARS IN THE EASTERN U.S.

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Fusarium Head Blight (FHB) epidemics have occurred frequently in many of the eastern soft winter wheat production regions of the U.S. where much of the wheat crop is planted directly into maize residue. Prior to 1990, few winter wheat breeding programs considered it necessary or placed a significant amount of emphasis on the identification and development of cultivars having resistance to FHB. Initially uncertainty prevailed as to whether cultivars having significant resistance to FHB could be derived from existing breeding populations in which parental lines had not been directly selected on the basis FHB resistance. As a result the initial goal of many breeding programs was to incorporate FHB resistance derived from Asian, South American, and other "Exotic" sources into adapted soft winter wheat backgrounds. Initial success was hindered by lack of reliable phenotypic and genotypic (DNA markers) selection capabilities and by the persistence of linkage of unfavorable traits, such as low yields, susceptibility to other prevalent diseases, shattering, poor winter hardiness, etc., to FHB resistance. Subsequently, very effective sources of "Native" FHB resistance were identified within the soft winter wheat germplasm pool. While native resistance remains the genetic base of most breeding programs for developing FHB resistant cultivars, the goal of many programs is to pyramid unique QTL or genes derived from both native and exotic sources to further enhance resistance to FHB and DON toxin accumulation.

FHB Resistance Identified in Native Sources: Upon evaluation of existing adapted winter wheat lines and cultivars in FHB nurseries, several of them were documented as having moderate to high levels of FHB resistance, and subsequently referred to as native resistance sources. Recent analysis of FHB resistance in the variety development program at The Ohio State University infers that genotypes having moderate resistance to FHB derived from native sources are fairly common. The data indicates that FHB resistance alleles come from many parents and exists at a relatively high frequency in soft winter wheat. Similar observations have been made in other soft wheat breeding programs. Many soft wheat lines evaluated in the Uniform Scab Screening Nurseries have moderate FHB resistance and lack or have little exotic parentage.

Soft wheat cultivars with native resistance have been released and some of their QTL have been mapped. The soft red winter (SRW) wheat cultivar Freedom, released by The Ohio State University in 1991, was among the first winter wheat cultivars identified with native resistance. The SRW wheat cultivar Ernie, released by the University of Missouri in 1994, was identified as having a moderately high level of native FHB resistance, which subsequently was mapped and reported to be conferred by QTL on chromosomes 2B, 3BSc, 4B, and 5A in greenhouse single-floret inoculation studies. Recent mapping studies using phenotypic data collected in inoculated, mist-irrigated field tests indicate that the QTL located on chromosome 4B in Ernie has a larger effect on FHB severity and *Fusarium* damaged kernels, while the awn inhibitor gene *B1* on chromosome 5A has a larger effect on FHB incidence. An even higher level of native FHB resistance was identified in the cultivar Truman, released by the University of Missouri in 2003, which currently is being mapped.

Mapping studies published to date indicate that the Asian QTL alleles for *Fhb*1 on chromosome 3BS appear to have been absent in native soft winter wheat prior to recent introgression efforts. Collectively, there have been nine chromosome regions implicated to confer FHB resistance in soft winter wheat with potentially coincident QTL on chromosome 2B in Ernie and Goldfield, chromosome 3B near the centromere in Ernie and Freedom, and chromosome 5AS in Ernie and Freedom. The QTL appear to have moderate to small effects with just two QTL producing an R^2 that exceeds 0.20.

During past eight years, more than 30 SRW and 2 SWW wheat cultivars having resistance to FHB have been released by public and private breeding programs. A majority of these cultivars were evaluated in the Uniform Scab Screening Nurseries and have native FHB resistance including: McCormick and Tribute (released in 2002); INW0304 (QTL on chromosome 2B), IL94-1653 (exclusive release), Neuse, and Truman (2003); INW0411 with QTL on 2AS and 2B (2004); Bess, Coker 9511, Jensen (SWW), USG 3342, and WestBred X00-1079 (2005); IL99-12976, IL00-8061, and IL00-8633 (exclusive releases, 2006); OH02-13567 (exclusive release) and Jamestown (2007); Bromfield, Coral (SWW), GA981621-5E34, IL00-8109, IL008530, IL01-11934, IL01-16170, IL02-19463 (exclusive releases), Malabar, and Pembroke (2008). Lines having native FHB resistant slated for release in 2009 include: B030543 and NC04-20814. While native FHB resistance currently comprises and will continue to provide a base level of resistance in winter wheat cultivars, only a few native sources has been genetically characterized and mapped. This remains a critical priority if genes in these potentially novel sources of resistance are to be effectively used, selected for and combined with genes from other unique native and exotic sources in cultivar development programs.

I***ncorporation of FHB QTL from Asian and European Sources:*** In an endeavor to incorporate novel FHB resistance and/or to enhance current resistance derived from native sources, many programs initiated efforts using a vast array of breeding methods to incorporate Type II FHB resistance, derived predominantly from a seemingly diverse array of Asian and other sources, into adapted winter wheat backgrounds. Subsequent emphasis has been placed on identifying diverse sources of Type II resistance as well as other unique types of resistance and their incorporation and combination in elite wheat lines. Of the QTL reported for FHB resistance, those located on chromosomes 1B, 2AS, 2B, 2DL, 3A, 3BS, 3BSc, 4BL, 5AS, 6B and 7B have been postulated as conferring resistance among current winter wheat cultivars and advanced elite lines. Winter wheat cultivars having FHB resistance derived directly from Asian (3BS and 5AS) and/or European (1B and 3A) sources or from diverse combinations of these with native sources include: Pioneer Brand 25R42 with *Fhb*1 (2001); 25R35 and 25R54 (2003); INW0412 (2004); Pioneer Brand 25R51 (2005) and; INW0801 (2008) having QTL on chromosomes 1B, 2AS, and 3A. While notably lower, the number of cultivars having FHB resistance derived from exotic versus native resistance sources has increased in recent years. This trend is expected to continue as more desirable cultivars and parental lines having FHB resistance derived from exotic sources have and are currently being used in breeding programs. Increased availability of more diagnostic and broadly applicable high-throughput PCR-based markers for validated FHB QTL is critical as this also will determine success in further enhancements of FHB resistance and variety development efforts.

Genotypic Assessment of FHB Resistance of Entries in Uniform Scab Nurseries: Initially (2001 – 2003) entries in the Southern Uniform Winter Wheat Scab Nursery (SUWWSN) were genotyped for markers (*Xgwm 493, Xgwm 533, and Xbarc133*) first reported to be associated with resistance conferred by *Fhb*1 (3BS). Few entries in these early nurseries had FHB resistance derived from Asian sources and only a few lines, such as VA01W-476 (Roane/W14) were postulated to possess *Fhb*1. Beginning with the 2005 SUWWSN, entries were genotyped using markers for both *Fhb*1 and the 5A QTL. Two entries (NC03-11457 and NC03-11458) were postulated to possess both QTL and three entries (NC03-11465, NC0311561, and VA04W-433)

likely possess *Fhb*1. Among entries in the 2006 SUWWSN, three (NC04-27617, NC04-27618, and NC04-27669) were postulated to possess both *Fhb*1 and the 5A QTL, and one entry (AR97002-2-1) putatively has the 5A QTL. In the 2007 SUWWSN, entries also were genotyped using markers for the 2DL QTL from Wuhan1. One entry (LA01096D-88) was postulated to possess both *Fhb*1 and the 2DL QTL, and three lines (NC05-25083, GA991109-6E8, and GA991109-6A7) putatively have the 5A QTL. In the 2008 SUWWSN, entries were genotyped for *Fhb*1 and for the QTL on 2DL, 3BSc, and 5A. One entry (LA01164D-94-2-B) was postulated to possess *Fhb*1 and the 5A QTL, six lines (LA01141D-138-4-B, LA01150D-79-7-B, LA01162D-131-8-B, NC05-25059, NC05-25062, and NC05-25066) likely have *Fhb*1, and two lines (M04-4715 and VA06W-608) putatively have the QTL on 3BSc.

Genotyping of entries in the Northern Uniform Winter Wheat Scab Nursery (NUWWSN) for FHB resistance was initiated by the USDA-ARS Genotyping Lab beginning with the 2007 nursery. One entry (MSU Line E6003) was postulated to possess *Fhb*1 and the 2DL QTL, MSU Line E6001 likely has *Fhb*1, MSU Line E6002 likely has the 2DL QTL, and two OSU lines (OH02-12678 and OH02-12686) putatively have the 5A QTL. In the 2008 NUWWSN, one entry (VA05W-775) was postulated to possess *Fhb*1 and four lines (MO-050921, VA05W-534, OH02-13567, and OH02-7217) likely have the 3BSc QTL.

Genotype assessment of the entries in the Uniform Scab Nurseries has been useful not only for determining if a particular line may carry a resistance QTL, but also in determining the potential usefulness of markers for conducting marker assisted selection in soft winter wheat populations. The results of the genotyping done on the nurseries are most reliable for lines resulting from crosses where the resistance can be traced by pedigree. For lines having Asian sources of resistance in their pedigrees, the markers linked to *Fhb*1 and the 5A QTL can reliably determine if a chromosome region was inherited from the resistant parent. This is due to the fact that closely linked markers are selected for genotyping; however, the frequency of the Asian alleles and/or haplotypes is very low in soft winter wheat. For the QTL mapped in native sources of resistance, it is more difficult to find these sorts of markers for genotyping. Therefore, using the results of marker analyses alone to predict the presence of a native QTL is less reliable. For instance, a survey of 250 soft wheat lines found that for markers linked to the 5A QTL mapped in Ernie, the frequency of the Ernie alleles ranged from 0.34 to 0.53. Thus, these markers are not suited for genotyping or for marker-assisted selection (MAS) and new more diagnostic markers linked to this QTL need to be identified. In contrast, the frequency of Ernie alleles for markers *Xgwm285* and *Xwmc612* in the 3BS centromere region ranged from 0.10 to 0.18 and the Ernie haplotype for markers across this region is not common. Thus, these 3BSc markers are better suited for MAS and are included in genotyping the Uniform Scab Nurseries. However, there is not yet conclusive evidence of an effect of this haplotype in conferring FHB resistance in lines unrelated to Ernie.

Availability of genotypic data for marker alleles associated with confirmed QTL governing FHB resistance in entries evaluated in Uniform Scab Nurseries is critical to the success and efficacy of variety development efforts in breeding programs. This information in combination with other agronomic data allows breeders to select parental lines having effective and unique FHB resistance and to implement marker assisted selection in the incorporation, pyramiding, and selection of FHB resistance in subsequent progeny and pure lines. Current lack of such information on a majority of FHB resistant lines identified in Uniform Scab Nurseries greatly hinders breeders' ability to select the best parental lines and subsequent progeny due to the lack of knowledge regarding the QTL/genes conferring resistance and markers to deploy in MAS.

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DISCLAIMER

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

RESISTANCE TO ACCUMULATION OF DEOXYNIVALENOL IN SOFT RED WINTER WHEAT.

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ABSTRACT

The ultimate goal of host plant resistance to *Fusarium graminearum* (Fg) is reduced concentration of the toxin, deoxynivalenol (DON), in the grain. Resistance to spread of infection by Fg (Type II = T2), and to a lesser degree resistance to initial infection (Type I = T1) have been documented in wheat. Combinations of T1 and T2 resistance do not provide complete resistance to Fg, and DON can be accumulated in grain of even our most resistant cultivars. In general, visual symptoms of FHB and are positively related DON, and selection for T1 and/or T2 resistance lowers DON. However, this association becomes highly variable in moderately resistant wheat genotypes. Resistance to accumulation of DON (Type V = T5) has been proposed, but not validated. In a FY07 USWBSI grant we attempted to assess T5 resistance in soft winter wheat. We collected grain from moderately resistant soft winter wheat breeding lines in field-grown, inoculated spikes with 0, 1, 2, or 3 infected spikelets per spike (approximately 0, 6, 12, or 18% infection based on visual symptoms). Fungal biomass was determined by quantitative real-time-PCR (qRT-PCR) for each grain sample from each infection level. For each wheat genotype, we regressed DON on estimated Fg biomass. The slopes were statistically different. In some genotypes, DON increased significantly as Fg biomass increased, while in other genotypes, the response of DON to increasing Fg biomass was negligible. The experiment was repeated in 2008 with three field replications of each wheat genotype. In 2008, Fg biomass ranged from <0.05 copies Fg/copy wheat to nearly 30 copies/copy wheat, and DON concentration ranged from < 0.3 ppm to 33 ppm. In the analysis of covariance, the 28 genotypes differed significantly for DON concentration ($p < 0.001$), and the response of DON concentration to Fg biomass was highly significant ($p < 0.001$). The interaction of genotype and Fg biomass also was highly significant. The 13 grain samples among the 345 characterized that had DON concentrations >20 ppm had an average Fg biomass of 9.0 ± 5.3 copies Fg/copies wheat; the 11 grain samples that had Fg biomass of >20 copies Fg/copies wheat had an average DON concentration of 8.8 ± 5.3 ppm. Overall, biomass did not effectively predict DON ($r^2 = 0.02$). Genotypes could be categorized based on response of DON concentration to Fg biomass, as well as by the level of Fg biomass developed, as some genotypes developed little Fg biomass despite visual symptoms of infection. Genotypes were identified that appear to suppress accumulation of DON, despite detectable fungal biomass. Resistance patterns observed in the preliminary 2007 study generally were consistent with the results of the replicated 2008 study. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-101. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

IDENTIFICATION OF WHEAT LINES WITH *FHB1* BY INJECTING DON INTO FLORETS AT FLOWERING.

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OBJECTIVES

To determine if the direct application of DON into florets is a useful method for identifying lines with *FHB1* in different genetic backgrounds and to determine if other FHB resistance genes confer similar resistance to DON.

INTRODUCTION

In North America, the deoxynivalenol (DON) chemotype of *Fusarium graminearum* is the primary cause of Fusarium head blight (FHB) in wheat. Because DON is toxic to humans and animals, wheat cultivars with low or no DON are desirable for wheat growers, processors and consumers (Bai and Shaner, 2004). DON also acts as virulence factor by enhancing the ability of *F. graminearum* to spread within a spike (Desjardins et al. 1996). Therefore, wheat lines more resistant to DON should be more resistant to FHB. Lemmens *et al*. (2005) applied DON directly into wheat spikes and counted the number of bleached florets. Resistance to DON was closely associated with *FHB1* that confers resistance to spread within a spike and with detoxification of DON to DON-3-*O*-glucoside.

MATERIALS AND METHODS

A susceptible check and 15 diverse FHB-resistant winter wheat lines were grown in the greenhouse. For each pot, two pairs of similar-sized spikes in the early flowering stage were selected and marked. One spike of each pair served as a control, and the other spike was treated with DON (250 µg in each of four primary florets) using a modification of the procedure described by Lemmens *et al*. (2005). The total number of florets per spike was counted at 7 days after treatment, and the number of DON-bleached florets below and above the treated florets was counted at 7, 14 and 21 days after treatment. Spikes were harvested at maturity and threshed by hand to retain all grain. The relative yield for the treated spikes was calculated as (yield of treated spike / yield of control spike) x 100. The experimental design was randomized design consisting of 16 wheat lines and five replications (pots) in run 1 and four replications in runs 2 and 3. Lines were treated as fixed effects and runs and replications as random effects. Wheat line means were separated using Tukey's HSD test at P=0.05.

RESULTS AND DISCUSSION

The six wheat lines with molecular markers linked to *FHB1* had fewer DON-bleached florets 21 days after treatment than the ten lines without *FHB1* (Table 1), indicating that presence or absence of this gene can be identified in diverse backgrounds by injecting DON into florets. All lines with *FHB1* had type II resistance as measured by the number of FHB-blighted florets (determined in previous experiments), but lines VA04W-433 and Fg 368 had the fewest FHB-blighted florets (Table 1), indicating that these lines may have additional genes for type II resistance. Nine wheat lines without *FHB1* had numbers of DON-bleached florets similar to the susceptible check but had fewer FHB-blighted florets than the susceptible check, indicating that these lines had type II resistance that was conferred by genes other than *FHB1*.

The resistance to DON conferred by *FHB1* did not protect plants from the phytotoxic effects of DON on kernel formation as measured by the relative yield of treated spikes. However, lines Fg 365 and Fg 368 had higher relative yield than other lines, indicating that they may posses one or more genes conferring toler-

ance to DON and ability to fill kernels in the presence of DON. Line Fg 365 was found to have the highest tolerance against FHB (Mesterházy et al. 1999), and therefore measuring the relative yield loss to DON injection may be a method for identifying lines with tolerance to FHB (type IV resistance). There was only a weak positive correlation ($R^2 = 0.16$) between relative yield and number of florets per spike, indicating that results were not overly influenced by the size of the spikes.

In conclusion, injecting DON into florets can readily detect lines with *FHB1*, which appears to be the only gene that confers resistance to DON. Furthermore, measuring relative yield after DON injection may be a useful method for identifying lines with tolerance to FHB (type IV resistance).

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DISCLAIMER

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Table1. Presence of *FHB1* in 16 winter wheat lines and the number of DON-bleached florets per spike after injection of DON, the number of FHB-blighted florets after single-floret inoculation with *Fusarium graminearum*, the relative yield of DON-treated spikes, and total number of primary florets per spike averaged across three experiments

Line[1]	*FHB 1* present[2]	# of DON-bleached[3] primary florets	# of FHB-blighted[4] primary florets	Relative yield[5] (%)	Total # of primary florets per head
VA04W-433	Yes	3.2 a[6]	1.9 d	29.8 cd	29.5
Fg 368	Yes*	3.7 a	2.4 d	54.0 a	42.0
NC03-11465	Yes	3.9 a	8.1 bcd	32.7 bc	36.7
SZ 13	Yes*	4.1 a	5.9 bcd	25.0 cde	31.3
SZ 14	Yes*	6.2 ab	4.6 bcd	25.5 cde	29.2
ARGE97-1047-4-2	2 Yes: 3 No	12.1 abc	8.0 bcd	16.5 cde	34.9
ARGE97-1064-13-5	No	16.7 bcd	9.9 ab	24.7 cde	38.2
AR97002-2-1	No	20.2 cde	3.7 bcd	18.3 cde	31.1
VA04W-628	No	20.4 cde	3.9 bcd	11.5 de	34.7
ARGE97-1042-4-5	No	21.3 cde	8.1 bcd	23.7 cde	42.0
ARGE97-1048-3-6	No	21.4 cde	8.9 bc	25.1 cde	40.2
Coker 9835 (Susceptible)	No	21.7 cde	15.8 a	8.1 e	31.9
Bess	No	22.0 cde	2.8 cd	24.1 cde	38.9
ARGE97-1033-10-2	No	22.8 cde	2.7 cd	30.9 c	37.8
Roane	No	25.2 de	3.9 bcd	18.4 cde	39.0
Fg 365	No	29.7 e	6.7 bcd	49.5 ab	48.3

[1] Lines were provided by E. Milus, C. Griffey, R. Bacon, A. McKendry, P. Murphy and Á. Mesterházy

[2] Five plants of each line were tested for molecular markers linked to *FHB1* by Gina Brown-Guedira. Yes = all plants had the markers, No = none of the plants had the markers, * = one plant was heterozygous for the markers, 2 Yes: 3 No = line was heterogeneous for the markers.

[3] Four primary florets were treated with DON (250 µg/floret), and DON-bleached primary florets were counted after 21 days.

[4] Data from a previous experiment using the single-floret inoculation method to determine the level of type II resistance. One primary floret was inoculated with a DON-producing isolate of *F. graminearum*, and the number of blighted primary florets was counted after 21days.

[5] The relative yield for the DON-treated spikes was calculated as yield for treated spikes divided by yield for control spikes and multiplied by 100.

[6] Means within a column followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05.

RESISTANCE IN WINTER WHEAT LINES TO INITIAL INFECTION AND SUBSEQUENT SPREAD OF DON AND NIV CHEMOTYPES OF *FUSARIUM GRAMINEARUM*.

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OBJECTIVE

To quantify the resistance of selected winter wheat lines to initial infection by deoxynivalenol (DON) and nivalenol (NIV) chemotypes of *F. graminearum* and to subsequent spread of these infections that may be a useful measure of combined type I and type II resistances.

INTRODUCTION

Resistant cultivars are believed to be the key component in any integrated management program for Fusarium head blight (FHB) caused by *Fusarium graminearum*. Five types of resistance to FHB in wheat have been described (Mesterházy, 1995), however, only two types (type I – resistance to initial infection, and type II – resistance to fungal spread within a spike) have been widely accepted and clearly defined. In wheat, type II resistance is considered to be a major component of the FHB resistance complex (Bai et al. 2000), and breeders more commonly utilize this type of resistance than type I resistance. However, as Bai and Shaner (2004) stated, adding additional type II resistance genes into lines with type II resistance may not increase their overall resistance performance. However, adding type I resistance into lines with type II resistance should increase their field resistance against FHB.

MATERIALS AND METHODS

A susceptible check and 15 winter wheat lines resistant to FHB were inoculated using a spray inoculation technique (1×10^5 macroconidia/ml and 3 ml per spike) with two *F. graminearum* isolates representative of DON and NIV chemotypes in the United States. At flowering, spikes were inoculated, covered with plastic bags and incubated in a greenhouse at 17 to 22°C. Bags were removed after 48 hr, and plants were incubated at 20 to 24°C for the rest of the experiment. The total number of primary florets per spike was counted 7 days after inoculation (dai), and the number of blighted primary florets was counted 7, 10, 14 and 21 dai. The area under the disease progress curve (AUDPC) from 0 to 21 days was calculated to estimate the combined effects of type I and type II resistances. The experimental design was a randomized design consisting of 16 wheat lines, one DON and one NIV chemotype, and 7 replications (cones) in runs 1 and 2. Lines and chemotype were treated as fixed effects and runs and replications as random effects. Means were separated using Tukey's HSD test at P=0.05.

RESULTS AND DISCUSSION

The line × chemotype interaction was not significant for percentage of blighted primary florets 7 dai (P=0.931) or AUDPC (P=0.685), indicating that lines ranked similarly for both DON and NIV chemotypes. Chemotypes were not significantly different for percentage of blighted primary florets 7 dai (P=0.061) or AUDPC (P=0.124). However, the DON chemotype averaged 15.2% blighted primary florets at 7 dai and 446 AUDPC, whereas the NIV chemotype averaged 9.9% blighted primary florets at 7 dai and 250 AUDPC. The DON chemotype was expected to have a higher AUDPC than the NIV chemotype because DON is a virulence factor that increases spread of infection within spikes. These results indicate that NIV might also be a virulence factor to a lesser extent than DON. Wheat lines were significantly different ($P \leq 0.0001$) for both percentage of blighted primary florets 7 dai and for

AUDPC. There was much statistical overlap among lines, but Roane, AR97002-2-1, SZ 13, and VA04W-628 were among the most resistant for both variables (Table 1). All except four lines had significantly lower percentages of blighted florets at 7 dai than the susceptible check, indicating that most lines had type I resistance. All lines had significantly lower AUDPC values than the susceptible check, indicating that all lines have type I and/or type II resistances. There was a positive correlation ($R^2 = 0.71$) between AUDPC and percentage of FHB-blighted primary florets 21 days after single floret inoculation in previous experiments to determine levels of type II resistance, suggesting that AUDPC was associated with type II resistance.

Possibilities for improving the ability to quantify resistance to initial infection include 1) counting the number of blighted primary florets at 4, 5, 6 and 7 dai, 2) incubating inoculated plants in a growth chamber to achieve a uniform post-inoculation environment, and 3) utilizing inoculum of similar aggressiveness on each date of inoculation.

In conclusion, most of the winter wheat lines have type I resistance that is effective against both DON and NIV chemotypes. AUDPC following spray inoculation appears to be a useful variable for quantifying the combination of type I and type II resistances. The most resistant lines for both variables were Roane, AR97002-2-1 and SZ 13.

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Table 1. Percentage of blighted primary florets 7 days after inoculation and area under the disease progress curve (AUDPC) for 16 winter wheat lines inoculated using a spray inoculation technique with one DON and one NIV chemotype of *Fusarium graminearum* and averaged across two runs of the experiment.

Line[1]	Pedigree	Blighted primary florets after 7 days (%)	AUDPC across 21 days[2]
Roane	VA71-54-147 (CI17449)/Coker68-15//IN65309C1-18-2	5.2 a[3]	145 ab
AR97002-2-1	AR396-4-2/NING 8026	5.9 a	133 a
SZ 13	Ringo Star / Nobeoka Bozu	6.8 a	233 ab
VA04W-628	Ernie//NING7840/Ernie	7.9 ab	210 ab
SZ 14	Ringo Star / Nobeoka Bozu	8.4 ab	277 abc
ARGE97-1042-4-5	Mason / Catbird	9.5 abc	281 abc
ARGE97-1033-10-2	Freedom/Catbird	10.2 abc	245 ab
ARGE97-1064-13-5	Mason//Freedom/Super Zlatno	10.3 abc	295 abc
ARGE97-1047-4-2	P2684 / 3 NING 7840 // Parula / Veery # 6	11.8 abc	363 abc
ARGE97-1048-3-6	Mason // SHA 3 / Catbird	12.3 abc	377 bc
Bess	MO 11769/Madison	12.6 abc	300 abc
Fg 368	Zugoly / Reka / Nobeoka Bozu	14.9 abcd	341 abc
NC03-11465	NING 7840/P2643//NC95-22426	16.7 abcd	480 c
Fg 365	Ságvári / Nobeoka Bozu // Mini Mano / Sum3	20.2 bcd	484 c
VA04W-433	NING 7840/PION2684//96-54-244 (CK9803/Freedom)	21.1 cd	479 c
Coker 9835	Susceptible check	27.3 d	930 d

[1] Lines were provided by E. Milus, C. Griffey, R. Bacon, A. McKendry, P. Murphy and Á. Mesterházy.

[2] AUDPC was calculated according to the formula AUDPC = $\sum [(y_i + y_{i+1})/2 * (t_{i+1} - t_i)]$ where y= percentage of primary florets blighted at time t and t=0, 7, 10, 14 and 21 days.

[3] Means within a column followed by the same letter are not significantly different according to Tukey's HSD test at P=0.05.

DEVELOPMENT OF SCAB RESISTANCE IN SOFT RED WINTER WHEAT.

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ABSTRACT

Fusarium head blight (FHB) is a potential devastating disease in the southeast region of the United States. Several native sources of Type II resistance (Truman, Roane, Ernie, OH02-12686, and IL00-8530) and from derivatives of Sumai 3 (INW0411, VA04W433, VA 01-461) have being incorporated into GA elite lines. Breeding for Type I resistance is also in progress with populations derived from Truman and Frontana. Several elite breeding lines, GA 991109-6E8, GA 031307DH, GA 031454DH, GA 981621-5E34 have been identified as moderately scab resistant. Marker assisted backcrossing of QTL from Sumai 3 (3BS, 5AS), Goldfield (2BS) and Ernie (5AS, 3BS, and 4BL) have been used to transfer resistance into AGS 2000 background. Several FHB resistant sources were evaluated for Type I resistance in the greenhouse. Bess and Tribute were the two checks that had a low infection spread (1.3 and 1.7, respectively). Lines that were equal to the checks for low infection spread were M04*5109, M03-3616B, NC05-25062, GA 03131354-DH30, GA981621-5E34, and LA01141D-138. Lines that had the lowest DON levels were Bess, Jamestown, M04*5109, M03-3616B, GA 03131354-DH30, and VA 05W-510.

HISTORY OF FHB RESISTANCE EVALUATION IN MICHIGAN STATE PERFORMANCE TRIAL.

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ABSTRACT

In 1996, the Michigan wheat industry suffered a crippling blow due to a widespread Fusarium Head Blight epidemic. Since this time, FHB has been a priority concern for MI wheat producers, millers and industry. Michigan State University has been conducting FHB screening on the Michigan State Performance Trial for the past 11 years (http://www.css.msu.edu/varietytrials/wheat/Variety_Results.html). Many varieties entered into the Michigan State Performance Trial are evaluated for more than one year, and several varieties were present in over half of these 11 years of trials, and in a few cases, for all of the 11 years. For each year, with the exception of 2003, field trials were successfully conducted and scores of incidence and severity were recorded. In 2003, severity scores were assessed in the greenhouse. Only more recently has toxin evaluation been reported for the State Performance Trial. Over the years that the FHB screening has been conducted, methods of inoculation as well as method of FHB symptom measurement have varied and/or been changed. Our associated poster will consider the trends of genetic resistance in the MI State Performance Trial over time, as well as the consistency of FHB data for genotypes present in the trial over multiple years.

PRELIMINARY SELECTION OF F3 AND F4 BREEDING LINES FOR FHB RESISTANCE AT MICHIGAN STATE UNIVERSITY.

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ABSTRACT

In 2008 we evaluated early generation (F3 soft reds and F4 soft reds and F4 soft whites) breeding lines for FHB resistance at Michigan State University. Our purpose in evaluating these lines was to examine the efficacy of selection in earlier generations, and to do this using a rough evaluation method. Each F3 and F4 breeding line that was evaluated was derived from a single F2 or F3 (respectively) plant. In addition, many of the F3 soft red lines originated from the same crosses as the F4 soft whites, having been generated from segregating F2 populations. During our FHB screening, the breeding lines were observed for FHB index multiple times over the course of approximately 2.5 – 3.5 weeks. Breeding lines that showed clear susceptibility were selected against as early as possible (i.e., not waiting until a specified time after flowering), while lines that showed greater levels of resistance were only identified after a reasonable level of resistance had been maintained until a few weeks after anthesis. Using this rough evaluation system, a line that may have initially appeared resistant could be selected against at a later date if the resistance had appeared to break down over time. For those that were selected, they were objectively categorized on a 1-5 scale, where 1 = very good resistance and 5 = borderline between moderate resistance and moderate susceptibility. 'Truman' was used as a resistant check and was planted regularly in the FHB nursery for comparison across all FHB trials. F3 lines selected this year will be re-evaluated for FHB resistance in 2009 to access the predictability of resistance based on 2008 evaluation. In the associated poster we will highlight trends that were observed in the populations for FHB resistance selection in these F3 and F4 early generations.

IDENTIFICATION OF MOLECULAR MARKERS FOR SCAB RESISTANCE IN WINTER BARLEY USING ASSOCIATION MAPPING.

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ABSTRACT

Two major approaches to identify molecular markers linked to important traits are traditional mapping in bi-parental populations and association mapping with a panel of germplasm lines. To date two Barley OPAs, consisting of 3,072 SNPs, have been genotyped in 1,920 barley breeding lines contributed from ten US barley breeding programs collaborating in the USDA-CSREES funded Barley Coordinated Agricultural Project (CAP). Ninety-six advanced lines from the Virginia Tech barley breeding program were screened for about 1536 SNPs in 2006. Phenotypic data for 24 traits have been collected on these 96 lines in Virginia over several years. This presentation focuses on molecular markers linked to scab resistance that were identified via association mapping. A subset of 46 lines having phenotypic data for scab traits collected in inoculated, mist-irrigated tests in two years was used in the association mapping study. Tightly linked markers were detected for resistance to scab incidence and DON in both the 2006 and 2007 studies and for scab severity in 2007. Six SNPs on chromosomes 2H and 7H were associated with scab incidence in 2006 ($P<0.001$) and explained 16.0% to 21.5% of the phenotypic variation. In 2007, eight SNPs on chromosomes 1H, 2H, and 3H were associated with incidence ($P<0.01$) and explained 10.2% to 13.2% of the phenotypic variation. Seven SNPs on chromosomes 1H, 3H, 4H and 7H were significantly associated with DON levels in 2006 ($P<0.0001$) and explained 12.7% to 17.8% of phenotypic variation. In 2007, four SNPs on chromosomes 1H, 4H and 5H were linked to DON level ($P<0.01$) and explained 12.2% to 14.8% of the phenotypic variation. Three markers on chromosomes 3H and 4H were associated with severity in 2007 ($P<0.01$) and explained 12.7% to 16.3% of phenotypic variation. Among these significant associations, a region containing six SNPs on chromosome 1H was associated with both incidence and DON level in 2007. A region on chromosome 2H with three SNPs was associated with incidence in both years. A region comprised of two SNPs on chromosome 4H was associated with both severity and DON levels in 2007, while another unique region with seven SNPs was associated with DON levels in 2006 and severity in 2007. Three regions on the short arm of chromosome 7H were associated with scab resistance. Region one, comprised of three SNPs was associated with incidence in 2006 and DON in 2007; region two with three SNPs was associated with incidence in 2006 and severity in 2007 and; region three with two SNPs was associated with incidence and DON in 2006. These SNPs associated with scab resistance in the current study will be compared with other known QTL for scab resistance in barley based on chromosome locations. Validation of linked markers for known QTL and identification of novel QTL will further facilitate efforts in breeding for scab resistance in barley.

ACKNOWLEDGEMENT AND DISCLAIMER

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MAPPING QTL FOR SCAB RESISTANCE IN THE VIRGINIA WHEAT CULTIVAR MASSEY.

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ABSTRACT

Fusarium Head Blight (FHB) or scab is a serious disease which reduces yield and quality of wheat in warm and humid production areas worldwide. Planting resistant varieties is an economically effective and environmentally sound way to manage this disease. Identifying new sources of resistance and characterizing native sources of resistance are both major components in developing scab resistant wheat varieties. Massey, a cultivar released by Virginia Tech in 1985, has adult plant resistance to powdery mildew as well as being moderately resistant to scab. A set of 589 DArT markers and SSR markers were mapped onto all 21 chromosomes in a Becker/Massey mapping population comprised of 152 RILs. Phenotypic data for FHB severity were obtained from a greenhouse test conducted in Virginia and FHB incidence, severity, and Index data were collected in field tests conducted in Virginia (2007, 2008), Missouri (2008), and Kentucky (2008). Average FHB severity data from greenhouse evaluations was not correlated with FHB field data. Correlations among data collected from multiple locations and years were not significant. Within each test, FHB incidence was significantly correlated to FHB severity ($P < 0.001$), and correlations between FHB severity and FHB index were the highest. Mapping data indicate that Massey has QTL on chromosomes 1D and 3B conferring resistance to FHB severity on the basis of single floret inoculation tests conducted in the greenhouse study. Eight QTL conferring resistance to FHB in Massey were located on chromosomes 1A, 2B, 2D, 3B, 4B, 4D, 5B, and 6A on the basis of field data collected from one location in 2007 and three locations in 2008. Among these ten QTL, the major QTL was mapped on chromosome 3B and was associated with greenhouse severity and scab index in field studies conducted in Virginia in both 2007 and 2008. The 3B QTL explained 7.1% and 23.4% of the phenotypic variation for greenhouse FHB severity in 2007 and 2008, respectively. It also explained 9.1% and 8.2% of the field FHB index in 2007 and 2008, respectively. Another QTL associated with greenhouse FHB severity in 2008 is located on chromosome 1D and explained 9.2% of variation. Three other QTL associated with FHB incidence and index in Virginia field studies are on chromosomes 4B, 4D and 6A with R^2 values of 9.9%, 14.3%, and 19.5%, respectively. The 4B QTL also confers resistance to Fusarium damaged kernels and explained 14.8% of the variation. A major QTL located on chromosome 2B and associated with field incidence (R^2=8.1%), severity, (R^2=12.4%), and FHB index (R^2=11.0%), was identified via analyses of Missouri FHB field data, while the two major QTL identified from analysis of Kentucky FHB field incidence were mapped to chromosomes 2D (R^2=8.0%) and 5B (R^2=7.1%). The 5B QTL region also was associated with field severity and FHB index in analysis of the 2007 Virginia data and explained 13% of the phenotypic variation. The number of QTL and variation explained among different experiments reflect the complex nature of both phenotypic characterization and quantitative inheritance of FHB resistance. The similarity between QTL mapped in Massey with other known QTL will be compared and potentially novel QTL and/or unique combinations of QTL will be discussed in the presentation.

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SATURATION MAPPING OF SCAB RESISTANCE QTL IN ERNIE AND APPLICATION TO MARKER-ASSISTED BREEDING.

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ABSTRACT

Fusarium head blight (FHB), is caused mainly by *Fusarium graminearum* in wheat and results in significant yield and quality losses in humid and warm areas of the world. QTL for scab resistance have been mapped in exotic and native sources. However, only a few QTL have been widely deployed in breeding programs using marker-assisted selection (MAS) due to the lack of diagnostic and tightly linked markers for most QTL. Four major QTL for type II resistance were previously mapped on chromosomes 5A, 4B, 3BSc and 2B of Ernie. A set of 243 Ernie/MO94-317 RILs were evaluated in inoculated, mist-irrigated scab nurseries at Columbia, MO and Blacksburg, VA. The 4B QTL region was associated with field FHB severity (R^2=4.2%), index (R^2=4.4%), kernel quality assessed as 100 grain weight (R^2=8.0%), and fusarium damaged kernels (FDK, R^2=6.2%). The awn inhibitor gene, B_1, is associated with field FHB incidence (R^2=4.5%) and index (R^2=5.3%) in the Virginia test and with FHB severity (R^2=4.2%) in the Missouri test. Another QTL associated with 100 grain weight is on chromosome 2DS (R^2=12.4%). There is one minor QTL for FDK (R^2=4.3%) on chromosome 5A that is separate from the major QTL for type II resistance and the B_1 gene. Tightly linked markers are being applied for marker-assisted selection in breeding populations for the four QTL in Ernie and the two major QTL on chromosomes 3BS and 6B of Sumai 3. This will facilitate the pyramiding of various QTL for FHB resistance using MAS in variety development.

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INHERITANCE OF FHB RESISTANCE IN SPRING VERSUS WINTER WHEAT GROWTH HABIT BACKGROUNDS.

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ABSTRACT

Fusarium head blight (FHB) is one of the important diseases of wheat in South Dakota. This study was conducted to determine the inheritance of FHB resistance QTLs in spring and winter growth habit backgrounds. Four genotypes consisting of susceptible winter wheat 'Nekota' and '2137' and moderately resistant spring wheat 'ND2710' and 'BacUp' were crossed and populations derived from the crosses were segregated into spring and winter types following cold treatment of seedlings at -7^{0}C for an hr. A total of six SSR marker (3BS QTL marker: Xgwm389, Xgwm493 and STS256; 5A QTL marker: Xgwm293, Xgwm304 and Barc186) were used to genotype the population. Chi-square analysis showed that there were significant differences in the percentage of the genotypes containing homozygous marker alleles for 3BS and 5A QTLs between spring and winter types in the population ND2710X2137, ND2710XNekota and BacUpX2137. The percentage of the genotypes with homozygous marker alleles for 3BS QTL was less in spring compared to winter growth habit backgrounds in the population ND2710X2137 and ND2710XBacUp. In contrast, spring type in the population ND2710XNekota showed higher percentage of genotypes containing homozygous marker alleles for 5A QTL than winter type. The results indicated that the 3BS QTL was less inherited in spring growth habit backgrounds whereas the 5A QTL was less inherited in winter growth habit backgrounds.

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MAPPING QTLS FOR FUSARIUM HEAD BLIGHT FROM NOVEL SOURCE - TOKAI-66.

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ABSTRACT

Breeding for resistance is the most effective approach for managing Fusarium head blight (FHB), an important disease on wheat in South Dakota. This study was conducted to identify QTLs linked to FHB resistance in a resistance genotype - Tokai-66. A cross was made between Tokai-66 and Jagalene and single seed descent was used to advance the population. The $F_{2:4}$ and $F_{2:5}$ populations were evaluated by artificially inoculating disease in a mist irrigated nursery in 2006 and 2007. Disease incidence, severity, fusarium damaged kernel (FDK) and deoxynivalenol (DON) content were recorded in the population. Diversity Array Technology (DArT) was used to genotype the population. Preliminary analysis using single marker analysis in MapManager found that each wPt-5672, wPt-7757, wPt-4125 and wPt-5556 markers at 2B explained 11% of the variation in disease index in 2007. For FDK, wPt-2757 and wPt-1081 markers at 3B explained10% of the variation in 2006 and wPt-7984 marker at 3B explained 12% of the variation 2007. Marker wPt-0398 at 3A explained 16% of the variation in DON content in 2006, whereas marker wPt-7984 at 3B explained 13% of the variation in DON content in 2007. We are planning to saturate more SSR markers in the chromosome 2B, 3B and 3A to identify more QTLs linked to FHB.

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MAPPING QTLS FOR FUSARIUM HEAD BLIGHT FROM SOUTH DAKOTA'S INDIGENOUS GENOTYPE - SD97060.

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ABSTRACT

Breeding for resistance is the most effective approach to manage Fusarium head blight (FHB), an important disease on wheat in South Dakota. This study was conducted to identify QTLs linked to FHB resistance in an indigenous genotype – SD97060. A cross was made between SD97060 and Jagalene and single seed descent was used to advance the population. The $F_{2:4}$ and $F_{2:5}$ populations were evaluated by artificially inoculating disease in a mist irrigated nursery in 2006 and 2007. Disease incidence, severity, fusarium damaged kernel (FDK) and deoxynivalenol (DON) content were recorded in the population. Diversity Array Technology (DArT) was used to genotype the population. Preliminary analysis using single marker analysis in MapManager indicated that wPt-3132 in 2B explained 22% of the variation for disease index in 2006. Marker wPt-9032 at 3B explained 29% for DON content in 2006. We are planning to saturate more SSR markers in the chromosome 2B and 3B to identify further QTLs linked to FHB.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-130. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

USING ASSOCIATION MAPPING TO IDENTIFY FUSARIUM HEAD BLIGHT RESISTANCE QTL WITHIN CONTEMPORARY BARLEY BREEDING GERMPLASM.

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ABSTRACT

Utilization of quantitative trait loci (QTL) for Fusarium head blight (FHB) resistance identified through bi-parental mapping has had limited success in barley. Previously described resistance QTL (identified in wide bi-parental mapping studies) often have been associated with negative agronomic traits such as late heading and taller plants, thus reducing their overall utility. Mapping within existing breeding populations, however, would identify resistance QTL segregating in elite populations, and represent genetic factors which are immediately available to breeders. A total of 768 breeding lines from 4 programs (UM, BARI, NDSU-2, NDSU-6) were evaluated in four environments over 2006 and 2007. At each location the lines were planted in a randomized complete block design with two replications, inoculated, and overhead mist irrigated to encourage disease development. Each line was genotyped using 1,536 SNP markers, and QTL were mapped using a mixed model approach. Phenotypic variation among lines for disease severity was significant ($p<0.0001$), but skewed toward resistant. Linkage disequilibrium extended beyond 4 cM on average, and indicated that whole genome mapping was feasible with the available marker density. Multiple QTL with generally small effects ($R^2 < 5\%$) were identified. Overall eight QTL for DON and four QTL for FHB were reproducibly identified. These loci should be useful targets for immediate implementation of marker assisted selection to improve disease resistance.

USING OPTICAL SORTING TECHNIQUES TO SELECT FOR LOWER SCAB DISEASE IN SEGREGATING POPULATIONS.

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ABSTRACT

Natural epidemics of the Fusarium head blight (FHB), caused by *Fusarium graminearum*, may result in severe yield losses, reduction in end-use quality, and contamination of the harvested grain by mycotoxins. FHB is an episodic disease in the hard winter wheat region of the Great Plains that is known for its diverse and highly variable climate. In order to diversify our FHB germplasm we created two populations from a soft winter wheat (MO980829) breeding line with a known native FHB tolerance and two hard winter wheat genotypes (Jagalene and NE00564). The objective of this experiment was to estimate FHB damage in the populations and to enrich the segregating populations for FHB tolerance using a kernel optical sorting technique. Our hypothesis was that in a segregating population grown under disease conditions, kernels from the susceptible genotypes would have a higher level of disease than kernels from tolerant genotypes. Of course, kernels with low disease levels might be due to tolerance or escapes and similarly kernels with high disease levels might be due to susceptibility or an overwhelmed tolerance. Because a soft wheat parent was used in these two populations, we first sorted the population for hardness (two classes: hard and soft) and protein content (four classes; low, low to medium, medium to high, and high). We retained for our experiments, the hard segregants. In 2007, the hard kernels of the two populations, each with four levels of protein, were planted in an inoculated scab field with a misting system at Mead, NE. The harvested kernels were then sorted into FHB free (no scab) and FHB infected (scabby) grain. In 2008, the two sorted kernel samples (no scab vs scabby) plus an unsorted kernel sample (control) from each of the four protein classes were grown in a replicated misted scab nursery at Lincoln, NE. The visual scores of incidence ($p=0.0017$), severity ($p=0.0001$) and FHB index ($p=<0.0001$) were significantly different among the 26 lines that consisted of 24 lines from the two populations and two checks (Overland and Wesley). Among the scab-free sorted kernels, scabby kernels, and unsorted kernels there was a slight significant difference for incidence ($p=0.045$), highly significant difference for severity ($p=0.0034$), and highly significant difference for FHB index ($p=0.0004$). The mean of incidence for the no scab and scabby kernels were 41% and 51%, respectively (standard error was 3.1%). There was no incidence difference between the scabby and unsorted kernels. The mean of severity for the no scab, scabby and unsorted kernels were 16%, 32%, and 23%, respectively (standard error was 2.8%). The FHB indexes for the no scab, scabby and unsorted kernels were 7, 15, and 12, respectively (standard error was 1.3). There was no significant difference among the sorted and unsorted kernels for DON test which may reflect the very high CV associated with this trait. Also there were no differences for incidence, severity, FHB index, and DON between the two populations and among the four protein levels within the populations. Based on this study, kernel sorting may be an effective method for in reducing the visual symptoms of FHB, but not for significantly reducing the DON content.

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DEVELOPMENT AND EVALUATION OF THE FIRST *FUSARIUM* INTERNATIONAL ELITE SPRING WHEAT NURSERY (FIESWN) AND THE FIRST *FUSARIUM* INTERNATIONAL PRELIMINARY SPRING WHEAT NURSERY (FIPSWN): PRELIMINARY RESULTS FROM MEXICO AND EUROPE.

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ABSTRACT

Two international spring wheat nurseries, 1st *Fusarium International Elite Spring Wheat Nursery* (FIESWN) and *1st Fusarium International Preliminary Spring Wheat Nursery* (FIPSWN) were prepared and distributed to 13 collaborators in 2008. Of the 188 entries initially received, 28 were included in the FIPSWN and 50 in the FIESWN based on the field performance and DON content evaluation in Mexico during 2006-2007.

In Mexico, both nurseries were inoculated with a mixture of 5 *F. graminearum* isolates and evaluated from May to Sept. 2008. Sumai 3 (resistant) and Wheaton and Flycatcher (susceptible checks) were used as controls. In the FIPSWN, FHB index ranged from 1.4% (Sumai 3) to 49.3 % (line 2.49, resistant for crown root rot in the West Asia). Ten lines over 28 scored below 10% and 2 lines from CIMMYT's Fusarium program, EMB16/CBRD//CBRD and SABUF/3/BCN//CETA/AE.SQUARROSA (895)*2/4/BCN, scored around 2.5% FHB index. In Austria, 7 lines over 28 scored below 10% FHB index. Sumai 3 resistance was confirmed and comparable in both locations, confirming the suitability of screening for FHB resistance at CIMMYT El Batan station. EMB16/CBRD//CBRD ranked among the 10 best entries in both locations. Several entries, such as INMIR/NING 8331//INIA BOYERO (Uruguay) and SABUF/3/BCN//CETA/AE.SQUARROSA (895)*2/4/BCN (Mexico) ranked differently in the 2 locations. In the FIESWN 19 out 50 limes scored below 10% FHB index. Line MN00274-2-6 (USA) top ranked at FHB index below 1%; 5 CIMMYT lines (2 of them selected in the Caspian Sea region), 9 USA (MSU and MN) and 5 Canada submitted lines were in this group. In Austria, there was a clear cut separation between resistant (< 15% FHB index) and susceptible lines (from 15 to 75% FHB index); 89% of the best 19 lines in Mexico were in the <15%FHB index group in Austria.

These first results show that resistant lines selected by collaborators in North America, South America and the Caspian Sea region were confirmed in Mexico and Austria. As scores of some lines did not correlate in the different regions further research on the pathogen, environmental conditions at screening sites and the level of DON production in the field are needed. Further information will be added from haplotyping research in USDA-ARS Fargo to confirm whether the source of the resistant lines found in these trials differ from Sumai 3.

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THE 2007-08 SOUTHERN UNIFORM WINTER WHEAT SCAB NURSERY.

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ABSTRACT

Most components of Fusarium Head Blight (FHB) resistance are greatly influenced by genotype by environment interaction which limits the heritability of resistance estimated by a single program in any given year. The Southern Uniform Winter Wheat Scab Nursery provides breeders in the public and private sectors with an opportunity for multi-environment, independent evaluations of FHB resistance in advanced generation breeding lines compared with the current most resistant check varieties Ernie and Bess. In addition, the nursery facilitates the sharing of the best resistant materials throughout the breeding community.

The 2007-08 nursery comprised 49 advanced generation breeding lines and three check cultivars, 'Ernie' and 'Bess' (partially resistant) and 'Coker 9835' (susceptible). Six U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State University, Univ. of Maryland, N.C. State Univ. and VA Tech.), and one private company, Agripro-Coker, submitted entries. The nursery was distributed to 11 U.S., one Hungarian, and one Romanian cooperator for field and / or greenhouse evaluations. In addition three USDA-ARS laboratories conducted evaluations for Hessian Fly resistance, milling and baking quality and haplotypes based on established SSR markers.

Copies of the full report will be available at the 2008 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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SEVEN YEARS OF PROGRESS IN THE NORTH AMERICAN BARLEY SCAB EVALUATION NURSERY (NABSEN).

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ABSTRACT

The North American Barley Scab Evaluation Nursery (NABSEN) is a regional uniform nursery established to screen in North America and China, elite two-rowed and six-rowed barley germplasm for resistance to Fusarium head blight (FHB). Participants include breeding programs from North Dakota State University (NDSU), University of Minnesota (UM), Busch Ag Resources (BARI), Agriculture Canada and CIMMYT/ICARDA. Each year approximately 50 entries plus resistant, moderately resistant and susceptible checks are grown in either inoculated-misted or dryland sites. Each year one site has been sown in each of Mexico, and Canada, as well as six sites in the upper Midwest of the United States. In the last three years a site has also been established in China. NABSEN participants use different criteria to select elite material for testing, the CIMMYT/ICARDA program is centered on pre-breeding of two-rowed barley and the Agriculture Canada entries includes mostly two-rowed material, either feed or malting barley from several Canadian breeding programs. This abstract reports on the resistance to FHB of entries submitted from the NDSU, UM and BARI six-rowed malting barley programs that were tested in irrigated inoculated experiments between 2002 and 2008. Environment and disease pressure affected the magnitude of DON over the years with 2005 having the lowest DON of 11.2 ppm on the susceptible check Stander, and 2008 having the highest DON of 33.2 ppm also on the susceptible check Stander. Similarly, environment and disease pressure affected disease severity, with the highest severity on Stander in 2008 (27% infected kernels). In contrast to the DON data, the lowest severities on Stander were recorded in 2003 and 2006 (both 11% infected kernels). These data support the hypothesis that DON and FHB severity are not always well correlated in barley, which indicates that active selection needs to occur concurrently for both characters. To reduce the effect of different FHB severities between years, FHB severity was expressed as a percentage of the moderately resistant cultivars Robust/MNBrite The average of all entries from the NDSU program over all years was 89% of Robust/MNBrite, from the UM program was 83% of Robust/MNBrite, and from the BARI program was 110% of Robust/MNBrite. There was variation within the entries from each program, and when only the best entry from each program was included, then the percentages were 63%, 62% and 82% of Robust/MNBrite for the NDSU, UM, and BARI programs, respectively. DON was also expressed as a percentage of the moderately resistant cultivars Robust/MNBrite. The average of all entries from the NDSU program over all years was 85% of Robust/MNBrite, from the UM program was 91% of Robust/MNBrite and from the BARI program was 115% of Robust/MNBrite. As with severity, there was variation within the entries from each program, and when only the best entry from each program was considered, then the percentages were 58%, 68% and 94% of Robust/MNBrite for the NDSU, UM, and BARI programs, respectively. Overall the average reductions in disease severity and DON have been slight since 2002. Importantly though, good levels of resistance have been maintained while the programs have selected for the yield, maturity and quality characteristics required in a commercial cultivar. Within the average however, are individual lines that are significantly improved in both resistance to infection by FHB and DON accumulation compared to early material, and entries with good resistance are now in the American Malting Barley Association Pilot Scale Evaluation Program in preparation for commercial release.

NIR OPTICAL CHARACTERISTICS OF DEOXYNIVALENOL.

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ABSTRACT

We have developed rapid near infra red (NIR) techniques for nondestructive automatic sorting of Fusarium damaged wheat kernels and for estimation of deoxynivalenol (DON) levels in single wheat kernels. We studied NIR optical characteristics of DON to identify NIR absorption bands and to assess the applicability of NIR technique for direct measurement of DON in order to improve the calibrations. NIR transmission spectra of DON (0.5 - 2000 ppm) dissolved in acetonitrile and that of water (0 - 640 ppm) in acetonitrile were studied to identify NIR absorption bands of DON and water and to see how strong NIR absorption bands of water interact with DON NIR absorption bands.

Deoxynevalenol crystals were dissolved in acetonitrile to prepare a 2000 ppm stock solution. It was thereafter serially diluted to prepare a series of DON solutions up to 0.5 ppm. The solutions in IR quartz (10 mm path length) cuvettes were scanned using an ASD spectrometer. Solutions were scanned three times to collect three different spectra per each DON concentration. Likewise, water was added to acetonitrile and spectra were recorded. The collected DON spectra were used to develop a calibration to predict DON levels in acetonitrile solution. Two spectra from each concentration were used for developing the calibration by PLS regression method and the other spectra used to validate the calibration. The optical density spectra of DON and water in various concentrations were used to study DON and water absorption peaks. Difference spectra and second derivative spectra of DON and water were used to identify and resolve absorption peaks.

In the 950 - 2200 nm range two DON absorption bands were identified at 1390 -1440 nm and 1880-1950 nm having peaks at 1410 and 1905 nm respectively. The absorbance at 1905 nm is approximately one magnitude stronger than the absorbance at 1410 nm. Water absorption bands were found around 970 and 1420 nm in increasing intensity. The water absorption bands above 1850nm were much stronger being unable to measure even at 40 ppm using 10 mm path length.

The calibration developed for DON in acetonitrile (R^2=0.995 SECV=38.8 with 6 PLS factors) predicted DON levels in acetonitrile with a R^2=0.998. This shows that NIR absorbance can be used to accurately estimate DON levels in acitonitrile. However, when it comes to predicting DON in cereal grains such an accuracy is difficult to achieve due to interference with stronger water absorption bands that overlap DON absorption bands. Our present SKNIR technique for scab sorting and DON estimation use 950-1650 nm waveband. Based on the observations of this study it may be possible to further improve calibrations by extending NIR scanning range above 1950 nm to include the stronger DON absorption band at 1905 nm.

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PROGRESS ON DEVELOPMENT AND APPLICATION OF SINGLE KERNEL NIR SORTING TECHNOLOGY FOR ASSESSMENT OF FHB RESISTANCE IN WHEAT GERMPLASM.

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ABSTRACT

Plant breeders working on developing *Fusarium* resistant wheat varieties need to evaluate kernels coming from a multitude of crosses for Fusarium Damaged Kernels (FDKs). We are developing Near Infrared (NIR) spectroscopic methods to sort FDKs from sound kernels and to determine DON levels of FDKs nondestructively to facilitate rapid varietal screening for *Fusarium* resistance by assessing proportions of sound and FDKs and estimating their DON levels. We report the progress and research highlights of the development and use of our single kernel NIR (SKNIR) scab sorting and deoxynivalenol (DON) estimation techniques since January, 2008.

We have improved the SKNIR scab sorting technique and its feasibility as an objective, rapid and nondestructive method for assessment of FDKs of wheat germplasm demonstrated. Depending on the kernel DON level, FDKs can be sorted into 2-3 fractions. This makes it possible to get an understanding of what fraction and how much each fraction contributes to the final DON level of a composite sample. Moreover, our studies with sorting of North Dakota State University (NDSU) germplasm showed that proportions of SKNIR sorted FDKs in wheat lines affected by FHB correlated fairly well with field FHB assessment indices. Therefore, this technique can be used by wheat breeders as a nondestructive, rapid and an objective method for comprehensive analysis of FDKs when wheat germplasm are screened for *Fusarium* resistance. Since April 2008 we have sorted 108 samples for NDSU and 405 samples for University of Nebraska, Lincoln (UNL) wheat breeders. Another set of samples from the above two institutions will be sorted in November-December, 2008.

A calibration was developed for estimation of DON concentration in single wheat kernels by SKNIR system. This can estimate DON levels in single kernels having more than 60 ppm DON. Experiments will be carried out in collaboration of UNL researchers to further test and refine this calibration to estimate DON levels of FHB affected wheat samples. NIR spectra of pure DON were also studied and DON absorption peaks identified. Results of these experiments are presented in a separate poster. A SKNIR wheat moisture calibration was also developed. It will be integrated to determine moisture content of kernels concurrently when DON levels are estimated so that it is possible to compare DON levels of kernels having different moisture contents or to express DON content of kernels with specific moisture content.

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THE EFFECT OF KEY CHROMOSOME SEGMENTS ON FHB RESISTANCE IN A CROSS OF SOFT-WINTER BY HARD-SPRING PARENTS.

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ABSTRACT

Resistance to FHB is multifaceted and quantitative. New sources of resistance need to be evaluated for their ability to compliment and enhance our current sources of resistance. One potential source of soft winter wheat is a line from CIMMYT termed CASS94 in this study that is derived from a cross of Mayoor by a synthetic hexapoid. CASS94 has better FHB resistance than Mayoor suggesting it may have some FHB resistance alleles from *T. Tauschii*. Previous greenhouse evaluations for Type II resistance suggested that a sib of CASS94 had a major QTL on chromosome 2DL. Our objective was to assess the effect of key chromosome regions from CASS94 on FHB resistance in the field. We crossed CASS94 to OH685, an adapted soft red winter wheat that has been susceptible to FHB and developed 167 F4-derived RILs. Data on FHB Index (IND) were collected in Ohio in 2007 and in Michigan and Ohio in 2008. Incidence (INC) and severity (SEV) data were collected in both locations in 2008. Heritability was moderate to low (0.49 for IND, 0.44 for severity, and 0.36 for INC). The allele frequencies for many markers were quite skewed in favor of the OH685 allele. We could not phenotype CASS94 in the field due to its spring habit. About 26% of the RILs had lower IND than OH685 which displayed moderate resistance in this test. Nearly 27% had a lower IND than Freedom and 2.4% had a lower IND than Truman. Markers from 3BS and 5AS were not significant indicating that known QTL from these regions were not segregating in this population. We used 13 markers from 2D and formed two linkage groups. A QTL was detected on a group corresponding to 2DL. This QTL accounted for about 10-30% of the genetic variation for IND. Genotyping work is continuing and final results for these regions will be presented at the Forum.

SHORTENING OF THE *LEYMUS RACEMOSUS* SEGMENT IN THE *FHB3* TRANSFER USING *PH1B*-INDUCED HOMOEOLOGOUS RECOMBINATION.

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ABSTRACT

Fhb3, a new gene conferring resistance to *Fusarium* head blight disease of wheat, was transferred from *Leymus recemosus* to wheat in the form of a compensating wheat-*Leymus* translocation T7AL·7Lr #1S. However, whole arm translocations are often associated with deleterious linkage drag, which usually results in yield reduction and inferior quality. Our previous research indicated that *Fhb3* is located in the distal region of the short arm of the *Leymus* chromosome 7Lr#1. This is encouraging because genetic recombination is known to be high in the distal regions of chromosomes. Therefore, further chromosome engineering aimed at reducing the size of the *L. racemosus* segment while still retaining the *Fhb3* resistance gene appears to be feasible. The translocation stock T7AL·7Lr #1S was crossed twice with the CS *ph1b* mutant. 154 BC_1 plants were screened from the cross T7AL·7Lr#1S / *ph1b* using molecular markers to assay for *ph1b* and T7AL·7Lr#1S. Sixty-one plants were homozygous *ph1b/ph1b* and heterozygous for the translocation chromosome T7AL·7Lr#1S/7A. These plants were either backcrossed with Overley or selfed. We have developed a large recombinant population of 1,400 BC_2 seeds and more than 8,000 BC_1F_2 seeds. In homozygous *ph1b* genotypes, the alien 7Lr#1S arm is expected to pair and recombine with the homoeologous 7AS arm of wheat. Meiotic pairing analysis in plants homozygous for *ph1b* and heterozygous for T7AL·7Lr#1S and 7A failed to detect any metaphase I association of T7AL·7Lr#1S with 7A in more than 500 PMCs analyzed, suggesting that the recovery of recombinants is very difficult. A total of 1118 BC_2 plants were screened by molecular markers and three putative recombinants were identified. These recombinants were further confirmed by genomic in situ hybridization using total genomic *L. racemosus* DNA as a probe. Rec124 is a proximal recombinant with about the proximal 80% derived from *L. racemosus* and the distal 20% of the arm derived from 7AS of wheat (T7AL·7Lr#1S-7AS), whereas rec679 and rec989 are distal recombinants with about the proximal 80% derived from 7AS and the distal 20% of the arm derived from 7Lr#1S T7AL 1S (T7AL·7AS-7Lr#1S). Once homozygous recombinant stocks have been obtained they will be evaluated for their resistance to scab and the resistant stock will be crossed with adapted winter wheat cultivars.

MAPPING OF FHB RESISTANCE IN THE JAPANESE WHEAT LANDRACE, PI 81791.

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ABSTRACT

The Japanese wheat landrace, PI 81791 (Sapporo Haru Komungi Jugo), has been shown to have consistent resistance to FHB in field and greenhouse studies, and marker analysis has shown that this accession does not contain *Fhb1*. Because of this, a population of 150 recombinant inbred lines was developed from a cross between this genotype and the susceptible spring wheat variety, Wheaton. Phenotypic data for resistance to initial infection (type 1) and resistance to the spread of infection (type 2) were collected in field experiments for three environments. Type 2 data was also collected from greenhouse experiments. More than 200 SSR markers have been mapped in this population to date. From these data, four markers were found to be significantly (P<0.01) associated with type 2 resistance in field experiments with one marker being significant in all three environments and three being significant in two of the three environments. The most consistent marker, BARC98, has previously been mapped to chromosome 4D, although it's exact position in the map of this cross has yet to be determined. The other markers have been previously located on chromosomes 1D, 3BL/3DL, and 5D. Six markers were found to be significant (P < 0.01) for type 2 resistance in greenhouse experiments, with all six markers being significant for both greenhouse experiments. These markers have been previously identified on chromosomes 1B, 1D, 2A, 2B, and 3A, although their exact locations have not been determined for this cross. Only one marker was found to be significant in multiple locations for type 1 field resistance. Eight RI lines showed high levels of type 1 and type 2 resistance in both field and greenhouse experiments and can serve as breeding parents.

COMBINING RESISTANCE TO YELLOW DWARF DISEASE (*BDV3*) FROM INTERMEDIATE WHEATGRASS, AND RESISTANCE TO FUSARIUM HEAD BLIGHT (*QFHS.PUR-7E*) FROM TALL WHEATGRASS, IN COMMON WHEAT.

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ABSTRACT

The objective of this research is to combine *Bdv3* that conditions resistance to yellow dwarf disease (YD) and *Qfhs.pur-7EL* that conditions resistance to Fusarium head blight (FHB). *Bdv3* was introgressed into wheat (*Triticum aestivum* L.) chromosome 7D from intermediate wheatgrass (*Thinopyrum intermedium)* and is located subterminal on the 7E segment that was translocated into 7DS.7DL-7EL. *Qfhs.pur-7EL* was introgressed from tall wheatgrass (*Thinopyrum ponticum*) and was mapped to the distal region of chromosome 7DS.7DL-7EL in wheat. *Bdv3* is located more proximal to the centromere of chromosome 7DS.7DL-7EL than *Qfhs.pur-7EL*. The wheat line, 216-67, was developed in which the introgressed 7E segment replaced the distal approximately 1/3 of 7DL. The wheat line, 275-4, was developed in which the introgressed 7E segment containing *Qfhs.pur-7EL* replaced the distal approximately 1/3 of 7DL. The wheat lines 216-67 and 275-4 were crossed resulting in plants heterozygous for the introgressed 7E segments. A large population of F_2 plants was genotyped with SSR markers gwm37, associated with *Bdv3*, and with *Qfhs.pur-7EL* flanking markers BF145935 and cfa2240 to identify plants that potentially have *Bdv3* and *Qfhs.pur-7EL*. Progenies of selected F_2 plants will be genotyped for presence of *Bdv3* and *Qfhs.pur-7EL*, and their resistance to YD and FHB will be verified by testing putative recombinant plants to YD and FHB.

POWER OF FAMILY-BASED QTL MAPPING: OPTIMIZING FAMILY TYPE, SIZE AND MARKER DENSITY FOR QTLS OF DIFFERENT MAGNITUDES.

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ABSTRACT

Family-based QTL mapping has been shown to be an expeditious method to map and validate molecular markers using core plant breeding populations. Utilizing the linkage based variance component analysis method, in a previous study; the QTL *Fhb1* was accurately mapped to the same chromosomal location identified by conventional QTL mapping methods. However, questions need to be addressed regarding the potential limitations of the Family-based QTL mapping approach. This research details a simulation study, utilizing previous data, which investigates population size (number and size of family) and marker density in relation to the variance explained by QTLs (major or minor) when mapped with the Family-based method. The simulated population consisted of a "typical breeding program" with families derived from three-way and four-way crosses, the computer software package MERLIN was used to perform variance component based linkage analysis on the simulated population. A total of 1,000 simulations were performed using POWQ, a software module based on the variance component engine of MERLIN. The average power to detect a QTL ranged from less than 1 to 100% depending upon family size, family type (three-way or four-way crosses), marker density, variance explained by the QTL and the level of significance. Overall a larger population size led to a higher power of detection. Increasing family size (number of individuals within a family) had higher returns to power gain than increasing the number of families. Thus using a small number of large families, rather than a large number of small families was beneficial in terms of power gain. There was greater power to detect major QTLs than minor QTLs. Also the power was higher for families derived from four-way rather than three-way crosses. As a general rule, a sample size of 500 (20 families with 25 individuals in each family) will provide higher power to detect minor QTLs. Power can also be increased by increasing marker density. As the parameters discussed here effect detection power, the researcher can calculate QTL detection power for a given set of conditions, based on the plant breeding program and the number of molecular markers available. The POWQ module can be used to calculate power in such situation, allowing the researcher to determine if their resources should go into increasing population size or marker density. The results of this study suggest that the family-based QTL mapping method is useful for mapping both major and minor QTLs.

SELECTIVE GENOTYPING IN FAMILY-BASED MAPPING OF FHB RESISTANCE QTLS IN HEXAPLOID WHEAT.

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ABSTRACT

Using main-stream plant breeding populations for mapping of QTLs has many advantages including quick mapping, reducing resource requirement and simultaneous validation of QTLs in multiple genetic backgrounds. Family based QTL mapping methods were validated to map *Fhb1* in a previous study. A reduced population size for genotyping will reduce resource requirement. Selective genotyping of individuals displaying "extreme" phenotypes has been considered important for saving costs and time without reducing the power of detection. This study investigates selective genotyping in family based QTL mapping methods, specifically; linkage analysis (variance component and pedigree-wide regression) and association analysis (quantitative transmission disequilibrium test, QTDT). The target QTL of this study was the well characterized *Fhb1* for Fusarium Head Blight (FHB) resistance in wheat (*Triticum aestivum* L.). Individuals with disease value scores falling in the top and bottom 20 percent were selected for genotyping and QTL mapping from a base population of 82 families and 793 individuals. The QTL was mapped to the expected chromosomal location with LOD values comparable to using all the individuals of the population. These results indicate that the selective genotyping approach can be applied to family-based QTL mapping to reduce cost and time requirements for genotyping.

ASSESSING PROGRESS TOWARD BREEDING BARLEY VARIETIES WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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OBJECTIVES

Use several performance measures outlined in the USWBSI Action Plan to assess progress toward breeding barley varieties with enhanced FHB resistance.

INTRODUCTION

The USWBSI Action Plan states that the primary goal of variety development is to "*Increase acreage planted with varieties exhibiting improved FHB resistance*". In the case of barley, the first potential releases of new varieties with enhanced FHB resistance could occur in January of 2010 (Smith et al., 2007). Since release and establishment of new varieties is a continuing and long term goal, it is essential that breeding programs assess progress at intermediate points along the way to that ultimate goal. This will provide critical feedback to breeders to assess whether their current practices are likely to meet goals. In goal #2 of the USWBSI Action Plan for varietal development **"** *Increase efficiency of individual breeding programs to develop FHB resistant varieties*.", several performance measures were established to assess progress. In this report, we present data from the University of Minnesota using three of those performance measures.

Performance Measures (from Action Plan VDHR Goal #2)

- Total number as well as percentage of crosses made involving FHB resistant parent (native or exotic resistance).
- Average performance of breeding lines (advanced, preliminary yield trial entries etc…) compared to appropriate check varieties for FHB and agronomics.
- Number of variety candidates entered into Uniform or Regional Yield Nurseries or industry quality evaluations with enhanced FHB resistance.

MATERIALS AND METHODS

Assessment of breeding lines entered into first year yield testing was done by assembling data from individual trials conducted from 2001 to 2008. In our program, these lines (PYT entries) are generally evaluated in 2-3 locations for yield and 2-3 misted and inoculated nurseries for FHB severity. Yield trials and FHB trials are arranged in a randomized complete block design with two and three replicates, respectively. Lines included in the analysis were from FHB crosses (ie. involving parents with an exotic source of resistance in the pedigree). The value of each line was normalized for each trial by dividing it by the performance of the mean of three common checks for FHB severity and four common checks for yield. The checks were usually entered twice in a trial so the mean of the checks for any one trial was based on at least 12 plots and as many as 27 plots. The normalized trait values were averaged for each year for FHB severity and yield, plotted over time, and a trend line fit using MS Excel.

RESULTS AND DISCUSSION

Population Development. We make most of our crosses in the fall greenhouse in each year. Crosses involving an exotic source of FHB resistance, or that

include at least one parent with an exotic source of resistance in its pedigree, are referred to as FHB crosses. Since all exotic sources are unadapted they require multiple cycles of breeding to recover enhanced resistance in an elite background. In 1998 about 1/3 of the FHB crosses were 1st cycle, 1/3 were 2nd cycle, and 1/3 were 3rd cycle. In 2007, most crosses were 4th cycle or higher. In the UM program, we have both increased the total number of crosses made each year and also increased the proportion that are FHB crosses (Table 1).

Evaluation of Preliminary Yield Trial (PYT) Entries. Two years after a cross is made, F4 generation populations are evaluated in inoculated and mist-irrigated FHB nurseries. Each line is grown in two locations with two replications per location. Selection is based on visual assessment of FHB severity and lines with better resistance are harvested and the grain assayed for DON. The most resistant lines are advanced to preliminary yield trials and 2nd year FHB testing in multiple locations. To assess the effectiveness of our first year selection for FHB, we assessed the performance of PYT entries for FHB severity and yield in replicated trials in multiple locations. The data is normalized to a set of common checks and shows significant decrease in FHB severity over the eight year period (Figure 1). During that same period the average yield performance was stable.

Entry of Variety Candidates into Industry Quality Evaluations. For a barley variety to occupy significant acres in the Midwest, it must be approved by the American Malting Barley Association (AMBA). Our program is allowed up to four entries in the AMBA pilot-scale malting evaluation each year. Our first two variety candidates with enhanced FHB resistance to enter the AMBA pilot program were M122 and M123 in 2005 (Table 2). We have entered at least two new entries in each of the following years. Three of the six entries that have completed pilot testing were rated satisfactory. M122 is now in plant scale testing and if AMBA plant-scale brewing evaluation is satisfactory it will be released as a new variety in January of 2010.

Performance measures presented here indicate that the University of Minnesota breeding program is making progress toward the release of new varieties with enhanced FHB resistance and lower DON. Similar progress is being made by the other Midwest barley breeding programs. Each of these programs uses different sources of resistance and slightly different selection and breeding strategies. Crossing of elite materials with enhanced FHB resistance among the Midwest programs is in progress and should further improve the level of resistance of new varieties.

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DISCLAIMER

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

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Table 1. Description of crosses made for FHB resistance breeding from 1998 to 2007.

	Number of Crosses[1]		
Year	**Total**	**FHB[2]**	**Percent FHB**
1998	49	29	59%
1999	71	37	52%
2000	85	61	72%
2001	76	36	47%
2002	136	71	52%
2003	60	43	72%
2004	61	46	75%
2005	120	93	78%
2006	98	83	85%
2007	134	120	90%

[1] number of crosses made in fall and advanced as F1's in the winter greenhouse.
[2] number of crosses involving a parent with an exotic source of FHB in the pedigree.

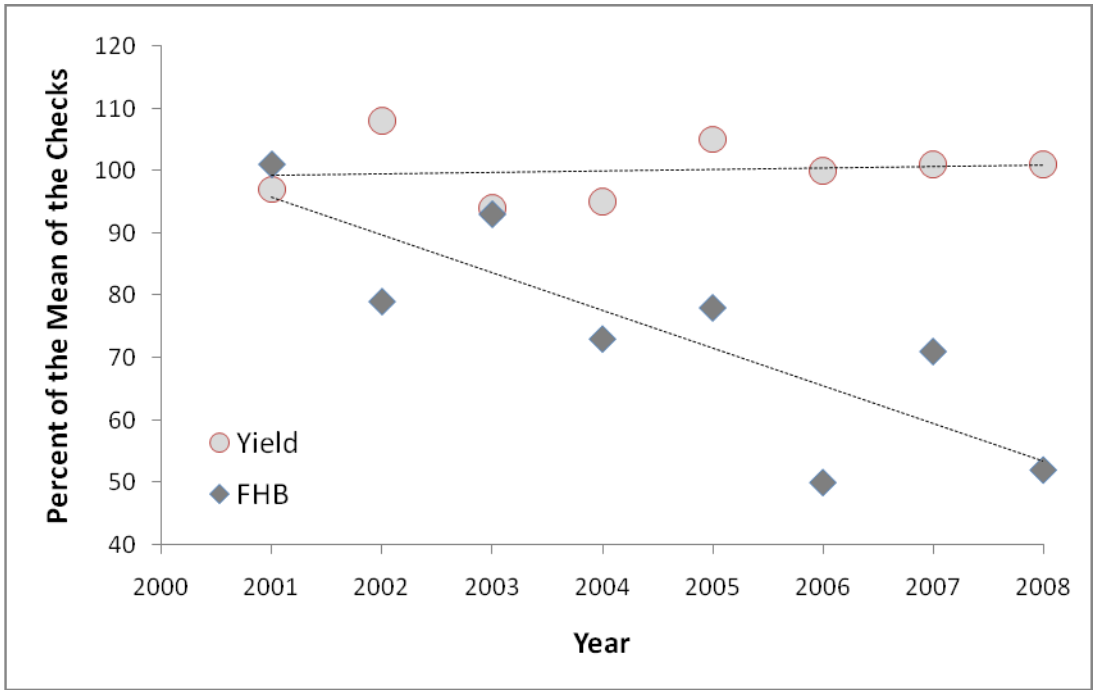

Figure 1. Mean performance of first year yield trial entries for yield and FHB severity from 2001 to 2008. Trend lines are linear fits using MS Excel 2007. Yield checks are Robust, MNBrite, Stander, and Lacey. FHB checks are Robust, MNBrite, and Stander. Each value is the mean of at least 46 and as many as 90 breeding lines. Lines are from FHB crosses.

Table 2. Description of UM barley variety candidates entered into AMBA Pilot Testing program.

Year Entered	Line	FHB Severity (% of Robust)	DON Conc. (% of Robust)	Evaluation Outcome
2005	M122	47	54	Plant-Scale
	M123	76	81	Unsatisfactory
2006	M128	70	78	Eligible for Plant Scale
	M129	56	83	Eligible for Plant Scale
2007	M130	63	76	Unsatisfactory
	M132	62	78	Unsatisfactory
2008	M134	56	85	In progress
	M135	50	75	In progress
	M136	53	93	In progress
	M137	58	78	In progress

REPORT ON THE 2007-08 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERIES (NUWWSN AND PNUWWSN).

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OBJECTIVES

This is a summary of the report on the 2007-2008 Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and the Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN). A full report will be available on the USWBSI web site prior to the 2008 forum. The objective of these tests is to screen winter wheat genotypes adapted to the northern portion of the eastern US for scab resistance.

MATERIAL AND METHODS

The traits assessed and locations that reported data are listed in Table 1. Entries for the NUWWSN came from 13 programs while the PNUWWSN entries came from 10 programs (Table 2).

RESULTS

This report presents seven traits using FDK in place of KR and PSS. NUWWSN entries with means that were not significantly different than the lowest mean for five or more FHB traits are shown in Table 3 (eg. entries with at least 5 "l"s). PNUWWSN entries with means that were not significantly different than the lowest mean for four or more FHB traits are shown in Table 4 (eg. entries with at least 4 "l"s). Only three entries had DON < 2 ppm (IL01-34159, KY02C-3005-25, and VA06W-553 from PNUWWSN) and seven had IND < 15% (IL01-34159, KY02C-3005-25, and P.0175A1-37-4 from PNUWWSN; Truman, DH22/24. E6003, and IL02-18828 from NUWWSN). The results for all traits and all entries for the two tests are in Tables 5 and 6.

Table 1. Traits assessed in the 2007-08 PNUWWSN and NUWWSN tests.

Code	Trait	Description	PNUWWSN Locations*	NUWWSN Locations*
SEV	Disease severity from field tests	% of infected spikelets in an infected head.	IL,IN,KY,MI,MO,ON,VA	IL,IN,KY,MD,MI,MO,NE,NY,OH, ON,VA
INC	Disease incidence	% of heads with at least one infected spikelets	IL,KY,MI,MO,ON,VA	ILKY,MD,MI,MO,NE,NY,OH, ON,VA
IND	Disease index	IND = (SEVxINC)/100	IL,KY,MI,MO,OH,ON, VA, RO	IL,KS,KY,MD,MI,MO,NE,NY,OH, ON,RO,VA
KR	Kernel rating	A visual assessment of the percent infected kernels	IL	IL,KS
PSS	Percent scabby seed	Percent of scabby seed by weight	KY	KY,MD,MO
FDK	Fusarium damaged kernels	Considers KR and PSS as equivalent estimates of kernel infection	IL, KY	IL,KS,KY,MD,MO
ISK	Composite of head and kernel traits	ISK Index = .3 (Severity) + .3 (Incidence)+.4 (% FDK or PSS)	IL,KY	IL,KY,MD,MO
DON	DON (vomitoxin)	PPM of vomitoxin in grain	IL,IN, VA	IL,IN,KS,KY,MD,NE,VA
GH	Greenhouse severity	Same as SEV except from greenhouse	IL	IL,MO

* ON and RO indicate Ontario Canada, and Romania, respectively

Table 2. Entries in the 2007-08 PNUWWSN and NUWWSN.

PNUWWSN ENTRY	PNUWWSN PEDIGREE
ERNIE	Moderate Resistant Check
TRUMAN	Mod Resistant/Resistant Check
FREEDOM	Moderate Resistant Check
PIONEER 2545	Susceptible Check
MSU Line E2043	Pioneer 2552/Pioneer 2737W
MSU Line E6059	D9070 / Pioneer 2552
MSU Line E6042	VA96W-403-WS / CJ9403
MSU Line E6038	VA96W-403-WS / CJ9403
MSU Line E5024	D6234 / Pioneer 25W33
P.992192A1-5-4-5-81	92145//201R/Patton
P.0172A1-12-1	97395/981129
P.0175A1-37-4	981419/97397
P.04281A1-4-5	INW0304/9811//92823/Ernie
P.04287A1-16	INW0316*2/INW0304//9346/CS5A
P.03630A1-18	99751/INW0315//981358/97462
MO050600	MO 960903/Bess 'S'
MO050261	MO 94-182/VA 91-54-219
MO051150	960815/IL 91-14163
MO050617	960815/IL 91-14163
MO041020	960429/960112
MO050917	Truman 'S'/960815
VA06W-553	(Too long, see final report)
VA06W-558	VA96W-348/P92823A1-1-4-4-5
VA06W-561	OH618//Roane/Sisson"S" (VA96W234)
VA06W-615	(Too long, see final report)
VA06W-622	(Too long, see final report)
TRIBUTE	VA92-51-39/AL870365 (CK747*2/Amigo)
BDLS. HONEY-6	(Too long, see final report)
SE98 1083-14	PION25R57/OH546
SEKY93 C-1699-14	MO800071-56/PION2545//KY88C
SE94 C-0480-2-2	84C-048-2-1/PION2510//FFR555
SE98 1106-6	OH546/SE1694-12
SE94-1012-25	T814/L880119
KY02C-3005-25	25R18/MCCORMICK
KY02C-3005-44	25R18/MCCORMICK
KY02C-3008-05	25R18/92C-0010-17
KY02C-3004-04	25R18/Tribute
KY01C-1542-07	Tribute/BL 940582//Tribute/91C-170-3
KY99C-1205-06-1	25R26/ USG 3209//2540
M04-4566	BRADLEY/ROANE
M04-4715	MASON/ERNIE
M05-1172	M94-1048-1/IO2552
M05*1589	GA871339/PIO2540
M05-1531	LA87167-D8-/P92118B4-2
OH04-213-39	HOPEWELL/IL94-6858
OH04-264-58	OH645/HOPEWELL
OH04-268-39	HOPEWELL/VA96-54-372
OH04-176-29	P.92227C5-1-1/BL930390
OH03-41-45	IL91-14167/OH599
DH ACF112103 -8T	AC RON x SD97060
RCUOGF110202D/4	SD97060 x Ringo Star
RCUOGDHACF1109O2D	SD97060 x Freedom
RCATTF174/1C	AC Ron x 25R18
RCATTF203/2	AC Ron x 25R18
RCATL31	(Too long, see final report)
IL01-34159	(Too long, see final report)
IL79-002T-B-B	IL94-6727 / IL96-6472
IL04-7874	G65201/ IL98-12212
IL04-8445	IL94-1653/ IL97-3578
IL04-17204	IL97-3578/ Ernie

NUWWSN ENTRY	NUWWSN PEDIGREE
ERNIE	Moderate Resistant Check
TRUMAN	Mod Resistant/Resistant Check
FREEDOM	Moderate Resistant Check
PIONEER 2545	Susceptible Check
MSU Line E6002	VA96W-403-WS / CJ9403
MSU Line E6001	Pioneer 25W60 / CJ 9306
MSU Line E6003	VA96W-403-WS / W14
MSU Line E5011	Caledonia / NY88024-117
P.99600A2-4-93	9560//9811/3/Fdm/201R
P.0179A1-17	Fdm/Gfd//92829/Patton
P.011010A1-15	97395/981129//INW0316
P.03112A1-7-3	97395//INW0315/99794
KS980512-2-2	(Too long, see final report)
KS05HW14-3	KS98HW452/CO960293//KS920709B-5-2
MO050143	MO 11769/Madison
MO050699	950016/3/950016//90X54-1-1/MO 91-1009
MO050921	Ernie/Truman "S"
MO050101	MO 11769/Madison
VA05W-425	Roane/3/Ning7840/Coker9904//Pioneer2552
VA05W-775	(Too long, see final report)
VA05W-777	Roane*2//W14/Roane /3/Roane, BC3F6
VA05W-534	Goldfield/Tribute//Gibson
MD01W233-06-1	McCormick/Choptank
MD01W233-06-16	McCormick/Choptank
MD99W483-06-11	VA97W358/RENWOOD3260
NYCalresel-L	Reselection from Caledonia
NY94052-9340	Pio2737w/Harus
NYW103-1-9100	Cayuga/Caledonia
NYW103-70-9232	Cayuga/Caledonia
NY93246SP-9070	(Too long, see final report)
SE911492-4	TAISHANG1/GR863//CARDINAL
SE89-1873-2	NASW84-345/Coker9835//0H419/OH389
SE98-1089-34	P25R57/SE1694-12
SE93-1094-8	OH489/OH490
NE05418	(Too long, see final report)
NE05549	(Too long, see final report)
NE05537	NI97435 /NE94632 /2/ KS89180B-2-1
NE03488	KARIEGA/PRONGHORN//Millennium sib
NE01643	Millennium sib//Seward/Archer
KY00C-2059-16	91C-170-3/2552
KY00C-2143-08	90C-048-59/90C-160-14
KY00C-2755-03	2552/Allegiance
KY97C-0321-05-2	Kristy/VA94-52-25//2540
M04*5109	VA94-54-479/PIO2628
M04-4802	FFR518//ELKHART/MV18
M03-3616-B11	HOPEWELL/PATTON
M03-3616-C10	HOPEWELL/PATTON
OH02-13567	(Too long, see final report)
OH03-235-2	OH552/HOPEWELL
OH02-12678	FOSTER/HOPEWELL//OH581/OH569
OH02-7217	P92118B4-2/OH561
DH 22/8	(Too long, see final report)
DH 22/24	(Too long, see final report)
DH 19/176B	AC RON/WEKO609H3 x AC RON
DH F/SF, 23	Frontana x Sumai 3
IL02-18228	Pio25R26/9634-24437//95-4162
IL02-19463	Patton / Cardinal // IL96-2550
IL04-10118	IL95-2516/ IL98-12212
IL04-10721	IL95-4162/ IL97-7010
IL04-10741	IL95-4162/ IL97-7010

Table 3. Best entries (top) and worst (bottom) from the 2007-08 NUWWSN. Summary statistics are for all entries.

NAME	INC	SEV	IND	FDK	ISK	DON	GH	#l	#h
IL02-18228	41.9 l	15.3 l	13.0 l	10.7 l	28.1 l	5.5 l	24.1 l	7	0
DH 22/24	42.4 l	19.5 l	12.5 l	17.0 l	31.6 l	7.8 l	9.0 l	7	0
TRUMAN	47.4 l	21.6 l	14.5 l	10.9 l	31.8 l	6.7 l	4.5 l	7	0
MSU Line E6003	48.5 l	17.6 l	10.5 l	9.1 l	28.8 l	6.7 l	7.0 l	7	0
IL04-10741	48.7 l	24.1 l	18.9 l	21.6 l	38.5 l	7.0 l	29.8 l	7	0
MO050921	49.6 l	24.1 l	16.4 l	13.4 l	29.8 l	6.1 l	4.9 l	7	0
M03-3616-C10	51.9 l	25.4 l	17.3 l	24.4 hl	39.1 l	10.7 l	17.9 l	7	1
VA05W-534	52.6 l	24.9 l	17.0 l	14.0 l	38.1 l	5.3 l	17.9 l	7	0
MO050143	52.6 l	28.7	19.4 l	11.5 l	33.7 l	7.1 l	6.7 l	6	0
M03-3616-B11	53.4 l	26.6	19.3 l	19.0 l	41.3 l	9.1 l	13.8 l	6	0
OH02-13567	54.1 l	28.7	18.3 l	22.8 hl	42.2 l	8.2 l	6.2 l	6	1
IL04-10118	55.9	24.7 l	18.9 l	10.5 l	35.7 l	8.2 l	14.5 l	6	0
MO050101	49.9 l	27.3	19.5	14.1 l	33.8 l	7.9 l	8.7 l	5	0
NE05418	52.0 l	26.3	19.7	24.5 hl	41.5 l	8.3 l	23.7 l	5	1
MD01W233-06-1	53.5 l	26.1	18.8 l	19.4 l	44.0	4.7 l	4.8 l	5	0
OH02-12678	54.4 l	27.0	20.3	16.2 l	41.9 l	8.1 l	23.3 l	5	0
VA05W-775	55.4	28.8	17.7 l	19.9 l	40.9 l	5.1 l	4.1 l	5	0
VA05W-777	57.2	28.1	18.5 l	16.9 l	38.7 l	5.7 l	4.9 l	5	0
DH F/SF, 23	64.7	50.1 h	39.1	37.6 h	55.7 h	33.8 h	43.1 h	0	5
PIONEER 2545	74.9 h	52.2 h	42.8	30.7 h	58.7 h	19	48.3 h	0	5
SE98-1089-34	78.5 h	60.7 h	52.1 h	38.9 h	63.5 h	20.7	40.7 h	0	6
AVERAGE	59.4	34.0	26.2	22.3	44.2	11.6	23.9		
MINUMUM	41.9	15.3	10.5	9.1	28.1	4.7	4.1		
MAXIMUM	78.5	60.7	52.1	38.9	63.5	33.8	70.0		
LSD(0.05)	12.8	10.6	9.0	16.3	15.5	7.3	31.0		

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 4. Best entries (top) and worst (bottom) from the 2007-08 PNUWWSN. Summary statistics are for all entries.

NAME	SEV	INC	IND	FDK	ISK	DON	GH	#l	#h
P.0172A1-12-1	49.7 l	17.4 l	9.4 l	13.1 l	29.6 l	3.8 l	13.3	6	0
IL01-34159	45.9 l	16.2 l	10.6 l	16.4 l	31.0 l	1.7 l	3.7	6	0
KY02C-3005-25	52.0 l	18.2 l	11.8 l	26.3 l	39.5 l	2.3 l	4.0	6	0
VA06W-558	55.3 l	25.4 l	15.3 l	19.7 l	38.8 l	3.9 l	43.5	6	0
TRUMAN	58.4 l	18.0 l	17.2 l	18.4 l	40.3 l	9.5 l	6.4	6	0
IL79-002T-B-B	60.2 l	26.3 l	17.6 l	26.4 l	42.1 l	3.9 l	4.5	6	0
ERNIE	57.9 l	24.2 l	18.5 l	20.9 l	41.9 l	10.0 l	10.5	6	0
P.03630A1-18	61.5 l	27.3 l	18.9 l	32.5 l	42.7 l	4.0 l	4.0	6	0
P.0175A1-37-4	63.3	23.6 l	15.7 l	29.3 l	43.9 l	5.9 l	4.5	5	0
KY02C-3004-04	63.6	24.6 l	16.7 l	24.6 l	42.1 l	3.3 l	7.7	5	0
VA06W-561	59.7 l	20.7 l	16.8 l	39.3	47.6	5.7 l	28.8	4	0
VA06W-553	65.1	29.7	16.9 l	21.5 l	41.4 l	2.9 l	5.3	4	0
KY02C-3005-44	64.6	25.7 l	18.3 l	35.8 l	47.9	9.5 l	4.0	4	0
SE98 1083-14	64.8	27.6 l	20.5	32.9 l	44.4 l	8.3 l	7.8	4	0
MSU Line E6042	50.8 l	30.2	20.7	15.2 l	33.3 l	8.8 l	49.7	4	0
IL04-17204	60.4 l	33.2	21.2	20.2 l	42.1 l	8.2 l	30.5	4	0
IL04-8445	59.8 l	30.5	21.7	28.7 l	43.8 l	6.0 l	28.2	4	0
RCUOGF110202D/4	59.5 l	31.1	23.2	27.9 l	44.3 l	10.1 l	7.0	4	0
SE94 C-0480-2-2	73.4 h	40.6 h	32.6	48.2 h	59.3 h	10.5 l	65.3	1	4
MSU Line E2043	84.5 h	39.1	33.2	64.0 h	66.2 h	24.8 h	10.5	0	4
M04-4566	74.9 h	45.0 h	34.5 h	50.6 h	64.3 h	13.0	65.5	0	5
M04-4715	70.1 h	41.5 h	34.7 h	42.1 h	52.7 h	14.6	76.3	0	5
OH04-213-39	73.2 h	40.6 h	34.8 h	56.5 h	65.6 h	9.7 l	36.5	1	5
SE94-1012-25	72.8 h	45.7 h	37.4 h	53.8 h	60.3 h	12.3	72.0	0	5
BDLS. HONEY-6	75.5 h	46.5 h	39.4 h	43.4 h	62.1 h	10.7 l	60.7	1	5
MSU Line E6059	85.5 h	40.6 h	33.8 h	50.5 h	64.2 h	27.7 h	24.8	0	6
PIONEER 2545	85.8 h	46.6 h	42.5 h	55.3 h	65.1 h	19.6 h	98.8	0	6
KY01C-1542-07	82.4 h	51.8 h	43.6 h	44.7 h	60.9 h	20.9 h	44.3	0	6
AVERAGE	68.3	32.7	25.6	36.8	50.5	10.1	29.2		
MINUMUM	45.9	16.2	9.4	13.1	29.6	1.7	3.7		
MAXIMUM	85.8	51.8	43.6	64.0	66.2	27.7	98.8		
LSD(0.05)	15.6	12.3	9.9	24.5	15.1	9.9			

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 5. Summary of results of the 2007-08 PNUWWSN.

NAME	SEV		INC		IND		FDK		ISK		DON		GH	#l	#h
ERNIE	57.9	l	24.2	l	18.5	l	20.9	l	41.9	l	10.0	l	10.5	6	0
TRUMAN	58.4	l	18.0	l	17.2	l	18.4	l	40.3	l	9.5	l	6.4	6	0
FREEDOM	67.5		34.0		24.9		47.9	h	57.5	h	8.7	l	12.5	1	2
PIONEER 2545	85.8	h	46.6	h	42.5	h	55.3	h	65.1	h	19.6	h	98.8	0	6
MSU Line E2043	84.5	h	39.1		33.2		64.0	h	66.2	h	24.8	h	10.5	0	4
MSU Line E6059	85.5	h	40.6	h	33.8	h	50.5	h	64.2	h	27.7	h	24.8	0	6
MSU Line E6042	50.8	l	30.2		20.7		15.2	l	33.3	l	8.8	l	49.7	4	0
MSU Line E6038	63.3		36.9		25.8		28.9	l	45.2		12.1		48.5	1	0
MSU Line E5024	76.0	h	37.1		29.1		29.3	l	54.7	h	12.9		26.7	1	2
P.992192A1-5-4-5-81	79.6	h	38.0		32.7		52.7	h	57.5	h	11.3	l	22.3	1	3
P.0172A1-12-1	49.7	l	17.4	l	9.4	l	13.1	l	29.6	l	3.8	l	13.3	6	0
P.0175A1-37-4	63.3		23.6	l	15.7	l	29.3	l	43.9	l	5.9	l	4.5	5	0
P.04281A1-4-5	74.9	h	34.3		27.7		42.5	h	54.7	h	10.8	l	6.6	1	3
P.04287A1-16	66.5		34.0		26.3		48.9	h	56.2	h	7.7	l	30.5	1	2
P.03630A1-18	61.5	l	27.3	l	18.9	l	32.5	l	42.7	l	4.0	l	4.0	6	0
MO050600	60.6	l	33.8		24.3		39.0		43.5	l	4.0	l	8.0	3	0
MO050261	65.6		31.2		22.9		41.0	h	51.3	h	6.5	l	7.5	1	2
MO051150	75.0	h	31.3		25.2		43.9	h	58.5	h	7.8	l	20.3	1	3
MO050617	78.8	h	37.9		32.0		33.0	l	55.8	h	10.1	l	16.3	2	2
MO041020	66.7		24.3	l	20.1		23.5	l	45.8		7.3	l	4.0	3	0
MO050917	77.0	h	35.2		28.1		36.8	l	55.9	h	11.5	l	5.8	2	2
VA06W-553	65.1		29.7		16.9	l	21.5	l	41.4	l	2.9	l	5.3	4	0
VA06W-558	55.3	l	25.4	l	15.3	l	19.7	l	38.8	l	3.9	l	43.5	6	0
VA06W-561	59.7	l	20.7	l	16.8	l	39.3		47.6		5.7	l	28.8	4	0
VA06W-615	70.3	h	36.8		25.5		31.0	l	48.6		5.1	l	12.7	2	1
VA06W-622	70.6	h	24.1	l	23.0		44.7	h	49.2		15.1		25.2	1	2
TRIBUTE	63.9		29.5		23.8		36.0	l	48.8		8.6	l	30.0	2	0
BDLS. HONEY-6	75.5	h	46.5	h	39.4	h	43.4	h	62.1	h	10.7	l	60.7	1	5
SE98 1083-14	64.8		27.6	l	20.5		32.9	l	44.4	l	8.3	l	7.8	4	0
SEKY93 C-1699-14	70.9	h	35.0		28.0		31.7	l	47.7		12.3		70.3	1	1
SE94 C-0480-2-2	73.4	h	40.6	h	32.6		48.2	h	59.3	h	10.5	l	65.3	1	4
SE98 1106-6	71.1	h	35.8		27.2		24.0	l	45.7		11.3	l	6.3	2	1
SE94-1012-25	72.8	h	45.7	h	37.4	h	53.8	h	60.3	h	12.3		72.0	0	5
KY02C-3005-25	52.0	l	18.2	l	11.8	l	26.3	l	39.5	l	2.3	l	4.0	6	0
KY02C-3005-44	64.6		25.7	l	18.3	l	35.8	l	47.9		9.5	l	4.0	4	0
KY02C-3008-05	68.4		22.3	l	16.0	l	41.4	h	50.7		9.4	l	3.0	3	1
KY02C-3004-04	63.6		24.6	l	16.7	l	24.6	l	42.1	l	3.3	l	7.7	5	0
KY01C-1542-07	82.4	h	51.8	h	43.6	h	44.7	h	60.9	h	20.9	h	44.3	0	6
KY99C-1205-06-1	75.0	h	36.1		32.2		55.5	h	59.4	h	13.7		28.5	0	3
M04-4566	74.9	h	45.0	h	34.5	h	50.6	h	64.3	h	13.0		65.5	0	5
M04-4715	70.1	h	41.5	h	34.7	h	42.1	h	52.7	h	14.6		76.3	0	5
M05-1172	72.1	h	33.1		26.3		43.1	h	56.7	h	10.1	l	29.8	1	3
M05*1589	67.6		31.1		25.3		23.7	l	44.1	l	9.1	l	21.5	3	0
M05-1531	64.4		24.5	l	24.0		43.0	h	52.4	h	7.0	l	22.7	2	2
OH04-213-39	73.2	h	40.6	h	34.8	h	56.5	h	65.6	h	9.7	l	36.5	1	5
OH04-264-58	77.0	h	38.3		31.1		55.0	h	62.9	h	12.2		23.8	0	3
OH04-268-39	83.9	h	39.2		33.6		52.1	h	60.6	h	12.3		53.3	0	3
OH04-176-29	69.3		34.0		27.3		29.4	l	50.4		6.3	l	29.2	2	0
OH03-41-45	67.6		35.3		29.7		36.5	l	47.1		9.6	l	24.5	2	0
DH ACF112103 -8T	73.8	h	35.7		31.2		46.9	h	60.7	h	10.1	l	7.2	1	3
RCUOGF110202D/4	59.5	l	31.1		23.2		27.9	l	44.3	l	10.1	l	7.0	4	0
RCUOGDHACF1109O2D	58.3	l	32.4		23.7		51.2	h	53.5	h	11.0	l	4.5	2	2
RCATTF174/1C	74.0	h	37.3		29.3		44.7	h	55.9	h	16.7		4.0	0	3
RCATTF203/2	83.3	h	37.6		29.3		30.5	l	51.2	h	17.9		11.0	1	2
RCATL31	71.0	h	38.4		30.1		33.7	l	44.8		20.0	h	61.2	1	2
IL01-34159	45.9	l	16.2	l	10.6	l	16.4	l	31.0	l	1.7	l	3.7	6	0
IL79-002T-B-B	60.2	l	26.3	l	17.6	l	26.4	l	42.1	l	3.9	l	4.5	6	0
IL04-7874	65.9		30.0		21.9		28.4	l	44.5	l	6.5	l	19.8	3	0
IL04-8445	59.8	l	30.5		21.7		28.7	l	43.8	l	6.0	l	28.2	4	0
IL04-17204	60.4	l	33.2		21.2		20.2	l	42.1	l	8.2	l	30.5	4	0
AVERAGE	68.3		32.7		25.6		36.8		50.5		10.1		25.3		
LSD(0.05)	15.6		12.3		9.9		24.5		15.1		9.9				
# Environments	7		6		8		3		3		3		1		

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

Table 6. Summary of results of the 2007-08 NUWWSN.

NAME	INC	SEV	IND	FDK	ISK	DON	GH	#l	#h
ERNIE	57.4	31.7	22.7	25.6 h	48.7 h	11.0 l	13.7 l	2	2
TRUMAN	47.4 l	21.6 l	14.5 l	10.9 l	31.8 l	6.7 l	4.5 l	7	0
FREEDOM	57.4	34.9	23.1	20.3 l	45.6	9.8 l	18.2 l	3	0
PIONEER 2545	74.9 h	52.2 h	42.8	30.7 h	58.7 h	19.0	48.3 h	0	5
MSU Line E6002	55.2	30.7	25.6	21.8 l	42.5 l	13.1	25.8 l	3	0
MSU Line E6001	61.2	35.1	25.7	25.7 h	48.2 h	10.7 l	6.7 l	2	2
MSU Line E6003	48.5 l	17.6 l	10.5 l	9.1 l	28.8 l	6.7 l	7.0 l	7	0
MSU Line E5011	68.1 h	45.1	40.2	26.1 h	50.5 h	13.0	15.3 l	1	3
P.99600A2-4-93	66.6 h	38.1	33.0	26.5 h	51.4 h	11.4 l	26.5 l	2	3
P.0179A1-17	63.6	36.3	26.3	30.2 h	49.0 h	13.7	18.6 l	1	2
P.011010A1-15	67.2 h	40.7	33.8	22.7 hl	51.7 h	11.5 l	15.5 l	3	3
P.03112A1-7-3	60.7	34.3	22.6	21.5 l	43.3 l	10.8 l	5.6 l	4	0
KS980512-2-2	60.9	40.2	27.9	21.5 l	48.2 h	17.6	16.1 l	2	1
KS05HW14-3	71.9 h	39.3	34.1	29 h	48.7 h	15.0	30.2 l	1	3
MO050143	52.6 l	28.7	19.4 l	11.5 l	33.7 l	7.1 l	6.7 l	6	0
MO050699	61.1	35.5	25.9	22.6 hl	42.8 l	9.7 l	5.0 l	4	1
MO050921	49.6 l	24.1 l	16.4 l	13.4 l	29.8 l	6.1 l	4.9 l	7	0
MO050101	49.9 l	27.3	19.5	14.1 l	33.8 l	7.9 l	8.7 l	5	0
VA05W-425	60.0	33.1	23.4	22.9 hl	40.9 l	6.9 l	10.3 l	4	1
VA05W-775	55.4	28.8	17.7 l	19.9 l	40.9 l	5.1 l	4.1 l	5	0
VA05W-777	57.2	28.1	18.5 l	16.9 l	38.7 l	5.7 l	4.9 l	5	0
VA05W-534	52.6 l	24.9 l	17.0 l	14 l	38.1 l	5.3 l	17.9 l	7	0
MD01W233-06-1	53.5 l	26.1	18.8 l	19.4 l	44.0	4.7 l	4.8 l	5	0
MD01W233-06-16	56.6	28.5	20.3	19.8 l	41.7 l	6.3 l	20.4 l	4	0
MD99W483-06-11	63.3	38.8	27.9	33.6 h	49.0 h	8.0 l	70.0 h	1	3
NYCalresel-L	60.0	40.2	33.1	33.2 h	53.7 h	14.8	51.1 h	0	3
NY94052-9340	59.1	31.7	26.2	24.1 hl	43.7	12.5	11.2 l	2	1
NYW103-1-9100	61.6	37.0	33.1	16.8 l	40.9 l	12.6	17.8 l	3	0
NYW103-70-9232	66.0 h	42.3	38.1	22.5 l	45.6	17.5	34.1 l	2	1
NY93246SP-9070	61.9	34.9	32.7	29.1 h	48.5 h	11.0 l	18.8 l	2	2
SE911492-4	64.1	33.2	25.5	23.3 hl	44.2	9.0 l	34.2 l	3	1
SE89-1873-2	65.5	39.4	31.9	23.6 hl	46.8	13.7	62.2 h	1	2
SE98-1089-34	78.5 h	60.7 h	52.1 h	38.9 h	63.5 h	20.7	40.7 h	0	6
SE93-1094-8	67.2 h	39.9	32.8	31.7 h	53.6 h	19.8	29.9 l	1	3
NE05418	52.0 l	26.3	19.7	24.5 hl	41.5 l	8.3 l	23.7 l	5	1
NE05549	68.2 h	46.6	41.0	23.1 hl	50.9 h	14.0	31.3 l	2	3
NE05537	54.8	36.7	24.7	22.3 l	41.5 l	11.5 l	32.2 l	4	0
NE03488	62.5	33.7	27.3	24.1 hl	42.4 l	13.1	58.8 h	2	2
NE01643	66.4 h	37.7	34.0	20.2 l	45.7	15.7	33.7 l	2	1
KY00C-2059-16	58.0	36.6	24.8	21 l	40.7 l	15.3	46.3 h	2	1
KY00C-2143-08	59.2	32.8	21.2	18.2 l	41.2 l	8.9 l	39.5 h	3	1
KY00C-2755-03	61.2	39.0	30.1	23.9 hl	49.1 h	12.6	36.4	1	2
KY97C-0321-05-2	67.2 h	46.7	41.6	35.9 h	57.8 h	18.0	49.5 h	0	4
M04*5109	59.5	33.5	26.6	25.9 h	49.4 h	12.0 l	44.6 h	1	3
M04-4802	65.5	41.0	32.8	32.3 h	53.1 h	14.5	45.5 h	0	3
M03-3616-B11	53.4 l	26.6	19.3 l	19 l	41.3 l	9.1 l	13.8 l	6	0
M03-3616-C10	51.9 l	25.4 l	17.3 l	24.4 hl	39.1 l	10.7 l	17.9 l	7	1
OH02-13567	54.1 l	28.7	18.3 l	22.8 hl	42.2 l	8.2 l	6.2 l	6	1
OH03-235-2	59.0	40.7	31.4	24.6 hl	50.3 h	13.8	24.5 l	2	2
OH02-12678	54.4 l	27.0	20.3	16.2 l	41.9 l	8.1 l	23.3 l	5	0
OH02-7217	59.4	28.3	21.2	16.3 l	39.6 l	11.5 l	15.5 l	4	0
DH 22/8	58.4	36.8	28.5	21.9 l	50.6 h	14.4	30.1 l	2	1
DH 22/24	42.4 l	19.5 l	12.5 l	17 l	31.6 l	7.8 l	9.0 l	7	0
DH 19/176B	67.4 h	42.4	36.1	23.6 hl	48.4 h	19.8	8.1 l	2	3
DH F/SF, 23	64.7	50.1 h	39.1	37.6 h	55.7 h	33.8 h	43.1 h	0	5
IL02-18228	41.9 l	15.3 l	13.0 l	10.7 l	28.1 l	5.5 l	24.1 l	7	0
IL02-19463	57.4	31.9	22.0	12.8 l	35.9 l	7.7 l	16.3 l	4	0
IL04-10118	55.9	24.7 l	18.9 l	10.5 l	35.7 l	8.2 l	14.5 l	6	0
IL04-10721	61.1	28.1	21.0	15 l	38.8 l	9.8 l	7.6 l	4	0
IL04-10741	48.7 l	24.1 l	18.9 l	21.6 l	38.5 l	7.0 l	29.8 l	7	0
AVERAGE	59.4	34.0	26.2	22.3	44.2	11.6	23.9		
LSD(0.05)	12.8	10.6	9.0	16.3	15.5	7.3	31.0		
# Environments	10.0	11.0	12.0	5	4.0	7	2.0		

l,h indicate a mean that is not significantly different than the lowest (l) or highest (h) mean in that column

TEN YEARS OF UNIFORM FHB TESTING OF SOFT WINTER WHEAT FROM THE NORTHERN U.S.

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OBJECTIVES

Soft winter wheat germplasm adapted to the northern part of the eastern US has been evaluated for resistance to Fusarium Head Blight (FHB) in two uniform trials: the Northern Uniform Winter Wheat Nursery (NUWWSN) and a preliminary version (PNUWWSN). Each breeder has different criteria objectives for the placing entries in the tests. Most entries are candidates for release as cultivars while others are being considered for release to meet other objectives. Some entries have gone through prior selection for FHB resistance while others have not. Our objectives were to summarize FHB testing from 1998 to 2007 and to assess trends over years.

MATERIALS AND METHODS

Each year the test are sent to multiple cooperators. Most grow the tests in inoculated nurseries and collect data on multiple traits. The number of locations reporting data on each trait varies by year and disease severity varies by location and year. From 1998-2007, three checks have been grown at each location and each year: Ernie (MR), Freedom (MR) and Pioneer 2545 (S). We analyzed incidence (INC, % of heads with at least one symptomatic spikelet), severity (SEV, % of symptomatic spikelets on heads showing symptoms), Index (IND, % = (INC*SEV)/100), deoxynivalenol content (DON, ppm), and percentage of seeds showing symptoms (FDK, %).

We standardized the trait value of the i^{th} genotype in the j^{th} environment (Y_{ij}) relative to the mean of the MR checks ($Y'_{ij}=Y_{ij}$–(mean of Ernie and Freedom)) to adjust for year and location effects. A second standardized trait value was obtained by dividing Y'_{ij} by the standard deviation from a particular environment and test ($Y''_{ij}=Y'_{ij}$/stdev of jth environment and test): Y, Y', and Y" are the means of Y_{ij}, Y'_{ij}, Y''_{ij} for a genotype over all environments. We obtained a best linear unbiased prediction (BLUP) of Y, Y', and Y" for each genotype using a mixed model where test, year, and location were considered fixed effects and genotype was considered random. The precision of the estimate of each effect varies as the number of testing environments varied by genotype. The variation in precision is incorporated in the BLUPs. For example, say genotypes A and B have equal means, but A was tested for one year while B was tested for five years. The value of A will regress towards the mean more than the value of B when obtaining BLUPs so their BLUPs will not be equal. The average BLUP for all genotypes in an analysis will be zero. To evaluate trends over years we regressed Y, Y', Y" and the BLUPs of each for all non-check genotype tested in a year on year of testing.

A principal component analysis using the correlation matrix among traits was conducted using BLUPS of Y' for all traits and all 462 genotypes.

RESULTS

Over the ten years, 462 unique genotypes were evaluated for FHB resistance. Significant genetic variation was found for each trait using Y, Y', or Y". There was a significant positive correlation among all traits for Y with the strongest correlations being among the three spike traits (INC, SEV, IND: all $r > 0.7$) and the weakest correlations being between the spike traits and DON (all $r < 0.47$). The principal component analysis of the BLUPs of Y' captured 64% of the variation in PC1 and 13% in PC2 (Fig. 1). PC1 modeled variation from all traits, but was dominated by the spike traits and FDK. PC2 primarily modeled a compo-

nent of DON that is independent of the first axis. The BLUPs of Y' for the genotypes with low PC1 and PC2 scores are shown in Table 1. Table 1 also has BLUPs of Y' for several other genotypes that had either low IND or low DON values. Only four genotypes were among the best 31 genotypes for IND, DON, FDK, and GH: IL97-6755, MO980829, Bess, NY87048W-7388, and OH904 (Table 1).

Using BLUPs of Y', genotypes that were superior to Truman were rare for except DON where 16.3% of the genotypes had lower DON values than Truman (Figs. 2 and 3). Genotype worse that the susceptible check Pioneer 2545 were also rare. More than 28% of all genotypes were superior to Freedom for all traits except GH.

There was no significant linear trend for increased resistance over time for any trait or measure (Y, Y', Y", or their BLUPs) except for Y" for Index (Table 2, Fig, 4). The greatest or second greatest Y' for all traits occurred in 2002 and this may have prevented finding a significant linear trend from 1998-2007 (Fig. 5). Performing regression using data from 2002 to 1998 decreased the slope of nearly all measures and traits and produced significant slopes for DON (Y), GH (Y', Y") and INC (Y") (Table 2). A trend for improved resistance from 2002-2007 was suggested for all traits.

DISCUSSION

A significant number of genotypes in the NUWWSN and PNUWWSN displayed a high level of FHB resistance as assessed by multiple traits. In general, resistance that is superior to that of Freedom was fairly common, while strong moderate resistance such as that displayed by Truman was rare. Seventeen (41%) of the 41 non-check genotypes in Table 1 have an exotic source of FHB resistance in their pedigree. This percentage is likely much higher than would be found among all 462 entries. Of the 10 best genotypes for IND, five had exotic parentage and putatively have some Asian QTL alleles that are known to improve IND (Table 1). Two of the10 best for DON had exotic parentage (Table1). The use of native or exotic parentage can lead to strong moderate resistance.

The data and tests are not well suited to investigate the effect of selection over time. The entries come from multiple breeders, each using different populations, different FHB selection pressure prior to submission, and have different objectives they are trying to attain with their entries. Entries are not necessarily the most FHB resistance material from each breeder. Rather they are their genotype most likely to meet their individual objectives such as improved yield, quality, or resistance to other diseases, as well as resistance to FHB. Despite these issues that would minimize directional selection for FHB resistance, trends for increased resistance for all traits over time were evident. This was most notable for IND from 1998 to 2007, and for DON and GH from 2002-2007.

Table 1. BLUPs of Y' values and rank of the best genotypes based on principal component analysis (PCA, Fig. 1) or ranking by Index (IND) or DON values.

	NAME	IND		INC		SEV		DON		FDK		GH	
PCA	*0128A1-36	194	-1.6	84	-4.6	57	-5.5	64	-2.6	17	-9.8	92	-8.1
PCA	*01931A1-5	134	-3.0	149	-2.6	53	-5.6	46	-3.0	11	-11.0	66	-10.0
PCA	*97395B1-4-2-7	240	-0.1	202	-0.5	118	-3.4	12	-4.5	47	-6.7	155	-4.8
PCA	*97417A1-3-4	83	-4.3	138	-3.1	111	-3.8	13	-4.3	96	-4.7	108	-7.0
PCA	9793A1-5	51	-5.3	69	-5.3	84	-4.4	24	-3.8	43	-6.9	201	-2.2
PCA	HONDO	7	-8.7	83	-4.7	16	-7.6	18	-4.1	113	-4.2	134	-5.8
PCA	IL00-8061	25	-6.6	18	-9.8	24	-7.0	33	-3.3	3	-13.0	84	-8.6
PCA	IL00-8530	210	-1.1	188	-1.0	188	-1.4	52	-2.9	9	-11.2	93	-8.1
PCA	IL01-11934	162	-2.4	53	-6.2	127	-3.1	77	-2.3	31	-8.0	102	-7.7
PCA	*IL01-34159	49	-5.4	72	-5.1	80	-4.6	71	-2.4	32	-8.0	31	-12.0
PCA	IL01-5943	43	-5.6	76	-4.9	38	-6.1	67	-2.6	58	-6.3	123	-6.4
PCA	IL02-7735	59	-4.9	27	-8.8	104	-4.0	117	-1.6	40	-7.2	295	4.4
PCA	IL95-4162	93	-3.9	23	-9.2	150	-2.3	60	-2.7	29	-8.1	138	-5.7
PCA	*IL96-24851-1	19	-6.8	100	-4.0	29	-6.5	14	-4.2	119	-4.0	18	-14.0
PCA	IL96-3073	24	-6.6	9	-12.2	43	-6.0	19	-4.1	15	-10.2	44	-11.3
PCA	IL96-6472	189	-1.6	90	-4.3	203	-1.0	7	-5.4	6	-11.7	110	-6.8
PCA	IL97-1828	16	-7.2	16	-9.9	17	-7.6	3	-6.9	7	-11.7	167	-4.1
PCA	IL97-2945	39	-5.8	11	-11.3	148	-2.3	25	-3.8	19	-9.2	189	-2.9
PCA	IL97-4228	228	-0.3	48	-6.4	262	0.7	11	-4.5	74	-5.7	98	-7.8
PCA	*3 IL97-6755	4	-9.6	6	-14.1	1	-13.0	2	-7.3	4	-13.0	13	-14.8
PCA	IL99-20756	173	-2.1	117	-3.5	109	-3.9	101	-1.9	1	-14.3	49	-10.9
PCA	IL99-27048	94	-3.9	55	-6.1	64	-5.2	27	-3.6	10	-11.2	135	-5.8
PCA	MO980829	1	-11.8	10	-11.6	11	-8.7	5	-6.3	28	-8.1	3	-17.4
PCA	Bess = MO981020	12	-8.2	25	-8.9	13	-7.9	10	-4.9	20	-9.0	7	-15.8
PCA	*3,6 NY87048W-7388	10	-8.4	42	-6.8	9	-9.0	31	-3.4	12	-10.9	14	-14.8
PCA	NY89064SP-7139	18	-6.8	87	-4.5	205	-1.0	1	-8.2	55	-6.4	141	-5.5
PCA	*2,3,5 OH902	23	-6.6	14	-10.3	128	-3.1	49	-2.9	42	-6.9	60	-10.3
PCA	*2,3,5 OH903	5	-8.9	2	-17.4	3	-10.1	23	-3.8	24	-8.8	130	-5.8
PCA	*2,3,5 OH904	6	-8.8	4	-16.3	7	-9.1	16	-4.1	25	-8.6	17	-14.2
PCA	*VA02W708	15	-7.7	46	-6.5	51	-5.7	15	-4.1	16	-10.2	80	-8.9
PCA	*3 VA04W-563	193	-1.6	141	-3.0	166	-1.9	37	-3.2	13	-10.6	53	-10.7
PCA	*VA05W-417	92	-3.9	62	-5.7	85	-4.4	59	-2.7	81	-5.3	70	-9.8
IND	89118RC1-X-9-3-3	11	-8.4	109	-3.7	25	-6.9	222	-0.7	78	-5.5		
IND	*981359C1-4	8	-8.6	130	-3.3	74	-4.8	232	-0.6	5	-11.7	121	-6.5
IND	*2,3,5 E6003	2	-10.5	1	-18.9	6	-9.3	213	-0.7	23	-8.8	39	-11.6
IND	MO011174	14	-7.9	37	-7.3	66	-5.1	53	-2.8	51	-6.5	16	-14.5
IND	OH618	9	-8.5	47	-6.5	8	-9.1	181	-1.0	138	-3.4		
DON	IL96-3514	272	0.7	147	-2.6	274	0.9	8	-5.1	65	-6.2	150	-5.0
DON	IL98-6718	243	-0.1	215	0.0	218	-0.7	4	-6.8	50	-6.5	299	4.5
DON	NY89082-7159	152	-2.6	213	-0.1	323	2.6	9	-5.0	201	-0.8	341	8.8
DON	*VA02W694	291	1.1	64	-5.6	191	-1.3	6	-6.2	177	-2.1	388	14.1
	TRUMAN	3	-10.0	7	-13.6	2	-11.9	75	-2.3	14	-10.3	1	-18.8
	ERNIE	68	-4.6	49	-6.4	32	-6.4	265	-0.2	48	-6.7	65	-10.1
	FREEDOM	138	-2.9	285	1.6	129	-3.1	228	-0.6	331	3.1	34	-11.8
	PIO2545	457	11.4	461	12.9	459	11.7	437	5.4	461	15.6	359	11.2

* indicates genotype with parentage from exotic sources of FHB resistance. 2, 3, 5, 6 indicate a genotype that likely has an exotic FHB resistance allele for QTL on 2DL, 3BS, 5AS, or 6BS based on haplotype.

Table 2. Regression coefficients (*b*) from regressing mean trait values (Y, Y', Y") of all non-check genotypes tested in a year on the year of test.

	All Years			**2002-2007**		
	Y	Y'	Y"	Y	Y'	Y"
INC	0.19	-.026	0.10	-0.95	-0.96	-0.08*
SEV	-0.29	-0.64	-0.05	-2.25	-1.81	-0.15
IND	-0.19	-0.32	-0.04*	-1.55	-0.16	-0.03
FDK	-0.45	0.62	0.08	-1.86	-0.42	-0.05
DON	-0.36	-0.01	0.02	-2.43*	-0.68	-0.07
GH	-0.98	-0.38	-0.02	-3.10	-1.92*	-0.11*

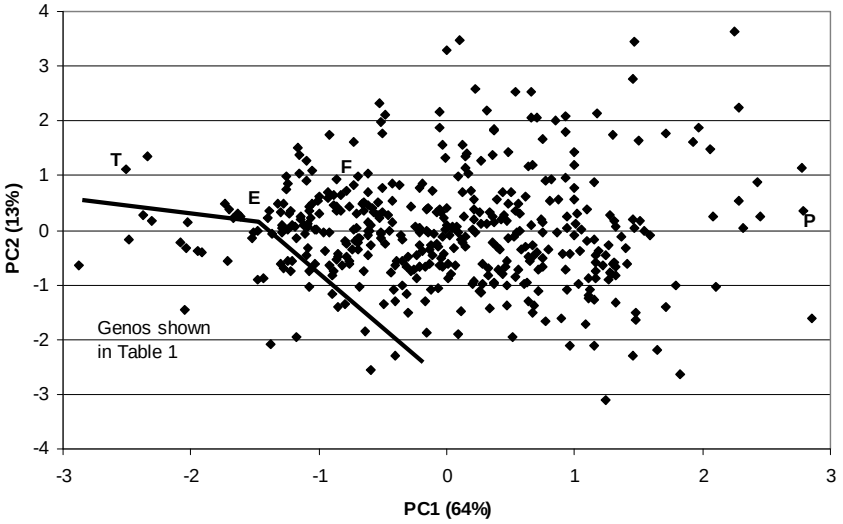

Figure 1. First two principal components from analysis of 462 genotypes and six traits. T (Truman), E (Ernie) ,F (Freedom), and P (PIO 2545) indicate position of checks.

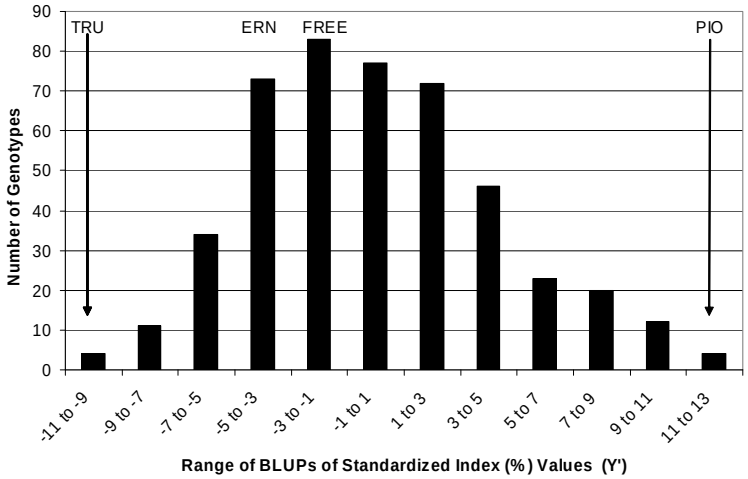

Figure 2. Distribution of the BLUPs of standardized Index values (Y') of all genotypes tested from 1998 to 2007.

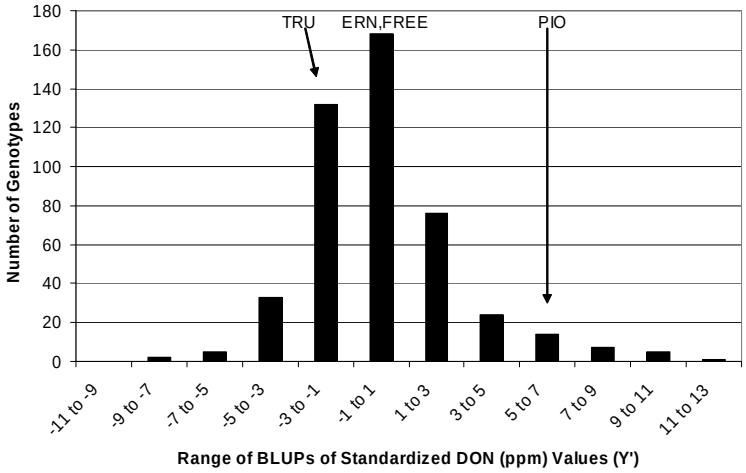

Figure 3. Distribution of the BLUPs of standardized DON values (Y') of all genotypes tested from 1998 to 2007.

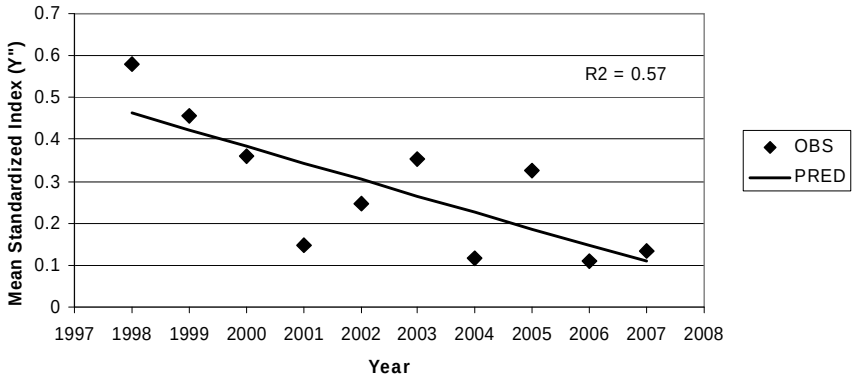

Figure 4. Regression of mean Y" of Index for non-check genotypes on year of test.

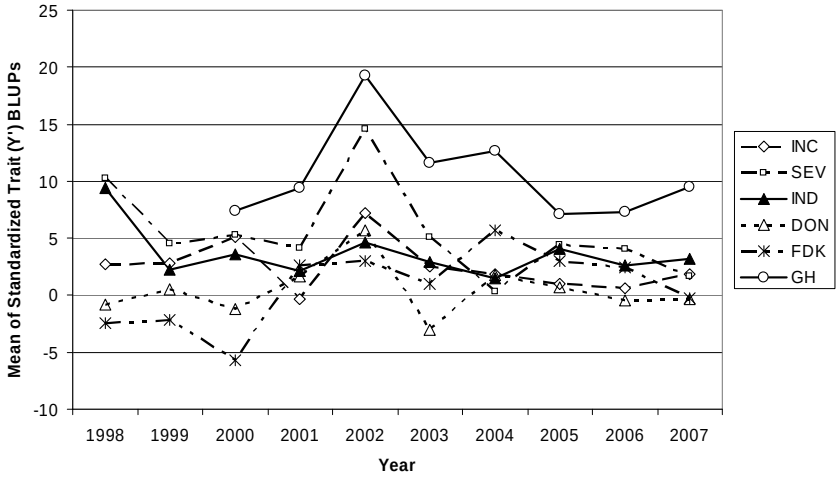

Figure 5. Mean Y' for each trait of non-check genotypes for each year.

WHEAT QUALITY EVALUATION OF FUSARIUM HEAD BLIGHT (*FUSARIUM GRAMINEARUM*) RESISTANT SOFT WHEATS AND THE EFFECT OF FUNGICIDE MANAGEMENT ON WHEAT QUALITY.

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ABSTRACT

Wheat quality was evaluated for Fusarium head blight resistant germplasm entered into regional FHB nurseries. A sub-set of lines were identified in the evaluation that had good soft wheat quality and significant resistance to Fusarium head blight. Results will be summarized in the poster. In a second study, wheat quality was evaluated for Fusarium head blight resistant cultivars and for the effect of fungicide vs. non-fungicide treatments on soft white wheats. To test the effect of fungicide treatment, milling and baking quality were compared in wheat grown at four locations in paired plots of five varieties with or without fungicide treatment applied at flowering. Contrary to previous European studies, we found that the application of fungicide did not change grain falling number values and had no significant implication in quality. Treatment with Prosaro reduced the FHB index by approximately 40%. Yet, even for susceptible varieties, the fungicide did not significantly improve milling and baking quality. We also tested the historical relationship between FHB resistance and quality. An examination of FHB resistance scores publicized by cooperating researchers against quality data generated at the USDA-ARS Soft wheat Quality Lab and compiled for 15 trials across the eastern US and comprising 377 entries, revealed no consistent trend between resistance and quality. The best sources of FHB resistance, such as Bess and Truman, are moderate in quality. Using inoculated trials, we also evaluated FHB resistance against milling and baking quality for matched uninoculated plots among 38 commercial cultivars in Wooster in 2007. In these trials at Wooster, several lines with very good quality were found to have moderate FHB resistance. AGI 401 followed by SC 1348 and SC 1358 fit these criteria and may be good choices for managing FHB infection while maintaining good soft wheat quality.

INTO THE WILD: FHB RESISTANCE IDENTIFIED IN *HORDEUM VULGARE* SUBSP. *SPONTANEUM*.

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, has devastated the malting barley industry in the Upper Midwest. The deployment of cultivars with resistance to *F. graminearum* and its associated mycotoxins (i.e. deoxynivalenol or DON) is the best means for combating the disease. Extensive evaluations of cultivated barley germplasm have identified only a few sources of partial resistance to FHB. The objective of this study was to expand the search for FHB resistance in the wild progenitor of cultivated barley, *Hordeum vulgare* subsp. *spontaneum*. An ecogeographically diverse collection of wild barley accessions (1,770 in total), primarily from the Fertile Crescent but also Central Asia and North Africa, was evaluated for reaction to FHB in screening nurseries at Zhejiang University in Hangzhou, China from 2003-2008. Accessions were planted in late October to early November, inoculated in March, and scored for FHB severity in May. Inoculations were performed using the "grain-spawn" method, and the nurseries were irrigated daily to promote infection. At the mid-dough stage, disease assessments were made on plants using a 1-5 scale, where 1 is most resistant and 5 is most susceptible. Of the 1,770 accessions tested, only 20 (1.1%) exhibited a resistance level comparable to Chevron, the six-rowed resistant control. Eleven of the 20 accessions were from Israel, suggesting that this country may be a center of concentration for FHB resistance. Other resistant accessions originated from Iran, Iraq, Syria, Jordan, and Azerbaijan. One of the most resistant accessions found (PI 466423) comes from Israel near the Jordan River and has a distinct morphology (a petite spike) compared to other wild barley accessions. In replicated tests conducted in Hangzhou, China in 2005-2007, PI 466423 exhibited FHB severities that were slightly lower (1.4 vs. 1.7) than Chevron. Being a unique wild barley accession, PI 466423 likely possesses alleles for FHB resistance that have not yet been exploited in breeding programs. Our ultimate goal is to reduce the losses caused by FHB, including quality discounts due to DON contamination. This can be best achieved by developing barley cultivars with the highest level of resistance possible. The next objectives for this project are to determine the number and chromosomal position of FHB resistance loci in PI 466423 and transfer them as quickly as possible into cultivated barley. This is now being done using the "advanced backcross QTL" method. The information generated from this study will lead to the development of malting barley cultivars with enhanced FHB resistance and low DON accumulation.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-6-073. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

AN UPDATE ON THE DEVELOPMENT OF FUSARIUM HEAD BLIGHT (FHB) RESISTANT WHEAT GERMPLASM WITH LOWER DEOXYNIVALENOL (DON) ACCUMULATION AT THE UNIVERSITY OF GUELPH, ONTARIO, CANADA.

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INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is an important wheat disease. Deoxynivalenol (DON) is the most frequent mycotoxin in wheat grain in Canada produced by *F. graminearum*. Different types of FHB resistance have been reported in wheat, including type I and II resistance (resistance to initial infection and spread of symptoms within the spike, respectively) and type III and IV resistance (resistance and tolerance mechanisms to trichothecenes mycotoxins including DON). The disease is strongly influenced by the environment; multiple locations screening with reliable checks, is needed to identify new FHB resistant breeding material.

MATERIALS AND METHODS

The wheat breeding program at the University of Guelph has participated in the collaborative Northern Uniform Winter Wheat Scab Nursery (NUWWSN) and Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN) with other breeders from USA for the past five and two years, respectively. Each partner has been contributing up to six wheat lines which are tested for different types of *Fusarium* resistance across all locations every year. The winter wheat lines were inoculated in the field with *F. graminearum* at anthesis, and rated two-three weeks later for visual symptoms of FHB infection (FHB index (%)= severity x incidence/100). Number of locations ranged from eight to fourteen from 2002 to 2007. Severity of wheat heads, point-inoculated with *F. graminearum* at anthesis in the greenhouse (GH), was also rated for each line included in the test. In mature grain, the DON content and percent of scabby seed (PSS) was estimated from each line from several locations each year. In addition, ISK index- % (resistance based on incidence, severity and percent of scabby seed) was calculated from each line from 2003 to 2007.

RESULTS

- Some lines showed low levels for *Fusarium* head traits recorded, but high for *Fusarium* kernel traits recorded and *vice versa*.

- In 2002-2003, our line RCATL33 was rated amongst some of the most resistant entries in the test (registered as germplasm-Crop Sci. 2006. 46:1399-1400), (Table 1).

- In 2004-2005, line RCAT31 was our most *Fusarium* resistant line tested (Table 1).

- In the 2005-2006 NUWWSN test, RCAT TF203/2 was among the best entries for Fusarium resistance (Table 1), in addition to excellent soft wheat quality traits (http://www.scabusa.org -NUWWSN Reports).

- In 2005-2006, line RCATTF 174/1C was among the best entries for all traits recorded across all locations in PNUWWSN test (Table 2).

- In 2006-2007, lines RCUOGF110202D/4 and RCUOGDHACF1109O2D were among the best entries for all traits recorded across all locations in NUWWSN test. Line RCUOGF110202D/4 had low mean DON level of 1.7 ppm (Table 1).

- In 2006-2007, line RCUOG10/18 had the lowest DON level (1.3 ppm- Table 2) among all lines developed in our program to date and was 1 out of 3 lines in PNUWWSN test with DON level <2.0 ppm.

CONCLUSIONS

- The results showed that is necessary to select for all types of FHB Resistance simultaneously.

- Multiple locations tests have been very beneficial for all participants.

- Different sources of FHB resistance have been used in our Breeding program with a goal to pyramid FHB resistance to type I, Il, III and IV in single cultivars.

- Our long term objective is to release winter wheat cultivars, adapted to Ontario, with improved FHB resistance, quality, and yield.

ACKNOWLEDGEMENTS

Financially supported by OWPMB and Canadian Adaptation Council. The contribution of the U.S. Wheat and Barley Scab Initiative and the cooperators who collected the data for NUWWSN and PNUWWSN test is greatly appreciated. Summary reports on the NUWWSN and PNUWWSN from Dr. Clay Sneller are gratefully acknowledged.

Table 1. NUWWSN test-average level for *Fusarium* traits recorded across all locations for RCAT/ RCUOG lines and check entries (2002-2007).

Year	Line	Source of resistance	FHB index (%)	GH severity (%)	DON (ppm)	PSS (%)	ISK index %)
2002-2003			10 locat.*	5 locat.	4 locat.	4 locat.	
	RCATL33	Frontana +Sumai 3	14.6 L**	46.2	13.2 L	39.3	
	RCATL10	SVP72017-17-5-10-1	27.6	34.7 L	16.3	48.9	
	RCATL13	Frontana	36.0 H**	65.9 H	29.6 H	51.5 H	
	RCATTF19/26	EX9806	25.4	51.9	8.9 L	50.4 H	
	RCATTF2/4	EX9806	19.3 L	56.2 H	14.1 L	49.1	
	RCATTF17/34		39.3 H	61.2 H	28.3 H	53.8 H	
	ERNIE-MR check		19.0 L	27.6 L	17.3	36.3 L	
	FREEDOM-MR check		21.6	37.8 L	14.6	49.9 H	
	PIONEER 2545-S check		34.4 H	43.5	21.2 H	58.6 H	
2003-2004	Line		14 locat.	4 locat.	6 locat.	5 locat.	5 locat.
	RCATL33	Frontana +Sumai 3	23.0	31.2	4.4 L	28.5	25.0
	RCATL10	SVP72017-17-5-10-1	26.5	51.0 H	11.5	43.0 H	32.7
	RCAT 24	Ena + Frontana	16.2 L	33.9	10.6	30.5	16.4 L
	RCATL12	AC Morley	24.5	26.1	6.7	44.5 H	27.5
	RCATL2	Frontana	28.5	23.6 L	5.4 L	25.1 L	24.0
	ERNIE		24.4	13.9 L	4.4 L	18.6 L	18.6 L
	TRUMAN-MR/R check		13.0 L	8.2 L	3.9 L	22.6 L	14.0 L
	FREEDOM		26.3	12.2 L	4.6 L	35.9	32.2
	PIONEER 2545		43.5 H	32.7	11.1	48.2 H	42.0 H
2004-2005	Line		11 locat.	3 locat.	4 locat.	3 locat.	4 locat.
	RCAT 13/18	Frontana	23.6	57.9 H	8.9 L	27.6	48.0 H
	RCAT 23/1	Ena	19.2	24.2 L	9.2 L	22.6 L	44.9
	RCAT 24	Ena + Frontana	21.7	12.1 L	17.6 H	20.1 L	40.1
	RCAT 28	Frontana	22.3	33.7	11.4 L	36.1 H	48.3 H
	RCAT 31	Frontana + SVP72017-17-5-10-1	14.2 L	25.1 L	2.7 L	20.3 L	35.6
	ERNIE		11.3 L	12.5 L	6.2 L	23.9	31.4 L
	TRUMAN		9.9 L	9.0 L	2.7 L	17.4 L	25.9 L
	FREEDOM		16.1	17.2 L	6.1 L	29.7	40.3
	PIONEER 2545		32.5 H	27.4 L	11.3	41.0 H	59.2 H
2005-2006	Line		13 locat.	3 locat.	7 locat.	4 locat.	4 locat.
	RCAT 202D/ 1	Freedom	21.3	22.5	7.0	15.0 L	42.5
	RCAT 32/157	Frontana +Sumai 3	14.7 L	55.0 H	7.5	25.0	31.6 L
	RCATTF 203/2	Sumai 3	14.9 L	15.8	5.4	8.2 L	27.4 L
	RCAT19/4c	AC Morley +Sumai 3	14.8 L	45.5	5.0	24.8	31.0 L
	ERNIE		20.2	24.3	5.5	7.4 L	25.4 L
	TRUMAN		12.4 L	3.3 L	4.5	5.2 L	24.1 L
	FREEDOM		16.4 L	13.3 L	7.0	17.8 L	38.4
	PIONEER 2545		31.9 H	38.2	10.4 H	44.0 H	57.9 H

Table 1 (cont).

Year	Line	Source of resistance	FHB Index (%)	GH severity (%)	DON (ppm)	PSS (%)	ISK index %)
2006-2007	Line		13 locat.	2 locat.	2 locat.	5 locat.	5 locat.
	RCUOG19/21	Sumai 3	15.8 L	31.2 L	7.8	14.2 L	29.5
	RCUOGF110202D/4	SD07060 + R. Star	14.7 L	10.7 L	1.7 L	11.1 L	23.5 L
	RCUOGF111202A/3	Freedom	21.9	25.5 L	5.9	22.4 H	42.5 H
	RCUOGDHACF1109O2D	SD07060 +Freedom	12.1 L	25.4 L	3.9 L	22.7 H	26.7 L
	RCUOGNS984-1		28.3 H	45.9 H	8.2	26.6 H	42.6 H
	ERNIE		16.8	18.0 L	6.2	4.3 L	30.7
	TRUMAN		6.1 L	3.4 L	3.9 L	12.6 L	16.6 L
	FREEDOM		15.2 L	20.7 L	5.8	7.1 L	34.9
	PIONEER 2545		30.0 H	50.5 H	11.6	18.5 H	50.3 H

*average (number of locations) for each trait; L**, H** indicate a mean that is not significantly different (LSD=0.05) than the lowest or highest mean.

Table 2. PNUWWSN test-average level for *Fusarium* traits recorded across all locations for RCAT/RCUOG lines and check entries (2005-2007).

Year	Line	Source of resistance	FHB index (%)	GH severity (%)	DON (ppm)	PSS (%)	ISK Index (%)
2005-2006			9 locat.*	2 locat.	4 locat.	2 locat.	3 locat.
	RCAT 32/35B	Frontana +Sumai 3	25.4 H**	35.2 L**	6.4 H	8.9 L	30.6 H
	RCAT F 13	Maringa	20.1 H	23.0 L	7.3 H	38.5 H	43.2 H
	RCATTF 174/1C	Sumai 3	9.1 L	8.1 L	4.8 L	21.3	22.6 L
	ERNIE-MR check		14.6 L	26.5 L	5.2 L	3.4 L	20.3 L
	TRUMAN-MR/R check		7.1 L	13.7 L	2.2 L	4.8 L	19.9 L
	FREEDOM-MR check		14.8 L	14.4 L	4.5 L	7.2 L	29.2 H
	PIONEER 2545-S check		27.7 H	58.4 H	8.9 H	15.4 L	38.8 H
2006-2007	Line		8 locat.	1 locat.	3 locat.	2 locat.	3 locat.
	RCUOG Golden Value		38.9 H	85.4 H	8.3 H	40.1 H	46.7 H
	RCUOGL15	Frontana + SVP72017-17-5-10-1	17.9 L	76.2 H	4.6 L	23.1	35.6 H
	RCUOGL4	EX9806	22.6	40.3 L	6.9 H	13.9 L	31.1
	RCUOGL17	SVP72017-17-5-10-1	17.7 L	66.7 H	4.5 L	11.2 L	25.6
	RCUOG10/18	Frontana +Sumai 3	16.8 L	17.4 L	1.3 L	6.8 L	16.9 L
	ERNIE		12.9 L	28.6 L	4.9	12.7 L	23.6 L
	TRUMAN		7.3 L	12.3 L	2.2 L	9.4 L	17.8 L
	FREEDOM		15.6 L	5.0 L	6.0 H	12.5 L	26.2
	PIONEER 2545		30.9 H	50.5 H	6.5 H	28.6 H	39.8 H

*average (number of locations) for each trait; L**, H** indicate a mean that is not significantly different (LSD=0.05) than the lowest or highest mean.

INTROGRESSION OF FHB RESISTANCE FROM ALIEN SPECIES-DERIVED LINES INTO SPRING WHEAT.

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ABSTRACT

We have produced and collected over 300 wheat lines derived from the crosses of wheat with wild species related to wheat. Evaluation of these lines for reaction to Fusarium head blight (FHB) identified 74 lines with resistance comparable to "Sumai 3" in two greenhouse seasons. Most of the resistant lines, however, cannot be utilized directly in wheat breeding because of the linkage drag associated with the alien chromatin from the wild species. We have been eliminating unwanted alien chromatin from the resistant lines by manipulating chromosomes and introgressing resistance into adapted spring wheat backgrounds through backcrossing and disease screening. To date, we have developed 285 alien introgression lines ($BC_{1\text{-}2}F_{6\text{-}9}$) that have consistently showed resistance in several greenhouse seasons. Some of the lines exhibited a level of resistance comparable to "Sumai 3". The most resistant lines (~150) were evaluated for FHB resistance and agronomic performance in the field at Langdon and Prosper, ND and Jianyang, China. About 20% of the lines maintained resistance under the high disease pressure in the fields. Most of the resistant lines contain minimal amounts of alien chromatin and do not have obvious linkage drag. We will continue improving the resistant lines with undesirable genes from wild species through chromosome manipulation. The resistant introgression lines were haplotyped at the molecular marker loci closely linked to several well-characterized FHB resistance QTLs. Some of the resistant lines were found to have the haplotypes different from those associated with the known QTL at the molecular marker loci investigated, suggesting the difference of the resistance QTL in the introgression lines from those known resistance QTLs. Currently, we have been preparing seed for DON testing and a larger scale of field evaluation to validate resistance of the introgression lines.

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U.S. Wheat & Barley

Scab Initiative