Proceedings of the 2015 National Fusarium Head Blight Forum

December 6-8, 2015
Hyatt Regency St. Louis at the Arch
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EFFECTIVENESS OF FHB INDICES IN ESTIMATING STRAW DON ACCUMULATION IN WINTER WHEAT CULTIVARS

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ABSRACT

Little is known about how deoxynivalenol (DON) accumulates in wheat straw tissue as a result of *Fusarium graminearum* infection in wheat heads. Finding a way to estimate DON concentration in the straw through the use of visual disease ratings and Fusarium head blight (FHB) indices is a challenge. Much research has focused on the advancement of FHB-resistant breeding lines through the use of the FHB index. FHB index has been used for the advancement of many wheat lines for FHB resistance. Results of recent research suggest that the incorporation of proportions of *Fusarium*-damaged kernels (FDK) with incidence and severity (known as the ISK index) offers a better index to evaluate and advance wheat lines. Research results have also suggested that incorporating grain DON along with incidence, severity, and kernel damage (known as the DISK index) might offer an even greater improvement in selecting for FHB-resistant lines. The two main purposes of this study were a) to determine the accuracy of different FHB indices to DON accumulation in the grain for the advancement of wheat lines and b) determine of these indices can be used to relate to DON accumulation in the straw.

This study was conducted over three growing seasons (2011, 2013 and 2014) and consisted of a selection of 16 to 20 winter wheat cultivars from the University of Illinois FHB nursery. FHB incidence and severity, and FDK were recorded for each cultivar. Immediately after harvest, the bottom 25 cm of straw was collected for each cultivar, dried in a forced air drier, and ground. DON concentrations were determined for both grain and straw.

All analyses were performed using PROC MIXED and PROC CORR in SAS with cultivar and resistance classes treated as fixed effects. Cultivars evaluated varied from year to year, and for this reason, years were analyzed separately. Evaluated cultivars ranged in their susceptibility to FHB. The FHB index was calculated by the formula Incidence*Severity/100, the ISK index was calculated by the formula (0.3*Incidence) + (0.3*Severity) + (0.4*FDK), and the DISK index was calculated by (0.2*Incidence) + (0.2*Severity) + (0.3*FDK) + (0.3*Grain DON). Correlation analysis was conducted to determine the relationships of the indices with DON in the grain and straw.

In all years, there was a large amount of variability in the DON concentrations in both grain and straw for all cultivars and classes, especially for the moderately susceptible and moderately resistant cultivars. The correlation between the DON in the grain and the DON in the straw was significant ($P < 0.05$) in 2011 and 2014 with a coefficient of 0.5 in both years, but this correlation was not significant in 2013.

In every year, resistant cultivars differed significantly ($P < 0.05$) from susceptible cultivars for all indices. In 2011 and 2014, each class was significantly different from one another in both ISK and DISK. Differences were not significant across all classes in 2013. The correlation between grain DON and FHB index was significant in all years with a correlation coefficient of 0.4 in all three years. The correlation between grain DON and the ISK index was significant in 2011 and 2014 with correlation coefficients of 0.7 in both years. The correlation between grain DON and DISK index was significant in all years, with a correlation coefficient of 0.8, 0.3, and 0.7 in 2011, 2013, and 2014, respectively.

The correlation between the DON in the straw and each of the indices may prove to be a useful indicator of which rating system provides the best estimate of the total DON accumulation in the straw. The correlation between straw DON and FHB index was significant in all years with a correlation coefficient of 0.4, 0.3, and 0.5 in 2011, 2013, and 2014, respectively. The correlation between straw DON and the ISK index was significant in all years with a correlation coefficient of 0.6, 0.3, and 0.7 in 2011, 2013, and 2014, respectively. The correlation between straw DON and DISK index was significant in all years with a correlation coefficient of 0.6, 0.3, and 0.6 in 2011, 2013, and 2014, respectively. For FHB index in all years, the correlation between FHB index and grain DON or straw DON was significant. However, the correlation coefficients indicated that these correlations were weak.

In conclusion, adding FDK and/or DON concentrations to the FHB index would provide some improvement in determining cultivar resistance to FHB and the accumulating DON toxin. The ISK index and DISK index were better measures of the resistance of a cultivar to FHB, though may fall short for MS or MR varieties. For the advancement of resistant lines, these indices are useful in determining which cultivars offer the best resistance to *F. graminearum* infection and the subsequent DON accumulation in both the grain and the straw tissue. For this reason, a rating that includes the FHB index and FDKs and DON accumulation may result in a more accurate assessment of FHB resistance to DON accumulation in both grain and straw.

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EFFECTS OF WINTER WHEAT CULTIVARS, FUNGICIDE APPLICATION TIMING, AND THE FUNGICIDES PROSARO® AND HEADLINE® ON FHB AND DON

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a disease of wheat whose frequency and severity have increased in Nebraska during the last 10 years. Major epidemics occurred in 2007, 2008, and 2015, and varying levels of the disease have occurred between 2008 and 2015. *Fusarium graminearum* produces the mycotoxin deoxynivalenol (DON). Therefore, in an FHB year, losses to the grower are manifested not only in yield and grain volume weight but also in discounts at the elevator if DON levels in grain exceed 2 ppm. Fungicide applications to control FHB are aimed at reducing disease intensity as well as DON. Because FHB infections occur on wheat heads mostly during flowering, optimal fungicide application is usually timed at early flowering. The narrow window (early flowering) of fungicide application presents challenges to the grower. Previous research has indicated that fungicides in the triazole class are more effective than those in the strobilurin class in suppressing FHB and DON, mainly because strobilurins are not as effective as triazoles in suppressing DON. The objectives of this research were to 1) Determine the effect of fungicide application timing at early flowering and 6 and 12 days later on FHB and DON in a susceptible and a moderately resistant cultivar and 2) Compare the effects of Prosaro® (a triazole) and Headline® (a strobilurin) on FHB and DON when applied at early flowering and 6 and 12 days later in a susceptible and a moderately resistant cultivar under Nebraska conditions. In 2015 two field trials (dryland and irrigated) were conducted in which Prosaro and Headline were applied at early flowering and at 6 and 12 days post early flowering (pef) (6 dpef and 12 dpef) to the cultivars Overley (susceptible) and Overland (moderately resistant). In the dryland trial in Overley, FHB index was similar between fungicides (Prosaro and Headline) and application timings (range 27-50%; 60% in the unsprayed check) and the same was true for DON (range, 33-46 ppm; 64 ppm in the unsprayed check). In the same trial in Overland, the results were similar except that FHB index (range 6-12%; 14% in unsprayed the check) and DON (range 6-14 ppm; 16 ppm in the unsprayed check) were significantly lower than in Overley. In the irrigated trial in Overley sprayed with Prosaro, FHB index was 42, 56, and 72% in the early flowering, 6 dpef, and 12 dpef treatments, respectively compared to 80% in the unsprayed check. The corresponding DON values were 19, 17, and 37 ppm, respectively compared to 91 ppm in the unsprayed check. In the same trial in Overley sprayed with Headline, FHB index was 62, 73, and 86% in the early flowering, 6 dpef, and 12 dpef treatments, respectively compared to 80% in the unsprayed check and the corresponding DON values were 41, 40, and 48 ppm, respectively compared to 91 ppm in the unsprayed check. In the irrigated trial in Overland, FHB index was similar between Prosaro and Headline (range 19 to 35% compared to 39% in the unsprayed check). Prosaro

DON values were 7, 11, and 18 ppm, in the early flowering, 6 dpef, and 12 dpef treatments, respectively compared to 46 ppm in the unsprayed check and the corresponding Headline DON values were 37, 18, and 29 ppm, respectively compared to 46 ppm in the unsprayed check. The results from this study can be summarized as follows: 1) FHB index and DON were significantly lower in Overland (moderately resistant) than in Overley (susceptible) as expected in both the dryland and irrigated trials, 2) the window of fungicide application to control FHB and DON can be widened from early flowering to 6 days later without loss of efficacy in suppressing FHB and DON, 3) Although Headline suppressed DON compared to the unsprayed check, DON in Headline treatments was significantly higher compared to the Prosaro treatments in the irrigated trial but not in the dryland trial, 4) Moderate resistance coupled with fungicide application can greatly reduce DON in grain.

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NATIONAL SURVEY OF U.S. WHEAT & BARLEY PRODUCERS ON SCAB MANAGEMENT PRACTICES

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ABSTRACT

In 2014, the USDA National Agricultural Statistics Service conducted a survey of wheat and barley producers' scab management practices that was commissioned by the U.S. Wheat & Barley Scab Initiative. The survey was carried out in 17 central and eastern U.S. states prone to scab and with significant acreages of wheat and/or barley. The purpose of the survey was to determine growers' perception of scab risk and best management practices (BMP), the extent of and barriers to BMP use, and how growers get their information about scab management.

The survey ultimately produced 5,107 usable questionnaires, with 4,683 farms that reported harvesting wheat for grain in the previous five years and 1,335 farms that reported harvesting barley for grain in the same period. Highlights of the findings:

Perception of scab as a problem varies geographically. On average, respondents in North Dakota, Minnesota, and the soft wheat states saw scab as a more serious problem than did those in AR, KS, NE, or SD. This was reflected in generally higher percentages in the former group of states who said that scab has reduced yields, caused problems with DON, and/or caused dockage or rejection of grain.

Use of varieties moderately resistant (MR) to scab. Overall, percentages of MR varieties varied significantly by market class. Out of the total acres reported by respondents in each class, the percentages planted to MR varieties were 47% for hard red spring (HRS) wheat, 29% for durum wheat, 21% for soft white winter (SWW) wheat, 15% for soft red winter (SRW) wheat, 11% for hard red winter wheat, and 8% for barley.

The percentages of respondents who said they reduced scab damage by growing moderately resistant (MR) varieties were not well aligned with the percentages of scab-resistant acres reported when growers were asked to name the top varieties they grew. An exception was in North Dakota and Minnesota, where respondents claimed relatively high rates of MR variety use (68% and 47%, respectively) and also reported relatively large percentages of specific MR variety acres (e.g., 46% and 67% of HRS acres, respectively). Also as an exception, New York respondents claimed a relatively low rate of MR variety use (37%) and reported relatively high percentages of variety acres that were MR (33% of SRW and 47% of SWW acres). By contrast, there was a substantial gap in many other SRW wheat states between the rate of perceived MR variety use (generally 35-70%) and the percentages of MR variety acreage reported (mostly <22%).

Use of a scab risk forecasting website. Growers were asked whether they had used a scab risk forecasting web site in the last five years. The highest percentage of positive responses was in North Dakota (18%), while in all the other states, the percentage was 8% or less. Of those who answered "yes," the large majority said the site was easy to understand and use, and useful for scab management.

Use of recommended fungicides to combat scab. The percentage of respondents who indicated they sometimes apply a recommended fungicide with scab as the target was highest in North Dakota (43%); intermediate in most soft wheat states (20-31%); and lowest in Kansas, Minnesota, Nebraska, New York, North Carolina, and South Dakota (9-17%). Growers were also asked which fungicide they applied the last time scab was the primary target. Overall, 1,222 responses of specific fungicide products were received, of which 54% were one of the most effective triazoles (Prosaro, Caramba, and Proline); 17% were another triazole; and 30% were strobilurins or strobilurin-triazole mixtures.

Rotation. There was a wide range in the percentage of respondents who said a graminaceous crop preceded wheat the last time the latter crop was harvested for grain. Percentages were relatively high (>50% of respondents) in Kansas, Kentucky, Maryland, New York, Pennsylvania, and Virginia. In the HRS states, as well as Indiana, Michigan, and Missouri, it depended considerably on district, with some parts of the state having low rates of wheat following an FHB host, and other parts having relatively high rates.

Barriers to use of BMP. Across all states, the barriers to using BMP that were most widely cited were weather (19% of respondents) and logistical difficulties (12% of respondents), such as problems engaging custom applicators, that prevent timely application of fungicides. The next most commonly experienced barriers were (1) the impracticality of rotations that keep wheat and barley from following other scab host crops, and (2) the difficulty of determining flowering dates in order to apply fungicides at the right time. This latter problem was cited by 10% of respondents overall, with the highest rates being 13-16% of respondents in Illinois, North Dakota, and Ohio. Information about scab resistance of varieties not being available or timely was a concern that varied significantly by state (e.g., 5-7% in Kansas, Indiana, Minnesota, Ohio, Pennsylvania, South Dakota, and Virginia, but 13% in New York and 21% in Kentucky).

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EVALUATION OF INTEGRATED METHODS FOR MANAGING FHB AND DON IN WINTER WHEAT IN NEW YORK IN 2015

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of the fungicide Prosaro on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) in New York.

INTRODUCTION

In response to the USWBSI goal to validate integrated management strategies for FHB and DON, the Disease Management RAC of USWBSI initiated a multi-state, multi-year, coordinated field study. In New York during 2015, we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with Prosaro fungicide at two timings.

MATERIALS AND METHODS

The trial was conducted at the Musgrave Research Farm in Aurora, NY in a Lima silt loam soil planted with four soft red winter wheat varieties, 'Otsego' (susceptible to FHB), 'Pioneer Brand 25R40' (moderately susceptible to Fusarium head blight (FHB), 'Emmit' (moderately susceptible to FHB), and 'Pioneer Brand 25R46' (moderately resistant to FHB), following corn harvest on 7 Oct 2014. The experiment was set up as a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the treatments as subplots, randomized in six replicated blocks. Main plots were sown with wheat at 118.8 lb/A with a 10 ft wide commercial grain drill. Subplots were 20 x 10 ft including 15 rows with 7-in. row spacing. The plots were fertilized at planting (200 lb/A of 10-20-20) and topdressed on 21 Apr (60 lb/A of urea, providing an additional 27.6 lb/A of nitrogen). The first Prosaro® application was at anthesis (Feekes growth stage, FGS 10.5.1) on 2 Jun including the surfactant Induce at 0.125% V/V. After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) to augment the development of FHB. The second Prosaro application occurred five days after anthesis on 7 Jun including the surfactant Induce at 0.125% V/V, and inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. Fungicide and *F. graminearum* treatments were applied with a tractor-mounted sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Incidence and severity (percent of symptomatic spikelets on symptomatic heads) of FHB in each plot were rated on 22 Jun and used to calculate FHB index, where FHB index = (FHB severity * FHB incidence)/100. Foliar diseases were rated on 22 Jun as percent severity on flag leaves (average rating for whole plot). Grain was harvested from a 20 x 5 ft area in each subplot using an Almaco plot combine on 23 Jul. Grain moistures, plot yields, and test weights were recorded. Yields and test weights were adjusted to bu/A at 13.5% moisture. *Fusarium*-damaged kernels (FDK) were evaluated post-harvest as a percentage

of kernels visibly affected by FHB out of a 100 kernel subsample from each plot. Analysis of deoxynivalenol (DON) content in grain was conducted by Dr. Yanhong Dong at the University of Minnesota. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test ($P = 0.05$).

RESULTS AND DISCUSSION

The incidence of FHB over all plots ranged from 4 to 46%. The impact of supplemental inoculation with *F. graminearum* was determined by comparing the non-inoculated and inoculum only treatment. Overall, inoculation resulted in significantly reduced yield and significantly increased FHB and DON as compared with the non-inoculated plots. FHB and DON development in 2015 were attributed primarily to supplemental rather than background inoculum.

Significant cultivar responses to inoculation were observed for yield, FHB and DON for the moderately susceptible variety Emmit and the susceptible variety Otsego, but only for FHB and DON for the moderately susceptible variety P25R40, and only for FHB for the moderately resistant variety Pioneer 25R46. These data support the current qualitative designations of varieties as moderately susceptible (Pioneer 25R40), moderately resistant (Pioneer 25R46). However, according to the results of this study, the quantitative susceptibility of Otsego, Emmit, and Pioneer 25R40 was indistinguishable.

Under moderately low disease pressure, significant differences were detected in yield among varieties, with both Pioneer varieties yielding significantly higher than Otsego and Emmit, regardless of treatment. Otsego had significantly higher FHB incidence and *Fusarium*-damaged kernels (FDK) than all the other varieties, regardless of treatment, but had FHB index similar to that of Emmit and P25R40. P25R46 had significantly lower FHB incidence, FDK and DON than all the other varieties, regardless of treatment, but had similar FHB index to that of P25R40. With excellent choices of high yielding varieties in the moderately resistant category, we counsel New York growers to no longer plant susceptible or moderately susceptible soft red winter wheats.

When results of all the cultivars were combined, the overall impact of each of the two Prosaro application timings was to significantly decrease FHB incidence, index, FDK, DON, and to significantly increase yield, as compared with the inoculum only treatment. Though not statistically significant, the Prosaro application at 7 days after the initiation of flowering resulted in the lowest FHB incidence, index and DON as compared with the Prosaro application at FGS 10.5.1. But it is also worth noting that sufficient fungicide remained on spikes from the FGS 10.5.1 Prosaro application to give significant suppression of FHB and DON resulting from fungal spores deposited on plants at 7 days after 10.5.1. It is unlikely that we would have seen any advantage of the late fungicide application over the earlier if spores had only been applied at the early timing. This underscores the necessity to apply supplemental inoculum corresponding to all timings that fungicides are applied in an unbiased experiment to assess comparative efficacy of fungicide timings.

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Table 1. Main effect of treatment on Fusarium head blight incidence, index, *Fusarium*-damaged kernels, deoxynivalenol contamination and grain yield at Aurora, NY in 2015.

Treatment	FHB incidence (%)	FHB index	FDK (%)	DON (ppm)	Yield (Bu/A)
Non-sprayed, non-inoculated control	17.5 b	2.7 b	7.3 b	0.9 c	67.8 a
Inoculated FGS 10.5.1, and inoculated 7 days later	31.3 a	8.6 a	11.7 a	1.9 a	60.9 b
Prosaro SC (6.5 fl oz) and inoculated FGS 10.5.1, then inoculated 7 days later	17.4 b	2.4 b	6.6 b	1.2 b	66.4 a
Inoculated FGS 10.5.1, then Prosaro SC (6.5 fl oz) and inoculated 7 days later	16.3 b	2.1 b	7.2 b	1.0 bc	67.7 a
LSD (*P*=0.05)	6.09	2.69	2.40	0.32	3.73
CV (%)	58.7	135.5	56.1	53.4	10.7

Table 2. Main effect of cultivar on Fusarium head blight incidence, index, *Fusarium*-damaged kernels, deoxynivalenol contamination and grain yield at Aurora, NY in 2015.

Cultivar	FHB incidence (%)	FHB index	FDK (%)	DON (ppm)	Yield (Bu/A)
Emmit	24.1 b	7.6 a	9.4 b	1.5 a	61.2 b
Otsego	29.8 a	5.2 ab	11.8 a	1.4 a	63.9 b
P25R40	21.0 b	2.8 bc	7.6 b	1.6 a	67.5 a
P25R46	7.6 c	0.3 c	4.1 c	0.6 b	69.9 a
LSD (*P*=0.05)	5.18	2.68	2.11	0.32	3.59
CV (%)	58.7	135.5	56.1	53.4	10.7

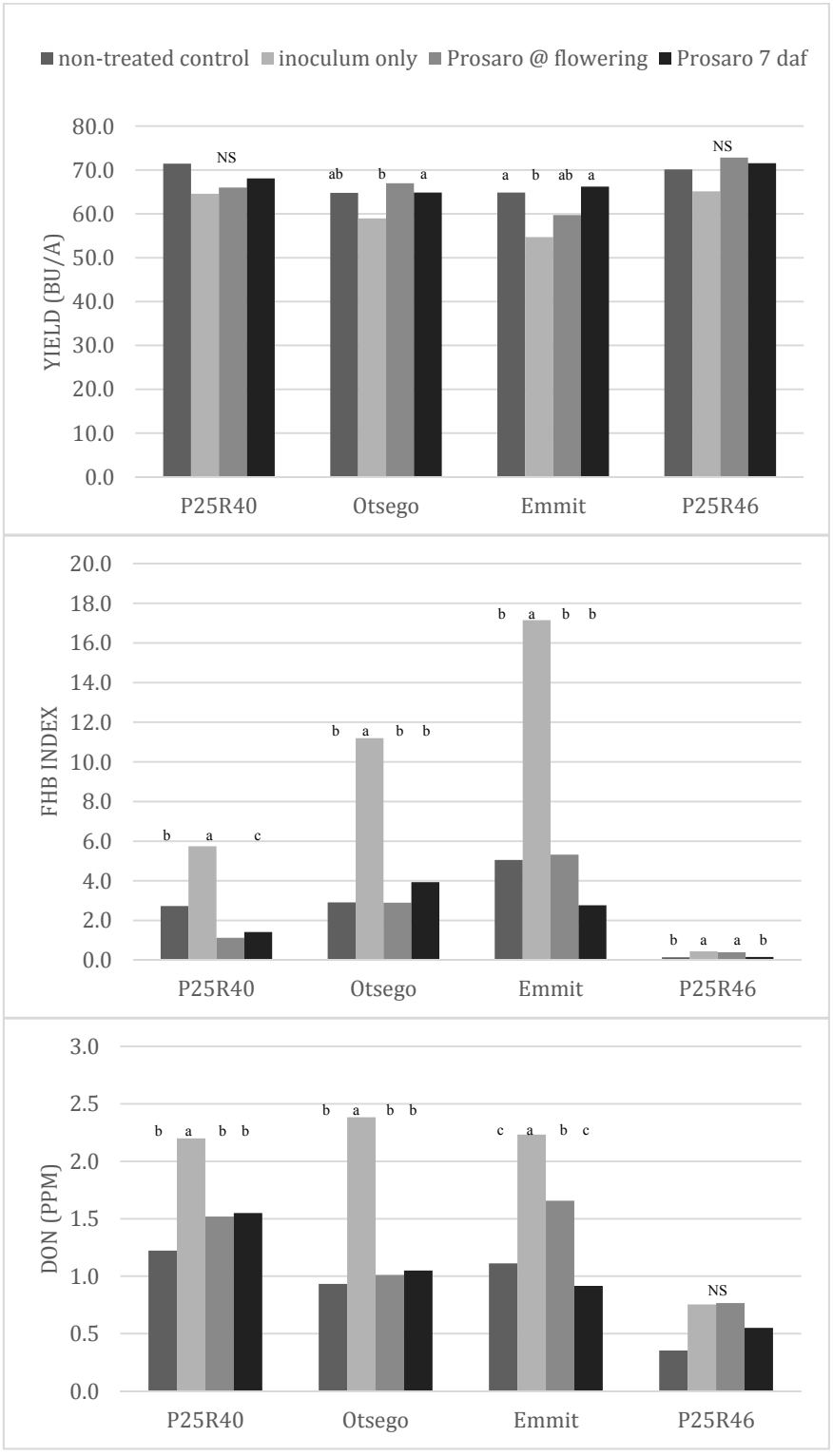

Figure 1. Effect of Prosaro® fungicide application and *F. graminearum* inoculation on yield, FHB index and DON contamination of four winter wheat cultivars in Aurora, NY in 2015.

IMPACT OF METEOROLOGICAL CONDITIONS AND PERITHECIA MATURITY ON *FUSARIUM GRAMINEARUM* ASCOSPORE RELEASE

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ABSTRACT

Cereal crops including wheat and barley will become increasingly stressed due to changing climate, growing populations, and harmful diseases. Fusarium head blight (FHB) is a harmful disease to cereal crops and is caused by the fungus *Fusarium graminearum*. The fungus produces ascospores capable of spreading the disease long distances (>500m) through the atmosphere. Information on the release of ascospores would be valuable in predicting FHB.

Our first research objective was to understand the meteorological conditions favorable for the release of ascospores. We investigated the numbers of ascospores released from perithecia and how far they traveled under controlled conditions. Ascospore release experiments were conducted with different combinations of temperature (15°C and 25°C) and relative humidity (60%, 75%, and 95%) for 12 hours in complete darkness. Ascospores released from perithecia were captured on microscope slides placed inside of 3D-printed spore discharge chambers. The number of ascospores released and the distances they traveled were quantified. The results showed that cold temperature and high relative humidity resulted in greater quantities and distances of ascospore release.

Our second research objective was to observe the relationship between the maturity of perithecia and the number of ascospores. A mechanical compression testing instrument was used to investigate the hardness of perithecia at various stages of maturity, resulting in a mean compression constant quantifying the uniaxial compression force required to rupture a perithecium. Results indicated that old (mature) perithecia contain the greatest amount of ascospores and require more force to rupture than young (immature) perithecia.

Collectively, our results may inform growers on the nature and timing of ascospore release, which could help inform FHB management decisions in the future.

VARIABILITY OF WHEAT TILLER GROWTH STAGES WITHIN SOFT RED WINTER WHEAT AND THEIR IMPACT ON FUNGICIDE TIMING DECISIONS

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ABSTRACT

A pilot field study was conducted in 2014 in West Lafayette, Indiana, to formally assess the range of growth stages across soft red winter wheat tillers during anthesis to determine the average growth stage of a plant when the primary tiller is determined to be at Feekes Growth Stage (FGS) 10.5.1. The goal of this research was to determine the potential contribution of secondary tillers to Fusarium head blight (FHB) in wheat and assess the growth stage at which fungicide is most effective at reducing disease incidence. This experiment was conducted within a larger fungicide timing experiment with a random complete block design. Tillers were evaluated from three replicates of the experimental plots, for a total of 21 plots. Within each plot, 3 plants were arbitrarily chosen on the pre-determined treatment date (anthesis + 0, 1, 3, 5, 8, 9, and 11 days after anthesis). The primary tiller was tagged, and then all tillers of the plant were growth staged. The growth stage of each tiller on the selected plants was determined in a clockwise direction, and was later rated for disease severity. Results indicated that when entire experimental plots were visually determined to be at 50% FGS 10.5.1, only 26% of rated tillers within the plot were at, or past, FGS 10.5.1. Not until 3 days after anthesis were over 50% of the rated tillers at or past FGS 10.5.1. Also, although tillers that were inoculated at FGS 10.5 to FGS 11 had the highest incidence of disease across all the inoculated, non-fungicide treated plots, tillers that had not yet reached FGS 10.5.1 when inoculated were able to become infected. This indicates that secondary tillers could be susceptible to infection past the optimum fungicide application timing and highlights the importance of including these secondary tillers in the determination of anthesis. Research will be conducted in 2016 to evaluate the contribution of secondary tillers to FHB and DON in wheat.

EVALUATING FUNGICIDE EFFICACY AND TIMING FOR MANAGEMENT OF FUSARIUM HEAD BLIGHT IN SPRING BARLEY IN NORTH DAKOTA

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ABSTRACT

The use of a well-timed fungicide is an important management tool when suppressing Fusarium head blight (scab) in barley production. With funding from the U.S. Wheat and Barley Scab Initiative, four fungicide trials were conducted on spring barley at two locations in North Dakota in 2014 and 2015. Two additional fungicide trials were conducted at another location. The primary objective at all three locations was to evaluate fungicide efficacy and timing on reducing disease and protecting yield in spring barley. Research sites were established at the Carrington Research and Extension Center (Carrington), North Dakota State University (Fargo) and Langdon Research and Extension Center (Langdon). Trials were conducted in a randomized complete block design with four replications. All plots were sown with the susceptible six-row barley variety Tradition. Trials were inoculated with *Fusarium* infested corn spawn at Fargo and Langdon; Carrington trials were seeded into wheat residue. Several fungicides and/or fungicide programs were evaluated and all trials included prothioconazole + tebuconazole (Prosaro®, Bayer CropScience) and metconazole (Caramba®, BASF). Other fungicides and fungicide rates evaluated varied among locations. All locations evaluated the application timing of Feekes 10.5 (fully-headed). Other timings evaluated were Feekes 9 (flag leaf), Feekes 10.3 (1/2 head emergence) and Feekes 10.5 + 4-5 days. Disease evaluations for foliar diseases and Fusarium head blight were conducted on all plots. DON levels were obtained from samples submitted to the NDSU Veterinary Toxicology Lab. Preliminary analysis indicates adequate disease pressure was achieved in five of the six trials as significantly lower DON levels were observed in fungicide treatments when compared to the non-treated control. Prothioconazole + tebuconazole and metconazole applied at Feekes 10.5 and at Feekes 10.5 + 4-5 days tended to have lower DON levels than all other treatment combinations. Future research to evaluate post-heading fungicide applications is needed to help strengthen current fungicide recommendations for scab management in barley.

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INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITYON DON PRODUCTION IN WHEAT AFTER FUSARIUM HEAD BLIGHT SYMPTOM DEVELOPMENT

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OBJECTIVE

Investigate the effects of temperature (cool-20ºC, warm-25ºC, and hot-30ºC) and relative humidity during the window between Fusarium head blight (FHB) visual symptom development and harvest on deoxynivalenol (DON) in grain from spikes with known levels of FHB index.

INTRODUCTION

FHB development and DON accumulation are strongly influenced by environmental conditions before, during, and even after anthesis. It is well known that major FHB epidemics and high levels of DON are associated with warm temperatures, high relative humidity, and adequate rainfall during the aforementioned periods (5, 6). However, very few studies have investigated the effects of potentially stressful environmental conditions on DON in diseased spikes during the post-anthesis window (1). In particular, the effects of different combinations of cool/hot and wet/dry conditions between visual symptom development and harvest on DON are still not fully understood. This constitutes a major knowledge gap in our understanding of the epidemiology of FHB. Producers and researchers alike have questioned the association between low FHB index and high DON or disproportionately low DON and relatively high index in some seasons. Anecdotal evidence and results from designed experiments have shown that post-anthesis environmental conditions may have contrasting effects on DON accumulation in harvested grain. For instance, in one study, moisture during the first 10 days after anthesis led to an increase in DON (2), but in a second study, a greater amount of total moisture between anthesis and harvest led to a reduction in DON (3). In addition, results from controlled-environment studies showed that post-anthesis moisture patterns may also play a role in DON exceeding critical thresholds even when FHB levels are relatively low. Andersen et al. (1) found that DON levels increased under certain patterns of intermittent moisture.

MATERIALS AND METHODS

Two different experiments were performed to evaluate the effects of temperature and moisture after FHB visual symptom development on DON production. In the first experiment, whole plants with FHB developed in growth chambers were used, whereas in the second, FHB-affected spikes were harvested from field-grown plants. For experiment 1, seeds of Cooper, an awnless, FHB-susceptible soft red winter wheat (SRWW) cultivar, were sown in batches, and after germination were allowed to vernalize in a cold (3°C) room for 10 weeks. Plants were then transplanted to individual containers containing autoclaved

silt loam and transferred to growth chambers with a constant temperature of 23°C and RH 70%, and photoperiod of 16 h of light and 8 h of darkness until inoculated. Plants were treated with 50% triadimefon for powdery mildew control, fertilized, and watered as needed. For experiment 2, field plots of Bravo, an awnless, susceptible SRWW cultivar, were planted on 25 September 2014 at OARDC Snyder Farm near Wooster, OH into a field previously cultivated with oats, and managed according to standard agronomics practices for Ohio.

In both experiments, plants were inoculated at early anthesis (Feekes 10.5.1) with a spore suspension consisting of a mixture of equal proportions of 10 Ohio isolates of *Fusarium graminearum*. For experiment 1, a set of 120 plants was point inoculated at anthesis as previously described (4). Immediately after inoculation, plants were placed in mist chambers and subjected to 1, 2, or 3 days of mist for 12 h during each 24-hour period to enhance infection and generate a range of FHB levels. For experiment 2, plants were spray-inoculated at anthesis with a 1:1 mixture of ascospores and macroconidia (50,000 spores. ml^{-1}).

In both experiments, temperature and moisture treatments were imposed after visual symptom development (which occurred 16 and 21 days after inoculation). The experiments were performed simultaneously in three programmable walk-in growth chambers set a 20, 25, and 30ºC. In each chamber, the experimental design was a randomized complete block, with moisture as whole-plot, and five FHB index categories (8-15, 20-40, 41-60, 61-80, 90-100%) as sub-plot. For the first experiment, seven subsets of 4 plants with spikes in each FHB index categories were exposed to each combination of moisture (dry and wet) and temperature. The wet treatment (>95% RH) was established by using a portable humidity chamber, equipped with humidifiers programmed to run 30 min every 60 min for 24 hours every day, and the dry treatment (70%) was established by leaving plants outside of the mist chambers under chamber-regulated RH conditions. There were 7 replications, consisting of different cohorts of spikes at anthesis.

For the second experiment, different saturated salt solutions and water were used to achieve four levels of relative humidity: 70% (1:1 mixture of NaCl + KCl), 80% ($(NH_4)_2SO_4$), and 90% ($BaCl_2$), 100% (distilled water) (7, 8). A fixed volume of 250 ml of saturated salt solution or water was placed into 17-by-12-by-6-cm transparent chambers and sealed airtight to reach and maintain the desired RH. Four arbitrarily-selected spikes in each of the five index categories were assigned to each humidity chambers. There were 5 replicate chambers of each RH level.

Spikes were harvested and threshed, and kernels were ground and assayed for DON at the U.S. Wheat and Barley Scab Initiative-funded laboratory at the University of Minnesota.

RESULTS AND DISCUSSION

In all cases, as expected, DON increased as mean index increased, with the high-index categories having the highest mean levels of the toxin (Fig. 1A and C). However, the rate in DON increase per unit increase in index (slope of the DON/index regression line) varied among temperature-moisture treatment combinations (Fig. 1C and D). At all three temperatures, slopes were higher for plants exposed to relatively dry conditions (70% RH) after symptom development (Fig. 1B) than under wet conditions (> 95% RH). At >95% RH, the highest slope was observed at 20ºC, whereas at 70% RH the slope was highest at 25ºC (Fig. 1B).

Interestingly, for detached spikes from the field (experiment 2), the rate of DON increase per unit increase in index at 100% RH was higher (at least numerically) at 20ºC than at 25 or 30ºC (Fig 1D). However, at 25º and 30ºC, similar trends to those observed for intact spikes (experiment 1) were observed, with the slopes tending to be higher at 70 and 80% than at 90 and 100% RH (Fig 1D). This interaction effect of temperature and RH on the relationship between index and DON is better depicted in the response surfaces in Figure 2. At 20ºC, the highest levels of DON were observed at 90-100% RH (Fig. 2A), whereas at 25 and 30 C, for a similar level of index, the highest levels of DON were observed at 70-80% RH. Follow-up experiments were conducted to determine the consistency of the results presented here, but at the time of this report, DON results were not yet available.

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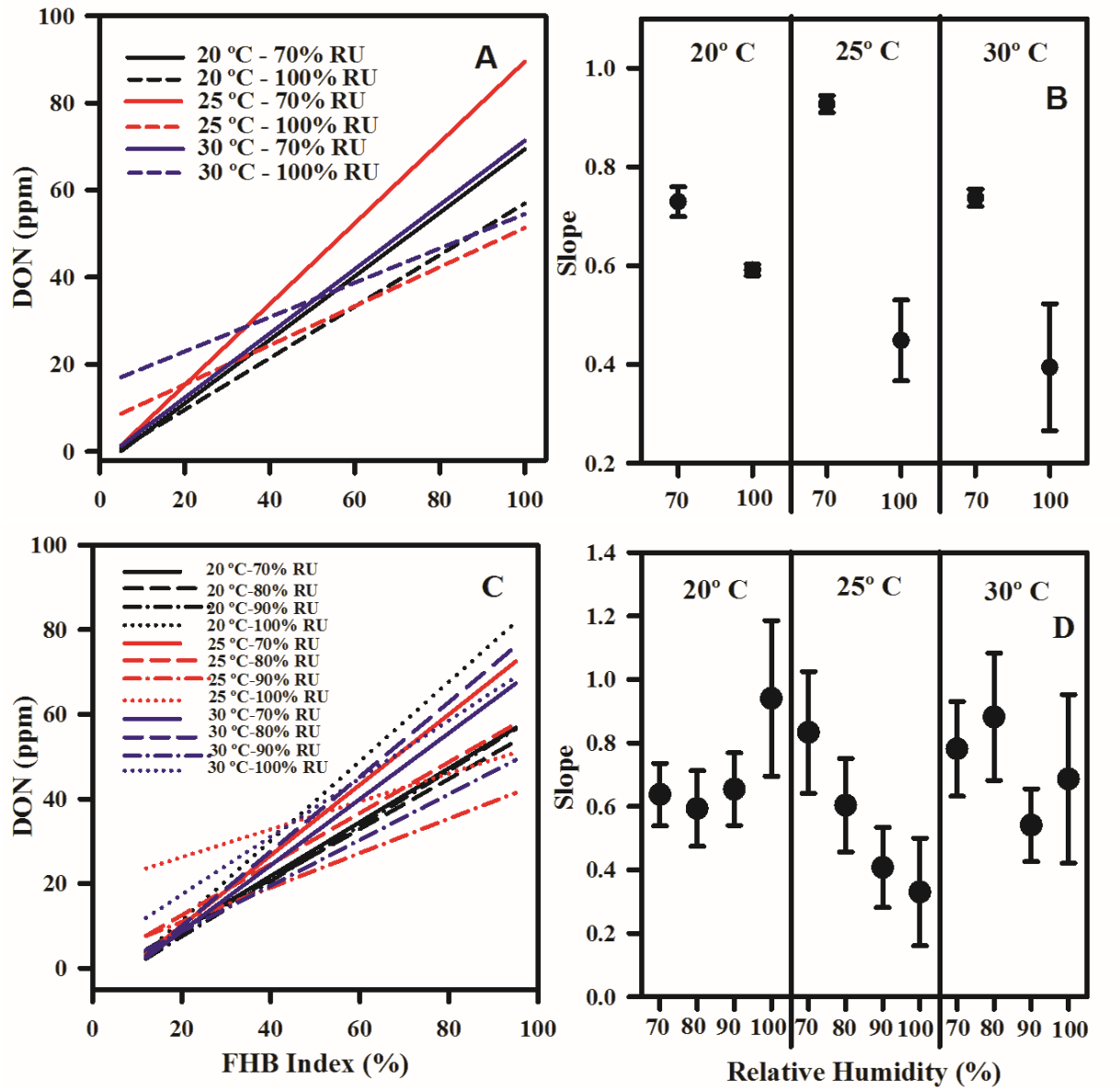

Figure 1. Relationship between Fusarium head blight (FHB) index and deoxynivalenol (DON) in spikes subjected to 70 to 100% relative humidity at 20 to 30°C after FHB visual symptom development. Diseased plants of Cooper were grown in growth chambers at 70 and > 95% RH (A, B), and detached diseased spikes of Bravo were harvested from the field after FHB symptom development (C, D) and placed in RH chambers. Lines are based on the estimated regression parameters from robust regression analysis (A, B). Slope for each temperature-RH combination are displayed in B and D along with their 95% confidence intervals.

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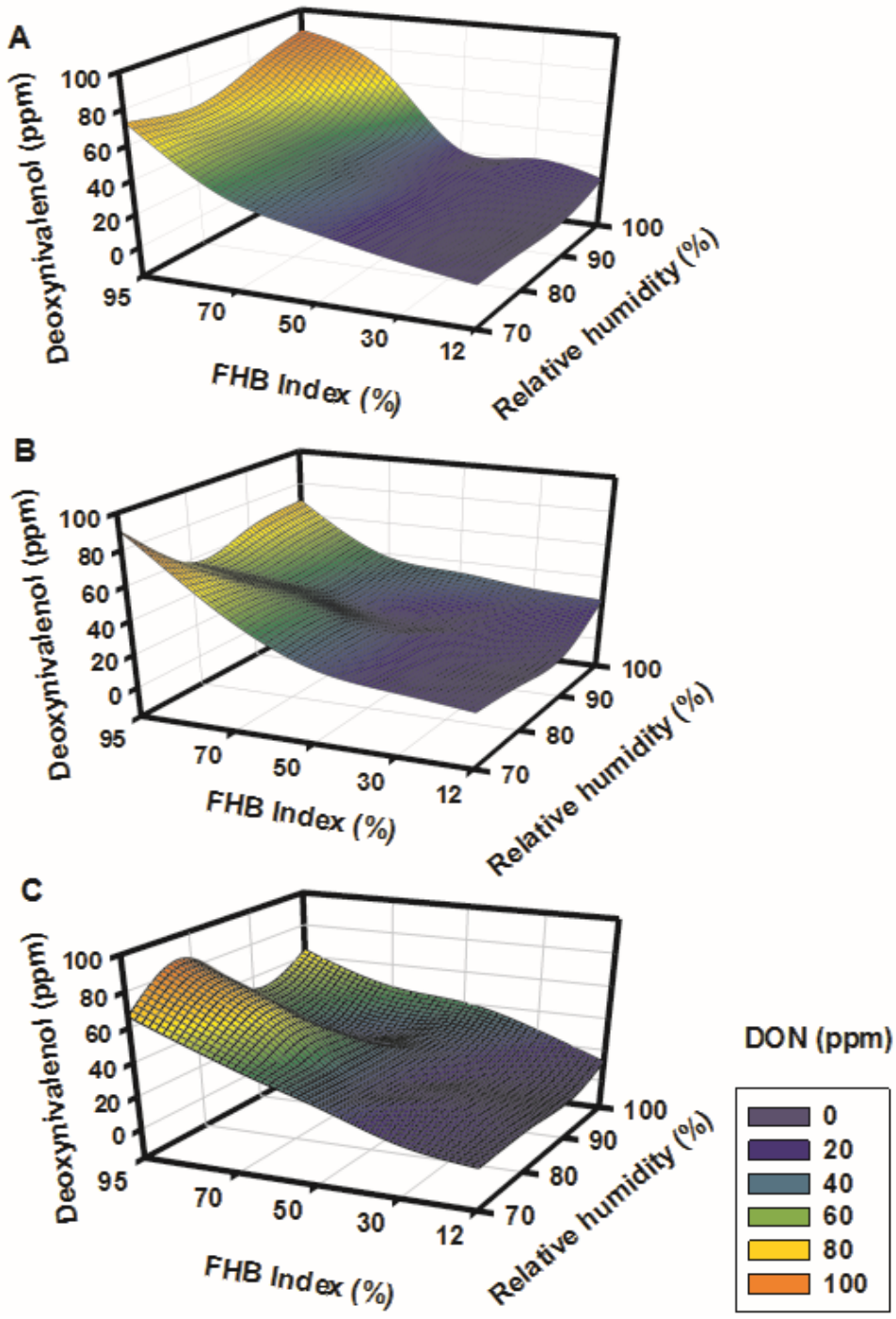

Figure 2. Response surfaces for deoxynivalenol (DON) as a function of Fusarium head blight (FHB) index and relative humidity. Detached diseased spikes of Bravo were subjected to 70 to 100% relative humidity at 20º (A), 25º (B) and 30°C (C) for 30 days. Plots were generated using values from the five repetitions.

2014 AND 2015 FIELD PLOT TRIALS FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA USING *BACILLUS AMYLOLIQUEFACIENS* STRAINS

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ABSTRACT

Fusarium graminearium (*Gibberella zeae*) is primarily responsible for Fusarium head blight (FHB) in Wheat and Barley. Economic losses due to FHB can be significant. Yield losses can be controlled or reduced through the use of fungicides alone or in combination with biological control agents (BCA). Field plot trials were conducted in 2015 in Brookings, South Dakota to analyze the efficacy of *Bacillus amyloliquefaciens* strains 1BA and 1D3 in biological control of FHB. Spray applications of *Bacillus* BCAs alone or in combination with Prosaro® (fungicide) and/or Induce NIS (non-ionic surfactant) and/ or colloidal chitin were done on Samson spring wheat heads at Feekes 10.5.1. Similar trials were conducted previously in 2014 using Briggs spring wheat.

Although multiple treatments had decreased DON (deoxynivalenol) and FDK (*Fusarium*-damaged kernels) levels in the 2014 field plot trials with Briggs spring wheat, statistically significant treatment differences (P=0.05) were not observed. Some treatments exhibited statistically significant treatment differences (P=0.05) for protein in comparison to the untreated control. Only the DON, FDK and protein results from 2014 plots are presented in this abstract, as the other results were presented at the 2014 FHB Forum.

In the 2015 field plot trials with Samson spring wheat, significant treatment differences (*P=0.05*) were observed for FHB incidence, disease severity and FHB index, in comparison to the untreated control. Statistically significant treatment differences were not observed for test weight and protein content, in comparison to the untreated control. Some 2015 treatments with *Bacillus* 1BA and/or 1D3 in combination with Prosaro, with/without colloidal chitin exhibited better results for FHB incidence, disease severity and FHB index than a single application of Prosaro alone. Significant differences (P=0.05) were observed with some BCA treatments for yield in comparison to untreated control. The combination of 1BA, 1D3, colloidal chitin, and Prosaro increased the yield to 54.49 bu/ac, which was 1.4 times more yield than the untreated control (38.67 bu/ac). Prosaro alone increased the yield to 43.93 bu/ac. The treatment combination of 1D3 amended with colloidal chitin and Prosaro increased the yield to 54.64 bu/ac; and the treatment combination of 1BA with colloidal chitin and Prosaro increased the yield to 54.54 bu/ac. The results for DON and FDK are pending and not yet available. These trials demonstrated that *Bacillus* strains 1BA or 1D3 in combination with Prosaro and/or colloidal chitin can not only reduce FHB in wheat, but can statistically increase the yield as well, compared to a single application of Prosaro alone.

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COMPARATIVE CONTROL OF *FUSARIUM VERTICILLIOIDES* INFESTING MAIZE USING *HYPTIS SUAVEOLENS* AND SYNTHETIC INSECTICIDE

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ABSTRACT

Pathological study on *Fusarium verticillioides* (Sacc.) infesting Maize, Zea mays, was investigated in a field trial. Comparative protection using a plant extract obtained via vaccum filtration using cold method and a synthetic fungicide was evaluated against *Fusarium verticillioides* (Sacc.) Nirenberg, causing great loss in maize production. Healthy maize plants were sprayed with botanical extract of *Hyptis suaveolens* and *Fusarium verticilliodes* prepared on the demonstration field of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The maize plants were inoculated with *Fusarium verticilliodes* inoculums at 5 weeks after planting. Subsequently on different maize plots, treatment of the maize plants was carried out using *Hyptis suaveolens* extract and synthetic fungicide (Redforce) separately in order to determine the efficacy of each treatment in the control of the disease. Remarkable control was established after the treatment of the infected maize with *Hyptis suaveolens* extract and synthetic fungicide as such, basis for comparison was subsequently recorded as there was no significant difference ($P < 0.05$) in the 1, 2, 3 and 4 weeks after planting before the inoculation. At 2, 3, 4 and 5 weeks after inoculation a significant difference ($P < 0.05$) was observed and at 7, 8, 9 and 10 weeks after planting, higher mean and standard error values were recorded for the parameters, stem girth, number of leaves and internodes under study in plants treated with *Hyptis suaveolens* extract while synthetic fungicide treated maize plants had lower mean and standard error values.

ROBUST MANAGEMENT PROGRAMS TO MINIMIZE LOSSES DUE TO FHB AND DON: A MULTI-STATE COORDINATED PROJECT

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OBJECTIVE

Evaluate the integrated effects of fungicide and genetic resistance on FHB and DON in all major grain classes, with emphasis on different application timings and new genotypes to develop more robust "*best-management practices*" for FHB and DON.

INTRODUCTION

FHB management programs that integrate multiple in-field, harvesting and post-harvesting strategies have been shown to be the most effective for minimizing FHB-associated grain yield and quality losses in wheat and barley (Wegulo et al., 2011; Willyerd et al., 2012; McMullen et al., 2012; Salgado et al., 2014). For instance, Willyerd et al (2012) demonstrated that the application of the DMI fungicide Prosaro® at anthesis combined with a moderately resistant cultivar resulted in more than 70% control of both FHB index and DON. However, weather and field conditions often prevent fungicides from being sprayed at the recommended anthesis growth stage. For instance, wet, soggy field conditions may prevent ground applications, and even if such applications are made, research shows the rainfall during or shortly after treatment may reduce fungicide efficacy (Andersen et al., 2014). Moreover, several other factors such as uneven crop development and variable anthesis window affect the ability of producers and crop advisors to correctly determine the anthesis growth stage when making a fungicide application to manage FHB and DON. To address these limitations, one of the primary goals of the USWBSI management action plan is to develop integrated management strategies for FHB and mycotoxins that are robust to conditions experienced in production fields.

MATERIALS AND METHODS

Field experiments were established in 12 US wheat-growing states (AR, DE, IL, IN, MD, MI, MN, ND, NE, NY, OH and SD) to investigate the effects of cultivar resistance and fungicide application timing on FHB and DON. Plots were established following host or non-host

crops of *F. graminearum*, according to standard agronomic practices for each location. At least three commercial wheat cultivars, classified as susceptible (S), moderately susceptible (MS), or moderately resistant (MR), were planted in most trials. However, some trials only included one or two of these resistance categories. Plots were planted in four to six replicate blocks. The standard experimental design was a randomized complete block, with a split-plot arrangement of cultivar as whole-plot and fungicide (Prosaro, 6.5 fl.oz./A + NIS) application timing as sub-plot (untreated or treated at anthesis [A] or 2 to 7 days post-anthesis [A+2 … A+7, respectively]). All plots were artificially inoculated with either *F. graminearum*-colonized corn kernels spread on the soil surface or spray-inoculated with a spore suspension of the fungus approximately 24-36 hours following the anthesis fungicide treatment. FHB index (plot severity) was assessed during the soft dough stage of grain development. Milled grain samples were sent to a USWBSI-supported laboratory for toxin analysis. For the purpose of this report, percent control of FHB index and DON was estimated for each cultivar x fungicide application timing combination relative to the untreated susceptible or very susceptible check (the reference treatment) for each trial/environment. However, in NY the untreated MS cultivar was used as the reference when estimating percent control.

RESULTS AND DISCUSSION

FHB index and DON results from 27 environments, representing 15 soft red winter, two soft white winter, three hard red winter, and seven hard red spring wheat classes were summarized. Estimated means and percent controls for FHB index and DON for S/VS, MS and MR cultivars treated with Prosaro at or after anthesis are shown in Table 1, 2 and 3, respectively. In some environments, FHB did not develop due to unfavorable weather conditions. In addition, DON data were not available for some trials at the time of this report, therefore trials with missing data or nominal disease and mycotoxin levels (< 3% index and < 1 ppm DON) in the untreated susceptible reference (S/VS/MS) were not used. Overall, mean FHB index and DON in the untreated susceptible check ranged from 3 to 54% and from 1.9 to 33 ppm, respectively. Relative to the untreated susceptible or very susceptible check, fungicide alone reduced FHB index by 1 to 97% and DON by 5 to 54% (Table 1). However, combinations of the fungicide treatment with a moderately susceptible (Table 2) or a moderately resistant (Table 3) cultivar were consistently more effective than fungicide alone at reducing FHB and DON in most trials, with percent control ranging from 4 to 99% for index and 11 to 89% for DON on the MS cultivars and from 42 to 99% for Index and 32 to 93% for DON on MR cultivars. Post-anthesis treatments were as effective as or more effective than anthesis treatments, particularly on MR cultivars. Based on these results, there is evidence suggesting that applying fungicides post-anthesis may be as efficacious against FHB and DON as treatments applied at anthesis in all wheat classes and environments.

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Table 1. Mean FHB index, DON, and percent control for different fungicide programs on FHB susceptible cultivars in 20 environments (ENV) representing different wheat classes (TYPE = SRW, SWW, HRW and HRS). Results are organized by fungicide treatment (untreated [UT] or treated at anthesis [A] or 2, 4, 5, 6 or 7 days post-anthesis [A+2…A+7, respectively]). Percent controls were estimated relative to the untreated susceptible or very susceptible (S_UT).

			Fungicide timing of application [a]							% Control Compared to Susceptible reference (S/VS[b])					
STATE	TYPE	ENV	S_UT	A	A+2	A+4	A+5	A+6	A+7	A	A+2	A+4	A+5	A+6	A+7
FHB Index (%)= mean proportion of disease spikelets per spike															
IL	SRW	1	7.3	8.5	3.8	0.7	--	1.1	--	-17.2	48.3	91.0	--	84.4	--
IL	SRW	2	12.5	7.0	3.0	8.3	--	7.5	--	44.0	76.0	34.0	--	40.0	--
IL	SRW	3[b]	31.3	17.8	16.8	8.8	--	16.8	--	43.2	46.4	72.0	--	46.4	--
IL	SRW	4[b]	22.0	5.4	6.1	7.6	--	2.4	--	75.5	72.1	65.3	--	89.2	--
IN	SRW	5	4.4	2.3	1.8	2.8	--	1.7	--	48.3	58.1	36.4	--	62.2	--
IN	SRW	6	29.5	16.8	8.2	22.1	--	18.4	--	42.9	72.3	25.0	--	37.4	--
OH	SRW	7	12.6	10.3	4.1	7.2	--	12.2	--	18.3	67.5	42.9	--	3.2	--
OH	SRW	8	40.6	22.1	23.5	30.2	--	26.2	--	45.6	42.1	25.6	--	35.5	--
MI	SWW	11[b]	8.7	2.3	1.3	1.5	--	2.9	--	74.0	85.6	83.3	--	67.1	--
DE	SRW	14	7.0	4.9	--	4.1	--	3.3	--	30.3	--	40.9	--	53.2	--
MD	SRW	15	13.2	12.4	--	12.0	--	10.5	--	5.9	--	8.5	--	20.2	--
DE	SRW	16	3.2	1.1	--	0.3	--	1.3	--	65.8	--	89.3	--	58.6	--
NE	HRW	17	3.6	6.0	--	--	2.4	--	2.0	-69.6	--	--	31.6	--	43.4
NE	HRW	18	27.5	11.6	--	--	--	--	16.9	57.9	--	--	--	--	38.7
SD	HRW	19	19.0	8.5	10.9	14.9	--	15.4	--	55.5	42.7	21.6	--	18.9	--
SD	HRS	20	48.8	37.8	38.4	33.3	--	50.6	--	22.5	21.2	31.7	--	-3.8	--
SD	HRS	21	21.1	13.2	13.0	12.3	--	15.6	--	37.6	38.5	41.9	--	26.2	--
SD	HRS	22	53.6	29.5	34.0	32.9	--	35.9	--	44.9	36.5	38.5	--	32.9	--
ND	HRS	25	10.1	4.5	--	--	2.1	--	--	54.9	--	--	79.1	--	--
ND	HRS	27	21.4	4.2	--	0.6	--	--	--	80.5	--	97.0	--	--	--
DON = Deoxynivalenol content of harvested grain in ppm															
IL	SRW	1	4.0	7.9	3.0	2.4	--	3.0	--	-97.2	25.1	38.9	--	25.1	--
IL	SRW	2	7.6	5.7	5.1	4.4	--	4.5	--	24.8	33.1	42.4	--	40.4	--
IN	SRW	5	7.1	7.3	4.5	4.1	--	4.1	--	-2.8	36.7	42.1	--	42.8	--
IN	SRW	6	7.2	4.9	4.9	4.7	--	4.5	--	31.5	32.2	35.0	--	36.6	--
OH	SRW	7	15.6	9.0	7.3	9.1	--	9.2	--	42.3	53.2	41.7	--	41.0	--
MI	SW/SR	10	2.4	1.6	1.1	1.5	--	1.4	--	35.4	54.2	37.5	--	43.8	--
DE	SRW	14	2.0	1.2	--	1.2	--	1.2	--	41.5	--	40.5	--	42.0	--
MD	SRW	15	1.9	1.6	--	1.1	--	0.9	--	13.7	--	42.6	--	51.6	--
NE	HRW	18	33.3	26.7	--	--	--	--	24.9	19.7	--	--	--	--	25.3
SD	HRW	19	6.9	5.4	5.4	5.0	--	5.3	--	21.9	21.2	27.0	--	23.7	--
SD	HRS	20	9.3	8.8	8.0	6.2	--	6.6	--	5.4	14.2	33.5	--	29.0	--
SD	HRS	21	9.6	7.7	7.2	6.3	--	7.6	--	20.3	25.3	35.2	--	21.2	--
ND	HRS	25	7.4	5.6	--	--	4.0	--	--	24.5	--	--	45.2	--	--

[a] Fungicide application = Prosaro applied at 6.5 fl. oz./A + NIS at or after anthesis

[b] Environments (ENV) where very susceptible cultivars (VS) were planted

Table 2. Mean FHB index, DON, and percent control for different fungicide programs on moderately susceptible cultivars in 15 environments (ENV) representing different wheat classes (TYPE = SRW, SWW, HRW and HRS). Results are organized by fungicide treatment (untreated [UT] or treated at anthesis [A] or 2, 4, 5, 6 or 7 days post-anthesis [A+2…A+7, respectively]). Percent controls were estimated relative to the untreated susceptible or moderately susceptible (S_UT or MS_UT).

			Fungicide timing of application [a]							% Control Compared to Susceptible reference (S/VS[b]/MS[c])						
STATE	**TYPE**	**ENV**	**MS_UT**	**A**	**A+2**	**A+4**	**A+5**	**A+6**	**A+7**	**MS_UT**	**A**	**A+2**	**A+4**	**A+5**	**A+6**	**A+7**
FHB Index (%)= mean proportion of disease spikelets per spike																
IL	SRW	3[b]	6.5	2.8	5.8	3.3	--	5.3	--	79.2	91.2	81.6	89.6	--	83.2	--
IL	SRW	4[b]	8.3	3.5	2.1	2.8	--	1.8	--	62.5	84.1	90.3	87.5	--	92.0	--
IN	SRW	5	0.5	0.4	0.4	0.4	--	0.2	--	88.4	90.4	90.7	90.0	--	95.4	--
IN	SRW	6	14.4	7.1	6.4	9.2	--	15.2	--	51.2	75.9	78.3	68.6	--	48.4	--
OH	SRW	7	8.4	6.5	3.3	4.6	--	7.8	--	33.3	48.4	73.8	63.5	--	38.1	--
OH	SRW	8	16.7	8.8	8.8	9.7	--	8.1	--	58.9	78.3	78.3	76.1	--	80.0	--
MI	SWW	11[b]	3.1	1.6	1.0	0.9	--	0.6	--	64.6	81.5	88.8	89.6	--	93.1	--
NY	SRW	12[c]	4.2	1.8	--	--	--	--	0.5	N/A	57.4	--	--	--	--	87.9
NY	SRW	13[c]	8.5	2.0	--	--	2.7	--	--	N/A	76.3	--	--	68.4	--	--
SD	HRW	19	30.6	11.3	10.4	15.9	--	18.3	--	-60.8	40.8	45.3	16.6	--	3.9	--
SD	HRS	20	11.3	9.8	13.5	9.2	--	14.1	--	76.9	79.9	72.3	81.2	--	71.0	--
SD	HRS	21	3.7	2.3	1.6	3.0	--	1.4	--	82.7	89.2	92.5	86.0	--	93.2	--
SD	HRS	22	31.4	22.3	16.7	19.3	--	24.5	--	41.5	58.3	68.9	64.0	--	54.3	--
ND	HRS	25	0.5	0.2	--	--	0.1	--	--	95.1	97.9	--	--	98.6	--	--
ND	HRS	27	1.3	0.4	--	0.1	--	--	--	93.9	97.9	--	99.4	--	--	--
DON = Deoxynivalenol content of harvested grain in ppm																
IN	SRW	5	2.4	2.3	2.1	2.0	--	1.8	--	65.7	67.5	70.3	71.3	--	74.9	--
IN	SRW	6	3.8	2.9	2.8	2.6	--	3.0	--	47.1	59.4	61.1	63.6	--	58.3	--
OH	SRW	7	6.1	5.3	4.6	4.4	--	5.0	--	60.9	66.0	70.5	71.8	--	67.9	--
MI	SW/SR	10	0.6	0.4	0.3	0.4	--	0.3	--	75.8	83.3	86.3	84.2	--	86.3	--
NY	SRW	12	3.2	1.3	--	--	--	--	0.7	N/A	59.1	--	--	--	--	76.7
NY	SRW	13	2.3	1.3	--	--	1.3	--	--	N/A	44.5	--	--	43.2	--	--
SD	HRW	19	7.4	5.9	5.9	6.1	--	4.5	--	-7.6	14.5	14.7	11.0	--	34.3	--
SD	HRS	20	3.6	2.6	2.5	2.6	--	3.2	--	61.5	71.7	73.7	72.0	--	65.8	--
SD	HRS	21	2.4	1.6	1.7	1.5	--	1.9	--	75.2	83.6	82.6	84.4	--	80.0	--
ND	HRS	25	1.0	0.8	--	--	1.0	--	--	86.4	88.7	--	--	87.1	--	--

[a] Fungicide application = Prosaro applied at 6.5 fl. oz./A + NIS at or after anthesis

[b] Environments (ENV) where very susceptible cultivars (VS) were planted

[c] Percent Control was estimated relative to moderately susceptible.

Table 3. Mean FHB index, DON, and percent control for different fungicide programs on moderately resistant cultivars from 22 environments (ENV) representing different wheat classes (TYPE = SRW, SWW, HRW and HRS). Results are organized by fungicide treatment (untreated [UT] or treated at anthesis [A] or 2, 4, 5, 6 or 7 days post-anthesis [A+2…A+7, respectively]). Percent controls were estimated relative to the untreated susceptible or moderately susceptible (S_UT or MS_UT).

			Fungicide timing of application [a]							% Control Compared to Susceptible reference (S/VS[b]/MS[c])						
STATE	TYPE	ENV	MR_UT	A	A+2	A+4	A+5	A+6	A+7	MR_UT	A	A+2	A+4	A+5	A+6	A+7
FHB Index (%)= mean proportion of disease spikelets per spike																
IL	SRW	1	1.8	2.4	1.5	1.1	--	0.8	--	74.8	67.2	79.3	84.4	--	88.6	--
IL	SRW	2	4.7	2.1	2.8	2.8	--	3.7	--	62.6	83.4	78.0	77.4	--	70.6	--
IL	SRW	3[b]	5.4	1.4	2.0	0.4	--	1.1	--	82.8	95.6	93.6	98.8	--	96.4	--
IL	SRW	4[b]	1.3	1.3	0.8	1.0	--	0.8	--	94.3	94.0	96.6	95.5	--	96.6	--
IN	SRW	5	0.4	0.2	0.2	0.2	--	0.5	--	90.0	94.5	96.6	96.1	--	87.9	--
IN	SRW	6	4.8	1.6	1.6	1.0	--	1.8	--	83.6	94.6	94.6	96.6	--	93.9	--
OH	SRW	7	4.1	3.2	1.3	2.5	--	3.4	--	67.5	74.6	89.7	80.2	--	73.0	--
OH	SRW	8	10.6	4.6	3.7	7.0	--	4.5	--	73.9	88.7	90.9	82.8	--	88.9	--
MI	SWW	11[b]	0.6	0.4	0.2	0.3	--	0.4	--	92.7	96.0	97.7	97.0	--	95.5	--
NY	SRW	12[c]	1.0	0.8	--	--	--	--	0.3	76.0	81.9	--	--	--	--	94.0
NY	SRW	13[c]	8.8	2.9	--	--	1.5	--	--	-3.9	66.2	--	--	82.6	--	--
DE	SRW	14	0.4	0.2	--	0.7	--	0.3	--	94.3	97.9	--	90.4	--	96.1	--
MD	SRW	15	5.2	0.8	--	3.7	--	2.4	--	60.8	93.6	--	71.6	--	81.5	--
DE	SRW	16	0.8	0.4	--	0.2	--	0.6	--	74.6	89.0	--	94.0	--	80.3	--
NE	HRW	17	1.8	1.2	--	--	1.5	--	0.8	49.4	65.3	--	--	57.3	--	78.7
NE	HRW	18	9.9	4.9	--	--	--	--	12.2	64.1	82.1	--	--	--	--	55.6
SD	HRW	19	7.2	1.8	3.0	4.2	--	3.9	--	62.4	90.4	84.1	78.2	--	79.5	--
SD	HRS	20	28.9	27.7	19.8	22.4	--	28.4	--	40.7	43.2	59.3	54.1	--	41.8	--
SD	HRS	21	2.9	1.4	1.9	2.0	--	1.6	--	86.5	93.6	91.1	90.6	--	92.3	--
SD	HRS	22	15.5	12.2	9.8	7.9	--	13.1	--	71.0	77.3	81.8	85.2	--	75.6	--
ND	HRS	25	2.8	0.9	--	--	3.1	--	--	71.9	91.3	--	--	69.7	--	--
ND	HRS	27	1.5	0.2	--	0.0	--	--	--	93.2	99.1	--	99.9	--	--	--
DON = Deoxynivalenol content of harvested grain in ppm																
IL	SRW	1	1.1	2.5	1.1	0.8	--	1.5	--	73.6	37.4	71.9	79.4	--	63.1	--
IL	SRW	2	2.2	2.4	2.1	2.5	--	2.1	--	70.3	68.5	71.9	66.6	--	72.7	--
IN	SRW	5	3.9	2.6	3.0	2.5	--	2.1	--	44.4	63.7	57.9	64.7	--	69.8	--
IN	SRW	6	3.9	3.0	3.3	2.8	--	2.8	--	45.0	57.5	54.0	61.0	--	60.4	--
OH	SRW	7	4.2	3.5	2.1	2.5	--	2.4	--	73.1	77.6	86.5	84.0	--	84.6	--
MI	SW/SR	10	0.4	0.3	0.2	0.2	--	0.2	--	85.4	89.2	92.1	91.3	--	91.3	--
NY	SRW	12	1.3	0.5	--	--	--	--	0.2	59.4	83.3	--	--	--	--	93.4
NY	SRW	13	1.5	1.2	--	--	0.7	--	--	34.9	47.2	--	--	68.6	--	--
DE	SRW	14	0.3	0.3	--	0.2	--	0.2	--	83.5	86.0	--	90.5	--	88.0	--
MD	SRW	15	0.6	0.5	--	0.4	--	0.4	--	67.4	73.7	--	77.9	--	81.6	--
NE	HRW	18	13.3	12.9	--	--	--	--	10.1	60.1	61.2	--	--	--	--	69.6
SD	HRW	19	4.8	4.7	3.6	3.8	--	2.6	--	30.2	31.7	48.4	44.3	--	62.2	--
SD	HRS	20	3.1	2.4	2.6	2.4	--	2.6	--	67.2	74.4	72.0	74.4	--	71.7	--
SD	HRS	21	2.3	1.4	1.4	1.2	--	1.5	--	76.7	86.0	86.0	87.2	--	84.6	--
ND	HRS	25	2.1	1.6	--	--	1.4	--	--	71.4	78.2	--	--	80.5	--	--

[a] Fungicide application = Prosaro applied at 6.5 fl. oz./A + NIS at or after anthesis

[b] Environments (ENV) where very susceptible cultivars (VS) were planted

[c] Percent Control was estimated relative to moderately susceptible.

A FUNCTIONAL EXPLORATION OF TEMPERATURE AS A PREDICTOR OF FUSARIUM HEAD BLIGHT EPIDEMICS

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ABSTRACT

Our focus is on weather-driven predictive models for Fusarium head blight (FHB) epidemics in the U.S. An epidemic was defined as having occurred when the FHB index ≥ 10%. The current FHB observational data matrix contains 865 observations representing site-years from 1982 to 2014. FHB epidemics were represented by 236 observations. Each observation was linked to a weather time series matrix consisting of daily estimates of temperature for the given location from which the FHB observation was collected. We looked at daily temperatures beginning 120 days pre-anthesis to 20 days post-anthesis. The raw daily temperature time series were then represented by smooth curves built from cubic B-spline basis functions with a roughness penalization. The mean smooth curves for epidemics and non-epidemics were estimated, along with their first and second derivatives, and the differences in these curves between epidemics and non-epidemics. The functional representations of the temperature time series indicated three potential periods where the separation between epidemic and non-epidemic temperature profiles may be greatest: 84 to 72 days pre-anthesis, 42 to 23 days pre-anthesis, and 7 days pre-anthesis to 10 days post-anthesis. We calculated (for each observation) the mean temperatures (T_m) estimated from the raw daily values within these three periods, and then looked at the distribution of T_m (for epidemics and non-epidemics) with histograms, density plots and empirical cumulative distributions. The plots showed some separation between epidemics and non-epidemics, and much overlap. Choosing $T_m = 11$ °C as a cut-point for classifying epidemics and non-epidemics gave an overall correct classification rate of 72%. Non-epidemics were correctly classified 88% of the time, but epidemics were correctly classified only 29% of the time. Therefore, the functional representation of the temperature time series was useful in identifying periods during which epidemics may be potentially discriminated from non-epidemics. Identified time periods (e.g. 84 to 72 days pre-anthesis) were much earlier than we had given thought to before (15 days pre-anthesis to anthesis) when formulating predictors of FHB epidemics. However, the mean temperature differences between the smooth curves for epidemics and non-epidemics was on the order of 1 °C, which was indicative of the difficulty of using temperature alone to separate the two classes. Current research is focusing on a functional analysis of moisture variables, such as mean relative humidity, a variable previously identified for its high discriminatory ability.

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THE EFFECT OF FUSARIUM HEAD BLIGHT AND STRIPE RUST ON GRAIN YIELD OF HARD WINTER WHEAT IN LINCOLN NE

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ABSTRACT

To determine the effect of fungal plant pathogens on grain yield in eastern NE, we initiated a study in 2015 to compare fungicide treated and untreated plots using our elite nursery. While it is well documented that diseases reduce grain yield and fungicide use is becoming more common, growers still debate the cost and value of using fungicides. The purpose of this experiment was to provide growers with information on the value of fungicides so they can make informed decisions and also learn about our advanced breeding lines and how they respond to fungicides in the presence of disease. The Nebraska elite nursery contains 60 lines (two historic check cultivars, 6 cultivars, and 52 unreleased elite lines). Two fungicide regimens, treated vs. untreated, were utilized. In the treated plots, Cruiser Max® was used to treat the seed before planting. Then at early spring green-up the plots were sprayed with Priaxor®. At flag leaf, the plots were sprayed with Twinline® followed by Caramba® at flowering. Seed treatments and fungicides were not applied to the untreated plots. Each fungicide treatment (treated and untreated) had 60 genotypes replicated twice in an alpha lattice design with an incomplete block size of 5 entries. Grain yield was harvested using a small plot combine and the grain was weighed after drying in the seed house. Eastern Nebraska receives on average 65 to 75 cm of rainfall annually. In 2015, the Lincoln research station received 42 cm of precipitation from 1 May to 15 June. The average flowering date for winter wheat in our elite trial was 24 May with a range from 20 May to 29 May. Hence the conditions were ideal for Fusarium head blight (FHB, incited by *Fusarium graminearum*.). The other major disease present was stripe rust (incited by *Puccinia striiformis* f. sp. *tritici*). Other diseases that are favored by cool moist conditions were present, but not to the extent of FHB and stripe rust. Average FHB index in the untreated plots was 56% (range 4% to 96%) compared to 10% in the treated plots (an 82% reduction in index; range 0% to 68%). Yield in the treated plots averaged 3460 kg/ha (range 4860 kg/ha to 1360 kg/ha) compared to 1940 kg/ha (a 44% reduction in yield; range 3500 kg/ha to 340 kg/ha). On average the diseases caused a 44% reduction in yield (excluding the two historic check cultivars which actually yielded higher in the untreated plots; yield loss due to disease ranged from 15% to 86%). There was a significant negative correlation between FHB index and yield in the untreated plots ($R = -0.38$; $P = 0.0034$) indicating that some lines had good FHB resistance whereas others were susceptible. In contrast, there was no correlation between FHB index and yield in the treated plots ($R = 0.04$; $P = 0.7454$), indicating the effectiveness of Caramba applied at flowering in suppressing FHB. The stripe rust reactions varied among lines from highly resistant to highly susceptible. In looking at those lines which had infection scores of 1-3 (on a 1= resistant to 9= susceptible scale) for stripe rust, the grain yield loss averaged 30% presumably due to FHB. In looking at those lines with infection scores of 7-9 for stripe rust, the grain yield loss averaged 50%. In

both the resistant and susceptible to stripe rust groups, lines varied in their response to FHB with the best lines having only a 15% or 27% yield loss, respectively. Though not measured, the effects on grain volume weight and seed germination were obvious in preparing and planting seed this fall. This experiment will be repeated to provide multi-year disease loss information and to ensure having high quality seed for planting. Growers in eastern Nebraska were warned of the scab epidemic and many decided to use fungicides despite the low price of wheat. Clearly this year fungicides were economically beneficial, especially when coupled with cultivars that also had some tolerance or resistance to FHB and stripe rust.

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UNIFORM FUNGICIDE TRIAL RESULTS FOR MANAGEMENT OF FHB AND DON, 2015

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ABSTRACT

Data from prior USWBSI-funded uniform fungicide trials have shown that DMI fungicides, Caramba® 90 SL and Prosaro® 421 SC, were two of the most effective products against FHB and DON. However, for the grower these products can be relatively expensive. Tebuconazole fungicide is now off-patent, and several "generic" formulations are available at relatively low costs (some reports of less than $3 per acre). In years where the risk of scab is predicted as low, and growers have fields planted to moderately FHB resistant varieties, many are making the economic decision to use generic tebuconazole products. The aim of this project was to compare generic formulations of tebuconzole products to evaluate any differences in efficacy and to examine biocontrol formulations for the suppression of FHB. Trials were conducted at multiple locations across five states (Illinois, North Dakota, South Dakota, Minnesota and New York) in 2015. All sites were inoculated with *Fusarium graminearum* infested corn spawn, infested residue, or spray inoculation with spores at flowering. In several locations, mist irrigation was used to promote disease development. Eleven common fungicide treatments were evaluated across locations; Prosaro, Caramba, Monsoon, Muscle, Onset, Orius, Tebustar, Toledo, Aproach, Aproach Prima and the biological control Taegro in combination with Prosaro. Additional rates of Caramba and/or Prosaro were tested. All treatments were applied at Feekes 10.5.1 (early anthesis). Preliminary analysis of the data revealed that all treatments helped reduce the incidence of FHB in comparison with plots that received no fungicide application. The fungicides Caramba and Prosaro appeared to provide the best control of FHB, yield increase and reduction in DON for the locations where DON was measured. Two new, products designated MAD1 and MAD2 also showed good FHB control at some locations.

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SPRING WHEAT CULTIVAR PERFORMANCE AGAINST FUSARIUM HEAD BLIGHT IN NATURAL INFESTATION

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ABSTRACT

Fusarium head blight (FHB) or scab, mainly caused by *Fusarium graminearum*, remains one of the most destructive fungal diseases affecting wheat and barley. The disease is responsible for economic losses stemming from reduced yield due to shriveled kernels and poor quality kernels as a result of mycotoxin accumulation. The minimum acceptable levels of mycotoxins, especially deoxynivalenol (DON), in finished wheat products for human consumption is 1ppm. Therefore, the market value of wheat kernels with high mycotoxin levels declines significantly. Use of resistant cultivars is the most cost effective approach to managing FHB. This study evaluated the performance of 19 released and popular cultivars under natural infestation. The trial was planted at the South Dakota State University (SDSU) Northeast research farm, near South Shore. Plot size was 6 m^2 and cultivars were replicated four times and were laid out in a randomized complete block design. Variables assessed included FHB incidence, FHB severity, FHB disease index (DI), yield, and test weight (TW). Significant differences ($p=0.05$) were observed in all assessed variables except for FHB severity. Amongst the top three cultivars for each assessed variable, at least one cultivar appeared in the top three of four out of five variables. Forefront, Brick and Sabin had the lowest FHB incidence and FHB index, while Brick, SY Ingmar and Norden had the lowest FHB severity. The cultivars which had low FHB index did not necessarily yield the highest. Norden, Sabin and SY Soren were the top yielding cultivars. Negative associations between FHB against TW and yield were observed although, in general, the magnitudes were higher for TW than for yield suggesting that TW was affected more by FHB than yield.

FOOD SAFETY AND TOXICOLOGY

ENZYMATIC SYNTHESIS OF B-GLUCOSIDES OF THE TRICHOTHECENE TOXINS DEOXYNIVALENOL, NIVALENOL AND HT-2 TOXIN

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ABSTRACT

Glycosylation is an important plant defense mechanism and glucoconjugates of *Fusarium* toxins often co-occur with their parent compounds in cereal based food and feed. Such derivatives have been termed "masked" mycotoxins, implying that they are not routinely detected and reconstitution of the parent toxins during food processing or digestion is possible. Of particular importance is deoxynivalenol-3-*O*-ß-D-glucopyranoside, but glucosides of other relevant trichothecenes such as nivalenol (NIV), T-2 (T2) and HT-2 (HT2) toxin have been identified as well. The toxicological relevance of trichothecene glucosides is not fully understood and it is crucial to synthesize such compounds in sufficient amounts to make toxicological studies possible. Standards of glucosides of NIV, T2 and HT2 toxins are so far not commercially available, which also limits screening of their occurrence in cereal samples. Better understanding the role of glycosylation in plant-pathogen interaction with strains producing different toxins may also be of interest for breeders aiming to increase *Fusarium* resistance of cereal crops. Previously, our group has identified and studied several DON-conjugating plant UDP-glucosyltransferases (UGTs). A rice UGT (OsUGT79) was expressed in *E. coli* and biochemically characterized. *In vitro* biochemical assays showed that the recombinant enzyme is able to equally conjugate DON, NIV and HT2, but is inactive with T2 toxin. Interestingly, preliminary assays with a related UGT from barley (HvUGT13248) indicate that this enzyme prefers NIV over DON as substrate. OsUGT79 was used to synthesize mg quantities of DON-3-glucoside, NIV-3-glucoside and HT2-3-glucoside. NMR spectroscopy confirmed the formation of a trichothecene-3-*O*-ß-D-conjugate in each case. We are now able to enzymatically synthesize DON-3-glucoside as well as the novel standards NIV-3-glucoside and HT2-3-glucoside, which is a crucial step forward in evaluating the natural occurrence and toxicity of these masked mycotoxins in animal feeding trials.

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TRICHOTHECENE MYCOTOXIN LEVELS DETECTED IN WINTER WHEAT IN ONTARIO, CANADA FROM 2009-2015

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ABSTRACT

Fusarium head blight caused by *Fusarium graminearum* is a serious disease of wheat (*Triticum aestivum* L.). Deoxynivalenol (DON) is the mycotoxin most commonly detected in contaminated wheat grain in Ontario. The objective of this study was to evaluate the level of trichothecene mycotoxins in winter wheat grain in Ontario from 2009, 2010, 2013, 2014 and 2015. The harvested grain was sampled to determine DON, 15-acetyl DON, 3-acetyl DON, nivalenol (NIV), T-2 and HT-2 toxins using a GC-MS system with a detection limit of 0.06, 0.05, 0.05, 0.12, 0.06 and 0.04 μg/g, respectively. In 2015, DON level was detected using ELISA method with a detection limit of 0.25 μg/g. The average DON level was 0.7 μg/g, 0.3 μg/g, 3.3 μg/g, 0.2 μg/g and 2.5 μg/g in 2009, 2010, 2013, 2014 and 2015, respectively. 15-acetyl DON and 3-acetyl DON were not detected in 2009, 2010 and 2014 in Ontario. However, they were detected in 2013 in soft white winter wheat at one or two locations. NIV was not detected in any sample in 2009, 2010 and 2014 while it was detected just in one sample in 2013 at level 0.14 μg/g. T-2 and HT-2 toxins were detected in one sample in 2009 at level 0.07 μg/g and 0.06 μg/g, respectively, while they were not detected in 2010 and 2014. In 2013, T-2 and HT-2 ranged from 0.08 μg/g to 0.14 μg/g and from 0.04 μg/g to 0.80 μg/g, respectively. In 2013, DON level was high in general, but lower mean levels of DON were detected in hard red wheat than in soft white wheat. DON level was low in general in 2014, and the highest in cv 'Wentworth' at Ridgetown location (1.6 μg/g). In conclusion, several times higher average levels of DON were detected in 2013 and 2015 compared to previous years, with some winter wheat lines showing a level of tolerance to mycotoxin accumulation. Future monitoring of trichothecene mycotoxins in winter wheat in Ontario is recommended.

STATE OF THE ART IN MULTI-MYCOTOXIN DETERMINATION BY LC-(HR)-MS/MS

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ABSTRACT

Methods based on liquid chromatography – tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of mycotoxins in different commodities are steadily increasing in popularity. In the presentation we will show applications using highly sensitive low resolution triple and quadruple mass spectrometers (QqQ), as well as the possibilities offered by high resolution quadruple time-of-flight (QTOF) instruments. All of the applications are based on a simple sample preparation procedure using acidified aqueous acetonitrile extraction, without further clean-up, and electrospray ionisation interfaces. Challenges in the development of such multi-analyte methods and the advantages and disadvantages of the different approaches will be discussed.

One of the obstacles in accurate quantification are matrix effects caused by suppression or enhancement of the analyte signal due to co-eluting matrix components. The use of stable isotopically labelled standards is an effective approach to compensate for these matrix effects. An accurate, reliable and fast method for the quantification of mycotoxins, currently regulated in the European Union in solid foodstuff, was developed, validated and will be presented.

A second method for the determination of several hundreds of mycotoxins and other fungal and bacterial metabolites in food and feed samples will also be discussed. In this approach, two specific transitions are optimized for each analyte and are acquired in a predefined retention time window. This method has been in-house validated for 295 analytes in four model food matrices, but currently comprises over 500 compounds.

Finally, the application of a database and spectral library using LC-QTOF-MS/MS will be presented. The acquired accurate mass spectra were corrected to their theoretical mass-to-charge ratio by a probabilistic approach before incorporation into the library. Once the MS/MS library has been created, standards are not required for the identification of compounds, moreover, this approach also allows post-acquisition data analysis.

GENE DISCOVERY AND ENGINEERING RESISTANCE

ANALYSIS OF THE SMALL INTERFERING RNA PROFILES OF RANDOMLY INSERTED PTRM-*TRI6* *FUSARIUM GRAMINEARUM* MUTANTS AND THEIR DON RELATED PHENOTYPES

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ABSTRACT

Deoxynivalenol (DON) production by *Fusarium graminearum* requires activation of the trichothecene pathway in which TRI5 catalyzes the first step of trichothecene synthesis and TRI6 is a transcription factor that activates the pathway. RNA interference (RNAi) has emerged as a useful fungal genetics tool for reducing the expression of specific genes such as *TRI6*. Reduced DON production and virulence on wheat has been demonstrated as a result of RNAi-induced reduction of *TRI6* expression via transformation of *F. graminearum* with the plasmid pTRM-TRI6. This plasmid contains the GDPA promoter driving *TRI6* linked to an inverted repeat of *TRI6* to generate a hairpin loop mRNA. Hairpin loop structures are known to be processed by the endoribonuclease dicer to produce a population of a specific type of non-coding small RNA approximately 20–30 bp long, called small interfering RNA (siRNA), that silence genes with homology to siRNAs. To verify and expand on the previous study, six additional fungal transformants containing pTRM-TRI6 were produced and evaluated by next generation sequencing for siRNA with homology to *TRI6* and for the genomic location of the pTRM-TRI6 insertions. The results support previous conclusions that expression of pTRM-TRI6 reduced DON production and virulence on wheat. Furthermore, experiments measuring the mycotoxin production capacity independent of host-pathogen interactions showed reduction in mycotoxin accumulation on rice cultures and lower expression of *TRI5* on toxin-inducing media (TBI) in association with the production of siRNAs with homology to *TRI6*. Understanding the resulting siRNA profiles from RNAi constructs is critical to optimizing RNAi applications. The results are being used to guide the development of vectors for barley transformation with the goal of elevating resistance to Fusarium head blight via host induced gene silencing, which is a promising application of RNAi.

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REACTIONS OF TRANSGENIC BARLEY LINES TO FHB INOCULATION IN 2015 NORTH DAKOTA FIELD TRIALS

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ABSTRACT

Transgenic lines were tested directly in the field in Langdon, ND. Lines and checks (Conlon, Quest, ND20448) were sown in hill plots in an augmented block design, with the checks repeated every 20 hills. Three replicates were planted in the inoculated, misted nursery and three replicates in the adjacent un-misted nursery. Ten seed were planted per hill with 30 cm hill spacing. Plots were bordered with wheat. The nursery was inoculated by grain spawn technique with a mixture of five *F. graminearum* isolates. From inoculation until grain ripening, the misted nursery was irrigated for 20 minutes early morning and again at late afternoon each day. FHB severity was evaluated at approximately three weeks after anthesis, by counting the total and infected number of seed on ten randomly selected spikes per hill. Samples from each plant were analyzed for DON content. For both misted and un-misted trials, there were significant differences for severity and DON content among lines. There were significant differences also among replicates for DON content. Pairwise comparisons ($p = 0.10$) between Quest, ND20448, and Conlon and their respective transgenic lines revealed few differences. In the misted nursery, ND20448-derived transgenic line ND2-6 had higher FHB severity and DON content than ND20448, and Quest-derived transgenic line 82Q3-6 had higher DON content than Quest. However, in the un-misted nursery, this line had lower severity. The other Quest-derived line, 82Q3-2 had lower DON content than Quest. 82DN2-6 again had higher severity and DON content than ND20448.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2015 FIELD NURSERY REPORT

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ABSTRACT

The 2015 field screening nursery consisted of 9 wheat and 12 barley entries evaluated in side by side experiments. Entries within each species experiment were arranged in a randomized complete block design with four replications in a field located at UMore Park, Rosemount MN. Trial entries and untransformed controls* were submitted by the University of North Texas (9 wheat lines + Bobwhite*) and the USDA (12 barley lines + Conlon* and ND20448*). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivar Alsen and the susceptible cultivars Roblin and Wheaton. The barley checks were the moderately resistant cultivar Quest and the susceptible cultivar Stander. Individual plots were 2.43 m long single rows. The trial was planted on June 2, 2015. All plots were inoculated twice with the exception of Wheaton and Stander non-inoculated controls. The first inoculation was applied at anthesis for wheat (July 13-July 22) and at head emergence (July 16-July 20) 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 40 *F. graminearum* isolates, applied 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 10 $ml.sec^{-1}$ at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on July 16 through August 6 to facilitate FHB development. FHB incidence and severity were assessed visually 18-24 d.a.i. for wheat and 17-21 d.a.i. for barley on 20 arbitrarily selected heads per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 heads observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets observed. Plots were hand harvested at maturity on August 17 (barley) and August 25 (wheat). Approximately sixty heads were harvested from each plot, threshed and the seed cleaned manually. The wheat grain was used to determine the percentage of visually scabby kernels (VSK) and then all samples (wheat and barley) were ground and submitted for deoxynivalenol (DON) analysis. In 2015, the disease severities were similar to the 2013 nursery and generally lower than the 2014 nursery. The mean FHB severity for the non-inoculated Wheaton control was 19%. Mean FHB severity for the untransformed wheat check Bobwhite was 31%. Mean FHB severities for the standard wheat checks, Alsen, Roblin and Wheaton were 9, 35 and 38%, respectively. The mean FHB severity for the non-inoculated Stander check was 17%, while the

untransformed barley check varieties Conlon and ND20448 had mean FHB severities of 25 and 15%, respectively. The barley standard checks, Quest and Stander, had mean FHB severities of 8 and 22%, respectively. The FHB severity data indicated that resistance was significantly ($P<0.05$) improved in some transformed barley lines compared to the untransformed checks. Preliminary analysis indicated that the FHB severities of several wheat entries may be better than the corresponding untransformed check, though the differences in FHB severities may not be statistically significant. The harvested grain is currently being analyzed for DON. The data are not yet available although they will be included in the poster presented at the forum.

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COARSE AND FINE MAPPING OF QUANTITATIVE TRAIT LOCI FOR FHB IN BARLEY

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ABSTRACT

Fusarium species cause Fusarium head blight (FHB) disease in wheat and barley. Resistance to the disease is controlled by quantitative trait loci (QTL) and previous genetic mapping studies have identified a number of resistant alleles from two- and six-rowed barley accessions. The aim of the current studies are to (1) identify additional QTL for resistance to initial infection (type I resistance); and (2) fine-map a QTL on chromosome 6H in barley. For the first study, a QTL mapping population comprising 93 $F_{6:7}$ recombinant inbred lines (RILs) derived from a cross between Rasmusson and PI383933 was developed. Rasmusson is a six-rowed malting barley cultivar released by University of Minnesota with moderately susceptible response to FHB. PI383933 is a six-rowed Japanese cultivar with susceptible response to FHB and often used as a susceptible check in FHB field screenings. The population was evaluated in FHB nurseries using a randomized complete block design with three replicates in St. Paul and two replicates in Crookston in 2015. Spray inoculation and grain spawn inoculation was applied at St. Paul and Crookston, respectively. Data for FHB severity, plant height, heading date and kernel density were recorded. The barley iSelect 9K SNP chip was used to genotype the RILs and a linkage map was generated with 1,394 SNPs and consisted of seven linkage groups. Composite interval mapping identified three QTL associated with FHB resistance on chromosomes 5H, 6H and 7H. The QTL on 5H and 6H were associated with PI383933 alleles and explained 6.2% and 5.5% of phenotypic variance, respectively. The 7H QTL was associated with a Rasmusson allele and explained 34.3% of phenotypic variance. Interestingly, the FHB QTL on 7H was coincident with a plant height QTL. To fine map the chromosome 6H QTL region, an F_2 population of 2,082 plants was derived from crossing lines carrying Chevron alleles in the 6H QTL region with the susceptible cv. Lacey. This population was genotyped with flanking SSR markers to identify 399 recombinants. These recombinants were further genotyped with 34 SNP markers covering the overlapping target region (2.8 cM), which resulted in the identification of 37 recombinants representing 13 recombinant classes. Selected recombinants will be tested for FHB response in 2016.

TRANSGENIC PLANTS EXPRESSING *HVUGT13248* EXHIBITS HIGH LEVELS OF RESISTANCE TO A WIDE SPECTRUM OF TYPE B TRICHOTHECENES

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ABSTRACT

Fusarium head blight (FHB) is a major disease problem in cereal crops. Trichothecenes produced by the main causal pathogen, *Fusarium graminearum*, play an important role in disease development and spread, and also pose threats to humans and animals that consume infected grains. However, research on FHB resistance has primarily focused on deoxynivalenol (DON), which is the major chemotype found in the United States. Other chemotypes such as nivalenol (NIV) are more prevalent in Asia, but have recently been identified in the US. Additional chemotypes include the 3-acetyldeoxynivalenol (3-ADON) and15-acetyldeoxynivalenol (15-ADON), and the newly identified NX-2. Previous research identified a barley UDP-glucosyltransferase gene, *HvUGT13248*, which provides high levels of type II resistance to FHB by converting DON to DON-3-O-glucoside (D3G) in transgenic wheat. Here, we report that this gene has a wide spectrum of resistance to different trichothecene chemotypes, including NIV, 3-ADON and NX-2. Transgenic wheat expressing *HvUGT13248* show high levels of type II resistance to a NIV-producing Fg strain in greenhouse point inoculation tests. The FHB severity of the transgenic events were reduced by up to 90% compared to the non-transgenic control, while NIV accumulation in the transgenic events were reduced up to 94% compared to the non-transgenic control. Transgenic wheat expressing *HvUGT13248* also exhibits type II resistance to Fg strains producing 3-ADON and NX-2. Using a root assay, we found that transgenic wheat and *Arabidopsis* expressing *HvUGT13248* show resistance to DON and 3,15-diA-NIV inhibited root growth. Taken together, *HvUGT13248* shows resistance to a wide range of type B trichothecenes.

EXPRESSION OF A LIPID TRANSFER PROTEIN IN WHEAT TO ALLEVIATE OXIDATIVE STRESS INDUCED BY TRICHOTHECENES - A POSSIBLE MECHANISM TO INCREASE RESISTANCE TO FHB

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ABSTRACT

Trichothecene mycotoxins, such as deoxynivalenol (DON), are potent virulence factors of *Fusarium graminearum*, a causative agent of Fusarium head blight (FHB). Exposure to trichothecenes can trigger reactive oxygen species (ROS) production at toxic levels. Overexpression of a non-specific lipid transfer protein (AtLTP4.4) in *Arabidopsis* was found to enhance resistance to trichothecene exposure and led to significantly attenuated reactive oxygen species (ROS) compared to nontransgenic controls. In addition, overexpression of the cysteine-rich nsLTP was found to increase the total cellular glutathione (GSH) content of *Arabidopsis* leaf tissue. These results demonstrate that trichothecenes cause ROS accumulation and overexpression of AtLTP4.4 protects against trichothecene-induced oxidative stress by increasing the GSH-based antioxidant defense. We previously showed that exogenous addition of GSH and other antioxidants enhanced resistance to Tcin while the addition of buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, increased sensitivity to the toxin, providing further evidence of the link between oxidative stress and trichothecene sensitivity. To determine if expression of AtLTP4.4 confers resistance to trichothecenes in transgenic wheat, we constructed a monocot codon optimized version of AtLTP4.4 with HA and His tags, cloned the insert into the Ubi expression vector pAHC17, and generated transgenic Bobwhite, Rollag, Forefront and RB07 lines. We are analyzing these lines for RNA and protein expression and determining if the overexpression of nsLTP impacts the GSH content in wheat. Lines that show high expression and enhanced resistance to trichothecenes relative to the parental genotypes in the greenhouse will be tested in the field for FHB resistance during the 2016 season.

ENGINEERING RESISTANCE AGAINST *FUSARIUM GRAMINEARUM* IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a damaging disease of wheat and barley. *Fusarium graminearum*, the principal causative agent of FHB, can also cause disease on leaves and flowers of *Arabidopsis thaliana*, a model plant for molecular-genetic studies. We have utilized the *Arabidopsis-F. graminearum* pathosystem to identify genes and mechanisms that can be targeted for promoting FHB resistance in wheat (Makandar et al. 2010, 2015, Nalam et al. 2015). Our results have demonstrated that defense regulatory genes as well as susceptibility factors can be engineered in wheat to enhance FHB resistance (Makandar et al. 2006, 2015; Nalam et al. 2015). Several of these transgenic wheat lines are undergoing field trials. Molecular-genetic studies have further demonstrated that the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) mechanism can be targeted for enhancing resistance against *F. graminearum* in wheat. These results will be presented. In addition, we will also describe the utility of a lipase-encoding gene, which is expressed at elevated levels in floral tissues, in enhancing resistance against *F. graminearum*.

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HOW DOES THE *FHB1* LOCUS OF WHEAT AFFECT THE ABILITY TO DETOXIFY DON – A HYPOTHESIS

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ABSTRACT

The mycotoxin deoxynivalenol (DON) is a virulence factor of *Fusarium graminearum* required for spreading of the pathogen in the infected wheat head. The major spreading resistance QTL of wheat, the *Fhb1* locus, has been associated with increased ability to detoxify DON by formation of a glucose conjugate, leading to the hypothesis that *Fhb1* either encodes a UDP-glucosyltransferase (UGT) or a gene regulating its activity. Within the *Fhb1* interval of the sequenced susceptible cultivar Chinese Spring no UDP-glycosyltransferase (UGT), which might catalyze this detoxification-reaction, was found.

We have determined the K_m value (concentration of half-maximal enzyme activity) of a recombinant UGT capable of detoxifying DON into DON-3-O-glucoside for the UGT cosubstrate UDP-glucose. The observed K_m value of 2.2 mM [1] is about 10x higher than the concentration found in wheat heads at anthesis in *Fhb1* lines [2], suggesting that the availability of the co-substrate UDP-glucose is limiting the detoxification ability. Wheat lines containing *Fhb1* show about 2.53x higher levels of UDP-glucose than lines lacking it. A gene biochemically linked to UDP-glucose was found in the *Fhb1* interval of Chinese Spring, potentially encoding a truncated UDP-glucose dehydrogenase (UGDH), presumably a nonfunctional pseudogene. Both enzymes, UGT and UGDH, use UDP-glucose as a cosubstrate - the first to detoxify DON into DON-3-O-glucoside, the latter to produce UDPglucuronic acid, a precursor for activated sugar donors for cell wall biosynthesis. Coexpression of functional UGDH and UGT in yeast leads to a decrease of UDP-glucose levels and to sensitivity against DON compared to yeast expressing the UGT alone. We were therefore interested to learn what is encoded in the *Fhb1* interval of a resistant wheat cultivar. A comparison of the corresponding region between the *Fhb1* containing cultivar CM-82036 with Chinese Spring revealed that the pseudo-UGDH is absent in the *Fhb1* cultivar due to a 41.5 kb deletion.

The mechanism how the pseudogene lowers the UDP-glucose level is under investigation. A hypothesis how an absent pseudogene can confer a dominant resistance phenotype will be presented.

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PATHOGEN BIOLOGY AND GENETICS

SURVEY FOR *FUSARIUM GRAMINEARUM* 15-ADON, 3-ADON AND NIV CHEMOTYPES IN WINTER WHEAT IN ONTARIO

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* (FG) is a serious disease of wheat (*Triticum aestivum* L.). Deoxynivalenol (DON) is the mycotoxin most commonly detected in contaminated wheat grain in Ontario, Canada. Grain samples from six winter wheat cultivars per year from 2013 and 2014 Ontario Performance Trial were included in the study. Grain samples were collected from Inwood, Elora, Woodslee, Palmerston and Ridgetown in 2013, and from Ottawa, Palmerston and Ridgetown in 2014 to assess the percentage of *Fusarium* infected kernels (FIK), the percentage of FG (identified as % of total *Fusarium* spp.) and the frequency of FG chemotypes (15-ADON, 3-ADON and Nivalenol-NIV). The cultivars were: highly susceptible to Fusarium head blight, moderately susceptible and moderately resistant. One hundred and fifty kernels of each cultivar were surface-sterilized in 0.16% NaOCl (dilute commercial bleach) for three minutes, air dried, and plated on acidified potato dextrose agar. The kernels were incubated for seven days under a 12:12 hr light: dark cycle at room temperature. Subsequently, single spore cultures of FG were recovered and identified morphologically. The FG isolates were also identified using molecular markers specific to *F. graminearum.* Genomic DNA was extracted from single spore isolates of FG. 15-ADON, 3-ADON and NIV chemotypes of the fungal strains were identified using *TRI3*- and *TRI-12* based molecular markers. In 2014, the highest percentage of FG was observed in highly susceptible cv. in Ottawa (83.3%) and Palmerston (90.9%), and moderately susceptible cv. in Ridgetown (94.6%). Percentage of FG 15-ADON chemotype was 63.9%, 95.8 % and 97.9 % from Ottawa, Palmerston and Ridgetown, respectively. 2.1% of NIV was detected at Palmerston and Ridgetown, and NIV was not detected in Ottawa. 3-ADON was detected at 36.1% in Ottawa, only 2.1% at Palmerston and was not detected at Ridgetown. We concluded that the frequency of the FG 3-ADON chemotype in winter wheat in Ottawa was much higher in 2014 (36.1%) than recorded previously (2%-7%) from anywhere in Ontario. The results from 2013 season will be presented at the conference.

GENERATION OF *FUSARIUM GRAMINEARUM* MUTANTS TO SCREEN CANDIDATE PATHOGEN-ASSOCIATED MOLECULAR PATTERNS

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ABSTRACT

Fusarium head blight (FHB) is a disease of wheat and other cereals caused predominantly by *F. graminearum* resulting in significant loss of gain yield and quality. Our study is aimed at screening candidate pathogen-associated molecular patterns (PAMPs) of *F. graminearum*. To this end, seven proteins (PAMP1-PAMP7) were selected from the *F. graminearum* secretome and knockout and overexpression mutants of *F. graminearum* were generated for the genes that encode these proteins. The selection of these proteins was based on their sequence homology to proteins having pathogenicity roles in other host-pathogen interactions. Mutants were generated by *Agrobacterium*-mediated transformation using plasmids designed for gene replacement with a hygromycin resistance gene for knockout (KO) mutants, and insertion of a hygromycin resistance gene adjacent to a fungal overexpression promoter for *in locus* overexpression (OX) of target genes. The mutants *PAMP1*-OX, *PAMP2*-KO, or *PAMP2*-OX were point-inoculated in Superb and GS-1-EM0040 ('CIMMYT 11'/'Superb'*2) that are moderately susceptible and moderately Type II resistant wheat lines respectively. *PAMP1*-KO appears to be a lethal mutant and hence was not evaluated here. The two wheat lines inoculated with the wild-type *F. graminearum* strain served as controls. Disease evaluations 18 days after inoculation showed that the number of infected spikelets was significantly lower for wheat lines inoculated with *PAMP1*-OX as compared to those inoculated with the wild-type *F. graminearum* strain. Similarly, *PAMP2*-OX inoculated wheat lines had fewer infected spikelets than wild-type inoculated wheat lines. Moreover, the number of diseased spikelets for *PAMP2*-OX inoculated wheat lines was significantly lower than those for *PAMP2*-KO inoculated wheat lines. From these preliminary results we hypothesize that PAMP1, and possibly PAMP2, activate wheat receptors triggering the basal immune response, known as PAMP-triggered immunity, in wheat. Generation of mutants for the remaining genes and their screening in additional wheat lines are currently underway.

EXPLORING THE FUNCTION OF GENES INVOLVED IN DISEASE INITIATION BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

During a study to identify genes involved in early penetration of host plants by *Fusarium graminearum,* we investigated the role of the three aquaporin genes in this process. Aquaporins are membrane bound transport proteins, and are best known as water transporters in animal cells. Their function in the growth, development, and pathogenesis of microorganisms is poorly understood. Strains harboring knockouts of the individual genes can grow and develop functional perithecia under controlled laboratory conditions, but do so with deficiencies or delays in conidial germination, development of aerial hyphae, and development of perithecia relative to the wild-type fungus. Likewise, the mutants are capable of infecting excised barley florets under high humidity conditions. However, all three individual mutants have greatly reduced ability to spread beyond the site of inoculation in susceptible wheat. Generation and analysis of double aquaporin mutants is ongoing. Although the relationship between these aquaporins is unclear, they appear to be functioning in a similar manner and all are essential. These genes may be good targets for development of control of early infection of *F. graminearum.*

STAGE-SPECIFIC A-TO-I RNA EDITING IN THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*

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ABSTRACT

Yeasts and filamentous fungi do not have ADAR orthologs and are believed to lack A-to-I RNA editing, which is the most prevalent editing of mRNA in animals. However, during this study with the *PUK1* pseudo-kinase gene important for sexual reproduction in *Fusarium graminearum*, we found that two tandem stop codons UA^{1831}G UA^{1834}G in its kinase domain were changed to UG^{1831}G UG^{1834}G by RNA editing in perithecia. To confirm A-to-I editing of *PUK1* transcripts, strand-specific RNA-Seq data were generated with RNA isolated from conidia, hyphae, and perithecia. *PUK1* transcripts were almost specifically expressed in perithecia and 90% of them were edited to UG^{1831}G UG^{1834}G. Genome-wide analysis identified 27,301 perithecium-specific A-to-I editing sites. Unlike those in animals, 70.5% of A-to-I editing sites in *F. graminearum* occur in coding regions and over two-thirds of them result in amino acid changes, including editing of 45 *PUK1*-like pseudogenes with stop codons in ORFs. Furthermore, *F. graminearum* differs from animals in the sequence-preference and structure-selectivity of A-to-I editing sites. Whereas As embedded in RNA stems are targeted by ADARs, RNA editing in *F. graminearum* preferentially targets As in hairpin loops, which is similar to the anticodon loop of tRNA targeted by ADATs, implying a potential evolutionary link between mRNA editing and ADATs in fungi. Overall, our results showed that A-to-I editing occurs specifically during sexual reproduction and mainly in the coding regions in filamentous ascomycetes, involving adenine deamination mechanisms distinct from metazoan ADARs.

A GENOME-WIDE VIEW OF POPULATION STRUCTURE AND GENETIC DIFFERENTIATION OF *FUSARIUM GRAMINEARUM* POPULATIONS FROM THE AMERICAS

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ABSTRACT

Population genomic studies of *Fusarium graminearum* (Fg) isolates provide an important complement to experimental studies that investigate the interaction of this important pathogen with its hosts and functional differences between different pathogen populations. Jointly, these studies can identify the genetic basis of functional differences between populations that can affect pathogen management and strategies for developing host resistance. Our broader aims include characterizing the variability along chromosomes of patterns such as variant density, genetic differentiation between populations, and genetic recombination to gain insight into evolutionary processes in this species. More specific goals include closer population genomics investigations of the fact that Fg isolates can be genetically clustered into groups that largely correspond to genotypes at the trichothecene gene cluster that determine whether 3-ADON or 15-ADON predominates, and observed population shifts in the prevalence of 3-ADON isolates in parts of North America.

Here, we provide an update of our FY14-15 USWBSI population genomics project that uses genotyping by sequencing (GBS) for the genetic analysis of Fg isolates from the Americas. We have expanded our sample to nearly 400 isolates, focusing on 3-ADON and 15-ADON isolates from New York and the upper Midwest and also including isolates from Uruguay. We demonstrate that by scanning along chromosomes, we can identify loci with the highest levels of genetic differentiation between chemotypes, geographic regions, or sampling years, highlighting loci with potentially important adaptive roles. We also describe patterns of linkage disequilibrium between variants in the populations and other patterns consistent with footprints of natural selection.

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A SMALL PILOT GWAS FOR THE GENETIC BASIS OF PATHOGENIC AND SAPROPHYTIC FITNESS IN A SAMPLE OF NEW YORK *FUSARIUM GRAMINEARUM* ISOLATES FROM WHEAT

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ABSTRACT

The dominant mycotoxin of Fusarium head blight in the U.S. is the trichothecene deoxynivalenol (DON), a known virulence factor, and *Fusarium graminearum* (Fg) isolates can be categorized based on which of two acetylated DON forms, 3-ADON and 15-ADON, each isolate produces in greatest quantity. Surveys of genetic markers have found that Fg isolates can be genetically clustered into groups that correspond to genotypes at the trichothecene gene cluster that determine which acetylated form of DON predominates. Reports of the increase in prevalence of 3-ADON isolates led to a series of studies aimed at identifying factors that give 3-ADON isolates an advantage over 15-ADON isolates. While several studies that focused on pathogenic traits found evidence for a 3-ADON advantage, a study of 50 isolates from New York that considered traits both pathogenic and saprophytic fitness on a susceptible wheat cultivar found no detectable advantage of 3-ADON isolates. Several important questions remain unanswered: what factors help explain the increase in prevalence of 3-ADON isolates; what is the relationship between trichothecene genotypes and fitness traits; and what other genetic loci are associated with differences in saprophytic and pathogenic fitness found among Fg isolates?

Our FY14-15 USWBSI population genomics project uses genotyping by sequencing (GBS) markers to characterize the genome of hundreds of U.S. Fg isolates, including the 50 New York isolates measured for pathogenic and saprophytic fitness as described above. Here, we aim to address the latter two questions above by screening for statistical associations between the fitness traits and genetic variation throughout the Fg genome. We have performed a pilot genome-wide association study (GWAS) for the 14 fitness traits that takes into account the known population structure of Fg populations (in particular, the subdivision between the 3-ADON and 15-ADON chemotypes). Our GBS markers provided genotypes at thousands of SNPs densely distributed throughout the Fg genome. These SNPs were filtered for quality and allele frequency, and then we performed imputation to infer the allele state of the remaining missing genotypes. Due to our small sample size, our preliminary analysis has low power to detect associated genetic variants. Yet this pilot study has the potential to identify the strongest signals of association and helps to identify the challenges for future GWAS in Fg populations.

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USING ENZYMES AND MICROORGANISMS TO MODIFY THE MYCOTOXIN DEOXYNIVALENOL

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ABSTRACT

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by the fungus *Fusarium graminearum* that contaminates staple crops such as wheat, barley, and maize when they are infected with this fungus. New strategies are needed to mitigate DON. We screened for microbes that could grow in the presence of 100 ppm DON and found two mixed cultures and two pure cultures that consistently detoxified DON in laboratory experiments. Sequencing analysis of the pure cultures indicated that they were *Pseudomonas* and *Achromobacter*. Nuclear magnetic resonance (NMR) analysis of one of the culture byproducts indicated that DON was converted to 3-keto-DON. In a second approach, we engineered yeast strains to be sensitive to 100 ppm DON and used them to screen library fragments generated from the mixed cultures and the *Pseudomonas* species and cDNA enzyme sequences created by Integrated DNA Technologies. Three library fragments and two cDNA enzyme sequences were identified that allowed the yeast to grow in the presence of 100 ppm DON. In future studies, microbes and enzymes that demonstrated DON detoxification will be tested on contaminated wheat and barley samples. Our research offers a unique approach to reduce DON in these grains, particularly in the context of ethanol co-products.

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VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

SCREENING FOR FHB SUSCEPTIBILITY IN BARLEY CULTIVARS IN THE WESTERN U.S.

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ABSTRACT

Fusarium head blight (FHB) has become a regularly occurring problem with economically significant impacts in Idaho. Until 2015, detectable DON levels in commercial barley production had remained below 0.5 ppm. Levels of DON are now occurring in malt barley that exceed acceptable levels. Identification of highly susceptible advanced lines and widely grown varieties will enable growers to manage risk of FHB and DON with best practices that reduce risk. An inoculated screening trial was conducted at Aberdeen, ID in 2015 to evaluate FHB resistance levels in barley varieties selected and released with or without prior screening. Fifty barley varieties were planted on May 8 with two replications. Nine local isolates of *F. graminearum* were collected, grown on potato dextrose agar and confirmed by PCR. Corn inoculum was prepared by placing corn kernels in spawn bags and autoclaving twice prior to inoculation of the *F. graminearum* isolates. Conidial inoculum was prepared by increasing macroconidial production in mung bean agar and storing spore suspension in one-liter bottles. Corn inoculum was applied at a rate of 30 g/m^2 approximately 2 to 3 weeks before heading while conidial inoculum at 100,000 spores/L was sprayed at heading. FHB incidence and severity were assessed 3 weeks after application of conidial inoculum. Significant differences in FHB index (P=<.0001), yield (P=0.0013) and FDK (P>.0001) among varieties were recorded. FHB indexes range from 0.2 to 19.9% The 6-row malt 'Quest', 2-row feed 'RWA 1758' and 2-row feed 'Conrad' were the most resistant lines. 'Transit', a hulless 2-row feed with high ß-glucan, and 'Goldeneye', a high-yielding 6-row feed, were the most susceptible lines. Yield ranged from 84.0 (2Ab09-X06F084-31) to 693.5 (Conrad) g/plot. FDK of hulless varieties were significantly higher than hulled varieties. DON levels will also be determined.

OBJECTIVE

The objective of this study was to determine FHB host resistance levels in barley varieties released for the arid irrigated production areas of the PNW.

INTRODUCTION

Ten years ago, the incidence of FHB in the irrigated west was regarded as a minor and relatively rare occurrence. With the substantial increase in corn acreage directly due to the increase in the dairy industry and with substantial changes in irrigation practices, FHB has become a regularly occurring problem with economically significant impacts for small grain producers. In barley, DON levels mostly have remained below detectable levels or below 0.5 ppm. However, in 2015 in Idaho, for the first time DON levels exceeded acceptable levels ranging from 0.3 to 4 ppm. Unacceptable levels of DON toxin have been found consistently in irrigated wheat and barley in areas of the PNW and

intermountain West in the past five years. Corn debris, where high levels of *Fusarium graminearum* reside, takes up to three or four years to degrade in arid west environments. Changes in crop rotation have shifted from the predominant *Fusarium spp.* to *F. graminearum*, which produce airborne ascospores that can disperse many miles in the wind. Disease management approaches must change and will depend on the degree of susceptibility of the varieties being grown. Control strategies must incorporate varieties that are less susceptible to FHB.

MATERIALS AND METHODS

Isolate collection. *Fusarium* species were isolated from infected hard white spring wheat WB-Pristea collected in 2014 from a local commercial field near Sugar City, ID. *F. graminearum* was confirmed using conventional polymerase chain reaction (PCR). Cultures were grown in potato dextrose agar (PDA) with streptomycin sulfate (50 mg l^{-1}). DNA was extracted using sodium phosphate buffer. Small amount of mycelia was scraped with a toothpick and mixed in 1000 µl of sodium phosphate in microcentrifuge tube. The extraction was incubated for 30 minutes and centrifuged at 8000 to 10,000 *g* for 1 min. Tubes were incubated at 85°C for 30 minutes and stored at -20°C until used for PCR analysis. Avoiding mycelia, 0.5 µl extraction was taken from top of the tube. PCR was performed using primer pairs (Fg16F and Fg16R).

Corn inoculum preparation. The amount of corn inoculum needed was calculated based on a rate of 30 g/m^2. Spawn bags were prepared by filling with 1500 g of once-autoclaved corn kernels and adding tap water to approximately an inch above the corn level. Corn kernels were allowed to imbibe water for 16 hours prior to draining and sealing the bags the following morning. Five bags were placed in an 11-gallon tub and autoclaved for 90 minutes at 30 psi. Using aseptic techniques, one PDA plate colonized with *F. graminearum* was used to inoculate each spawn bag. An additional 25-30 ml of sterile water amended with streptomycin sulfate (0.2 g per 150 ml water) was added to each spawn bag, sealed and mixed. Inoculum was incubated for 2 to 3 weeks at room temperature, dried for 5-8 days in the a laminar flow hood, and stored at 4°C until needed for field inoculation.

Conidial inoculum preparation. Inoculum was prepared following the protocol provided by Dr. Ruth Dill-Macky (*personal communication*). To increase macroconidial production, spore suspensions obtained from PDA plates were transferred to mung bean agar (MBA) plates and incubated for 10-14 days at room temperature. Inoculum was stored at a standard concentration (800,000 macroconidia per ml) in 1 L Nalgene bottles. Bottles were stored at 4°C and -20°C for short-term and long-term storage, respectively. A diluted concentration (100,000 macroconidia/L) was prepared for field application.

Field nursery establishment. An irrigated field nursery was established at the University of Idaho Aberdeen Research and Extension Center in the 2015 growing season. Fifty barley lines and varieties were tested for level of FHB susceptibility. Eight-foot plots consisting of two rows were planted on May 8 in a complete block design with two replications per variety. Approximately 60 g of corn inoculum was applied per plot on June 22. In addition, each plot was flagged with color-coded flags based on heading date and were sprayed with spore suspension (Table 1). A CO_2 backpack sprayer with 8003 VS nozzle tips calibrated at 40 psi was used to apply inoculum at a rate of 1 sec/ft. A second inoculum spray was repeated one week after the first. From the time of the earliest conidial inoculation and two weeks following, plots were irrigated once a day for 2 hours. Symptomatic heads were picked from Idagold II and Goldeneye plots for reconfirmation of infection by *F. graminearum*

using a PCR assay on infected kernels. DNA was extracted by mashing a single infected kernel in 100-200 µl sodium phosphate buffer with a wooden applicator stick. The extraction was incubated for 30 minutes at 85°C and centrifuged at 14,000 rpm for 5 minutes. Avoiding debris, 0.5 µl was taken from the top extraction for PCR using the same primer pairs detailed above.

Data collection and analyses. Plots were assessed for FHB incidence and severity 20-22 days after conidial inoculation (Table 1). Twenty heads were arbitrarily selected from each plot. Disease severity was determined by visually estimating percent area blighted for each head. Disease incidence was calculated by dividing the number of infected heads by the total number of sampled heads. The FHB index was calculated based on the formula: (% severity x incidence)/100. Percent stand per plot was also noted (data not shown). Plots were harvested on September 23 using a small plot combine. Harvested grains were cleaned using steel mesh sieves and weight recorded. Ten heads per plot were randomly harvested by hand and threshed. *Fusarium*-damaged kernels (FDK) was recorded by counting diseased kernels from a 100 seed sample. Data were analyzed using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS (version 9.2).

RESULTS AND DISCUSSION

FHB indexes significantly differed (P>.0001) from 0.2 to 19.9% (Table 2) among barley varieties. The 6-row malt 'Quest', 2-row feed 'RWA 1758' and 2-row malt Conrad', were the three most resistant varieties. Quest was released for resistance to FHB and resistance to DON accumulation in the grain. The 2-row malt 'Goldeneye" and 2-row feed 'Transit' were two of the most susceptible varieties.

Yield significantly differed (P=0.0013) from 84.0 g/plot in 2Ab09-X06F084-31 to 693.5 g/plot in Conrad (Table 2). Low yields of 2-row lines 'Julie', 'Clearwater', 2Ab09-X06F084-31 (low ß glucan lines) and 'Sawtooth' (low phytate lines) were also affected by low stands. The highest-yielding varieties were the 2-row malt 'Conrad', 2-row feed 'Vespa', 6-row malt 'Celebration' and 6-row feed 'Goldeneye'. Accurate yields for production purposes should be obtained from the Small Grains Research Report published at http://www.uidaho.edu/extension/cereals/scseidaho.

FDK significantly differed between varieties (P>.0001) and ranged from 98.0 % in 'Transit' to 2.0 % in 'CDC Copeland' (Table 2). Hulless, 2-row feed varieties had significantly higher FDK than hulled varieties. Levels of DON will also be tested at a later date.

The planting date was moved later in the season to increase the chances that heading occurred when warm temperatures were favorable for disease development. The barley plots also were inoculated using corn spawn and by spraying a conidial suspension twice to increase chances of infection. In 2014, very little FHB was seen in barley, although a direct comparison with 2014 results is difficult due to inoculum in 2014 being derived from cultures of *F. culmorum*. In addition, temperatures at anthesis were much more favorable for disease development in 2015.

Corn inoculum was spread in the field about three weeks prior to heading of earliest maturing varieties. However, the barley varieties used in this trial have different heading dates and estimating head emergence based on growing-degree days can be difficult to predict. An additional inoculation using a spore suspension helped to even out the effect of differential maturities. However, there still may be bias resulting from heavier disease pressure on later maturing varieties.

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Table 1. Conidial inoculation and disease assessment schedule of spring barley varieties in Aberdeen, ID in 2015.

Heading date	Flag color	Number of plots	Rating date	Days after inoculation
2-Jul	white	8	23-Jul	21
6-Jul	red	18	26-Jul	20
8-Jul	blue	3	29-Jul	21
10-Jul	green	21	1-Aug	22
13-Jul	yellow	25	4-Aug	22
15-Jul	pink	24	6-Aug	22

Table 2. FHB Index, yield and FDK results of spring barley varieties in Aberdeen, ID in 2015.

Variety	Class	Index		Yield (g)		FDK (%)	
Goldeneye	6-row feed	19.9	a	586.8	a-d	20	d-g
Transit *	2-row feed	17.8	ab	170.3	hij	98	a
UT10901-66	6-row feed	17.5	ab	412.1	b-h	17	e-h
2Ab09-X06F084-51	2-row feed	14.8	ab	315.5	e-i	13.5	e-i
Julie *	2-row feed	11.8	bc	145.3	hij	90	ab
Tetonia	2-row feed	11.7	bc	316.4	e-i	16.5	e-h
2Ab09-X06F084-31*	2-row feed	11.5	bc	84	j	84.5	ab
UT2183-85	6-row feed	10.8	bcd	460.9	b-f	21	def
Harriman (08ID2661)	2-row feed	8.3	cde	352.6	e-i	10.5	f-i
Lenetah	2-row feed	8	c-f	456.3	b-f	13.5	e-i
CDC Meredith	2-row malt	8	c-f	433	b-g	7.5	f-i
Herald	6-row feed	7.5	c-g	406.9	b-h	20.5	def
Idagold II	2-row feed	7.1	c-h	263.1	f-j	38.5	c
Oreana (BZ509-448)	2-row feed	6.6	c-i	365.1	d-i	14.5	e-i
LCS Odyssey	2-row malt	5.8	c-j	509.4	a-e	14.5	e-i
2Ab08-X05M010-82	2-row malt	5.8	c-j	374.3	c-i	16.5	e-h
08ARS206-17	2-row feed	5.7	c-j	519	a-e	11	f-i
Sawtooth (081D1549) *	2-row feed	4.8	d-j	103	ij	77	b
Millennium	6-row feed	4.7	d-j	411.1	b-h	11.5	e-i
ABI Balster (B0811)	2-row malt	4.6	e-j	406.8	b-h	12	e-i
LCS Overture	2-row malt	4.5	e-j	538	a-e	9.5	f-i
CDC Fibar *	2-row feed	4.1	e-j	211.9	g-j	85	ab
ACC Synergy	2-row malt	4.1	e-j	313.4	e-i	8.5	f-i
Merit 57	2-row malt	3.7	e-j	392.8	b-h	9.5	f-i
Merem (02Ab17271)	2-row malt	3.4	e-j	470.2	a-f	6.5	ghi
LCS Genie	2-row malt	3.3	e-j	432.8	b-g	13	e-i
Moravian 69	2-row malt	3.2	e-j	330.2	e-i	8	f-i
ABI Voyager	2-row malt	3.2	e-j	398.8	b-h	11	f-i
CDC Copeland	2-row malt	2.9	e-j	480.1	a-f	2	i
ABI Growler (2B09-3425)	2-row malt	2.8	e-j	355.6	e-i	14.5	e-i
Claymore (BZ509-216)	2-row feed	2.8	e-j	523.2	a-e	14.5	e-i
Celebration	6-row malt	2.8	e-j	600.6	abc	25	cde
Baronesse	2-row feed	2.7	e-j	413.1	b-h	12.5	e-i
Tradition	6-row malt	2.5	e-j	436.1	b-g	19.5	d-g
Clearwater *	2-row feed	2.4	e-j	95.8	ij	89.5	ab
AC Metcalfe	2-row malt	2.3	e-j	340.1	e-i	5.5	hi
Xena	2-row feed	2.3	e-j	430.4	b-g	20	d-g
03ARS391-34	2-row feed	2.1	f-j	347.4	e-i	7.5	f-i
Champion	2-row feed	1.8	f-j	540.7	a-e	31.5	cd
Menan (01Ab9663)	6-row malt	1.8	f-j	393.5	b-h	17.5	e-h
Vespa	2-row feed	1.7	hij	610.6	ab	13.5	e-i

Table 2 cont.

Variety	Class	Index	Yield (g)	FDK (%)
ND Genesis	2-row malt	1.6 hij	344.1 e-i	8 f-i
Lacey	6-row malt	1.5 hij	454.6 b-f	17 e-h
2Ab04-X01084-27	2-row malt	1.5 hij	497 a-e	12 e-i
Hockett	2-row malt	1.4 hij	495.5 a-e	11.5 e-i
Harrington	2-row malt	1 hij	533.7 a-e	11 f-i
2Ab07-X031098-31	2-row malt	1 hij	453.4 b-f	15 e-i
Conrad	2-row malt	0.6 ij	693.5 a	5.5 hi
RWA 1758	2-row feed	0.6 ij	363.8 d-i	16.5 e-h
Quest	6-row malt	0.2 j	408 b-h	9 f-i
Pr > F		<.0001	0.0013	<.0001

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SCREENING SPRING WHEAT FOR SUSCEPTIBILITY TO FUSARIUM HEAD BLIGHT IN THE PACIFIC NORTHWEST

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, has become a recurring problem for wheat producers in Idaho. The occurrence of FHB is directly related to the increase of corn acreage under irrigation. FHB is of particular concern not only due to yield losses, but because of *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) accumulation. Determining FHB susceptibility in widely grown wheat varieties is crucial to providing the best management recommendations in irrigated systems in the Pacific Northwest (PNW). A field trial was conducted at Aberdeen, ID in 2015 to evaluate FHB susceptibility of wheat varieties. Forty-four wheat varieties were planted on May 8 with two replications. Nine local isolates of *F. graminearum* were collected, confirmed by using species-specific PCR primers, and used to develop corn spawn. Corn kernels were placed in spawn bags and autoclaved twice prior to inoculation of the *F. graminearum* isolates. Plots were inoculated at a rate of 30 g/m2 approximately 2 to 3 weeks before heading (Feekes 10.1). FHB incidence and severity were assessed 3 weeks after flowering (Feekes 10.5.1) of the earliest maturing variety. Significant differences in FHB index (P=<.0001) and yield (P=<.0001) among varieties were found. FHB indices range from 3.2 to 44.4. Hard white cultivars Klasic and WB-Paloma were highly susceptible while soft white wheat elite lines M12001 and IDO 851 were least susceptible. Yield and FDK ranged from 61.5 (Alpowa) to 318.1 g/plot (Dayn) and 63.5 (UI Stone) to 98.5 % (HRS3530), respectively. Varieties will also be tested for DON levels.

OBJECTIVE

The objective of this study was to determine the level of FHB resistance in wheat varieties released for irrigated production systems in the PNW.

INTRODUCTION

Ten years ago, the incidence of FHB in the irrigated wheat in the Pacific Northwest (PNW) was regarded as a minor and relatively rare occurrence. With the substantial increase in corn acreage directly due to the increase in the dairy industry, and with substantial changes in irrigation practices, FHB has become a regularly occurring problem with economically significant impacts on spring wheat producers. Unacceptable levels of deoxynivalenol (DON) toxin have been found consistently in irrigated wheat in areas of the PNW and intermountain West in the past five years. Corn debris, where high levels of *Fusarium graminearum* reside, takes up to three or four years to degrade in arid western environments. Changes in crop rotation have shifted the predominant species of *Fusarium* to *F. graminearum*, which produce airborne ascospores that can disperse many miles in the wind. Disease management approaches are

changing depending on level of susceptibility of the varieties being grown. Control strategies must also incorporate varieties that are less susceptible to FHB.

MATERIALS AND METHODS

Isolate collection. *Fusarium* species were isolated from infected hard white spring wheat WB-Pristea collected in 2014 from a local commercial field near Sugar City, ID. *F. graminearum* was confirmed using conventional polymerase chain reaction (PCR). Cultures were grown in potato dextrose agar (PDA) amended with streptomycin sulfate (50 mg 1^{-1}). DNA was extracted using a sodium phosphate buffer protocol modified by Zhang et al (2010). A small amount of mycelia was scraped with a toothpick and mixed in 1000 µl of sodium phosphate in a microcentrifuge tube. The extraction was incubated for 30 minutes and centrifuged at 8000 to 10,000 *g* for 1 min. Tubes were incubated at 85°C for 30 minutes and stored at -20°C until used in PCR analysis. Avoiding mycelia, 0.5 µl extraction was removed from top of the tube. PCR was performed using primer pairs (Fg16F and Fg16R) and optimization protocol developed by Nicholson et al (1998).

Inoculum preparation. Inoculum preparation was modified from a protocol used by Gilbert and Woods (2006). The amount of corn inoculum needed was calculated based on a rate of 30 g/m^2.

Spawn bags were prepared by filling with 1500 g of once-autoclaved corn kernels and adding tap water to approximately an inch above the corn level. Corn kernels were allowed to imbibe water for 16 hours prior to draining and sealing the bags the following morning. Five bags were placed in an 11-gallon tub and autoclaved for 90 minutes at 30 psi. Using aseptic techniques, one PDA plate colonized with *F. graminearum* was used to inoculate each spawn bag. An additional 25-30 ml of sterile water amended with streptomycin sulfate (0.2 g per 150 ml water) was added to each spawn bag, sealed and mixed. Inoculum was incubated for 2 to 3 weeks at room temperature, dried for 5-8 days in the a laminar flow hood, and stored at 4°C until needed for field inoculation.

Field nursery establishment. An irrigated field nursery was established at the University of Idaho Aberdeen Research and Extension Center in the 2015 growing season. Forty-four wheat lines and varieties were tested for level of FHB susceptibility. Eight-foot plots consisting of two rows were planted on May 8 in a complete block design with two replications per variety. Approximately 60 g of corn inoculum was applied per plot on June 22. The field nursery was irrigated 2 hours every day after 5 PM for 2 weeks. Symptomatic heads were picked from Klasic fill plots for reconfirmation of infection by *F. graminearum* using a PCR assay on whole infected kernels. DNA was extracted by mashing a single infected kernel in 100-200 µl sodium phosphate buffer with a wooden applicator stick. The extraction was incubated for 30 minutes at 85°C and centrifuged at 14,000 rpm for 5 minutes. Avoiding debris, 0.5 µl was taken from the top extraction for PCR using the same primer pairs detailed above.

Data collection and analyses. Plots were assessed for FHB incidence and severity three weeks after flowering (Feekes 10.5.1) of the earliest maturing variety (Klasic). The first and second replicates were rated 21 and 22 days after flowering, respectively. Twenty heads were arbitrarily selected from each plot. Disease severity was determined by visually estimating percent area blighted for each head. Disease incidence was calculated by dividing the number of infected heads by the total number of sampled heads. The FHB index was calculated based on the formula: (% severity x incidence)/100. Percent stand per plot was also noted. Ten heads per plot were randomly

harvested by hand and threshed. FDK was recorded by counting diseased kernels from a 100 seed count. Plots were harvested on September 23 using a small plot combine. Harvested grains were cleaned using steel mesh sieves and the weight recorded. Data were analyzed using the generalized linear mixed model procedure (PROC GLIMMIX) in SAS (version 9.2).

RESULTS AND DISCUSSION

FHB indices significantly differed from 3.2 in the advanced line IDO851 to 44.4 in the hard white Klasic (Table 1). Hard white wheat varieties WB-Paloma, Snow Crest, LCS-Atomo and WB7328 were highly susceptible. Jefferson and IDO862E were two of the most susceptible lines among the hard red wheat and HRS 3419, HRS3504, HRS3530, WB9411 and WB9229 were the least susceptible. UI Pettit was the most susceptible among soft white varieties and the highest level of resistance in the soft whites were Seahawk, Alpowa, M12001 and IDO 851.

Yield significantly differed from 61.5 g/plot in soft white Alpowa to 318.1 g/plot in hard white Dayn (Table 1). The low yield of Alpowa was influenced by a low stand. HRS 3504 and 3419 were two of the highest yielding hard red wheat varieties. Although highly susceptible, Snow Crest and LCS Atomo had the highest yields among the hard whites next to Dayn and LCS Star. UI Stone, Alum and M12001 were the three highest-yielding soft white wheat varieties. Lines WB9411, HRS3504, HRS 3419, IDO1202S were some of the least susceptible varieties that also produced high yields. Accurate yields for production purposes should be obtained from the SmallGrains Research Report published at http://www.uidaho.edu/extension/cereals/scseidaho.

FDK significantly differed from 63.5 % in soft white UI Stone to 98.5 % in hard red HRS3530 (Table 1). UI Stone was selected for FHB resistance prior to release. Higher yielding lines 'Dayn' and IDO1202S also had the lowest FDK for hard white and hard red varieties, respectively. Other varieties with high yields and low FDK were hard reds WB9411, HRS3504, IDO862E, UI Winchester and HRS3419. DON levels will also be tested at a later date.

The planting date was moved later in the season to increase the chances of anthesis occurring when warm temperatures were favorable for disease development. Interestingly, the susceptible varieties hard white UI Platinum, soft white Alturas and durum wheat Alzada had lower FHB indices than was recorded in 2014, although direct comparison with 2014 results is difficult due to inoculum in 2014 being derived from cultures of *F. culmorum*. In addition, temperatures at anthesis were much more favorable for disease development in 2015.

Corn inoculum should have been spread in the field three weeks prior to heading. However, the wheat varieties used in this trial have different heading dates and estimating head emergence based on growing-degree days is more difficult to predict when based on the heading date of the earliest varieties. This may introduce bias resulting in heavier disease pressure on later maturing varieties.

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Table 1. Yield and FHB Index results of spring wheat varieties in Aberdeen, ID in 2015.

Variety	Class	FHB Index		Yield (g)		FDK (%)	
Klasic	hard white	44.4	a	197	b-k	90	a-g
WB-Paloma	hard white	42.3	ab	133.9	j-m	91.5	a-f
Snow Crest (W)	hard white	39.2	abc	261	a-f	93	a-e
UI Pettit	soft white	37	abc	119.5	j-m	90.5	a-g
WB7589 (W)	hard white	34.1	a-d	182.6	c-l	93	a-e
Jefferson	hard red	33.6	a-d	159.9	h-m	90.5	a-g
IDO862E	hard red	32.2	a-e	217.5	b-j	81.5	d-i
LCS Atomo (W)	hard white	31.5	a-f	255.4	a-h	93.5	a-e
SY3001-2	hard red	29.4	a-g	168.7	e-l	90.5	a-g
WB7328 (W)	hard white	27.6	b-h	160.7	g-m	97.5	ab
WB9668	hard red	27.1	b-h	161.4	f-m	91.5	a-f
WA 8214	soft white	26.9	b-i	83.3	lm	93	a-e
WB6430	soft white	25.8	c-i	178.4	d-l	89.5	a-g
Babe	soft white	25.5	c-j	98.7	klm	89	a-g
LCS Star (W)	hard white	23.9	c-k	278.6	abc	97	ab
UI Winchester	hard red	22.6	c-k	236.3	a-i	83	c-h
Diva	soft white	22	c-l	160.1	g-m	93	a-e
Bullseye	hard red	21.2	c-m	146.7	i-m	96	abc
Alum	soft white	20.7	c-n	184.8	c-k	95	abc
Kelse	hard red	20.4	c-n	104.3	j-m	98.5	a
SY Basalt	hard red	20	d-n	207.7	b-j	94	a-e
IDO1203 (W)	hard white	19.9	d-n	251.2	a-h	88.5	a-g
Dayn (W)	hard white	19.9	d-n	318.1	a	77.5	ghi
SY-10136 (W)	hard red	19.4	d-n	259.9	a-g	92	a-e
Alzada (D)	durum	17.7	e-o	105.8	j-m	96	abc
SY-40292R	hard red	16.3	f-o	268.1	a-e	98.5	a
UI Platinum (W)	hard white	15.8	g-o	189	c-k	96.5	ab
WA 8189	soft white	14.9	g-o	135.9	j-m	95	abc
10SB0087-B	hard white	14.5	g-o	187.5	c-k	91.5	a-f
UI Stone	soft white	14.3	g-o	188.5	c-k	63.5	j
IDO1202S (W)	hard red	13.9	g-o	261.9	a-e	72.5	hij
Alturas	soft white	12.5	h-o	148	i-m	87	a-g
WB9229	hard white	11.6	i-o	97.6	klm	87	a-g
WB9411	hard red	10	j-o	224.1	a-j	78.5	f-i
HRS3530	hard red	9.5	k-o	115.5	j-m	98.5	a
Cabernet	hard red	9.3	k-o	112.3	j-m	94.5	a-d
11SB0096	soft white	8.9	k-o	215.2	b-j	87.5	a-g
HRS3504	hard red	8.7	k-o	289.7	ab	81	e-i
HRS3419	hard red	7.1	l-o	271.9	a-d	85.5	a-h
Seahawk	soft white	6.6	l-o	101.5	klm	69	ij
Alpowa	soft white	6.2	mno	61.5	m	86	a-g
M12001	soft white	5.2	mo	181.2	c-l	88	a-g
IDO 851	soft white	3.2	o	114.2	j-m	85	b-h
Pr < F		<.0001		<.0001		0.0004	

*LCS Kiko not analyzed

MOLECULAR MAPPING OF QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT

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ABSTRACT

Genetic resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum*, is essential to reduce losses of grain yield and quality. This study was conducted to identify DNA markers linked to important genes controlling FHB resistance in adapted germplasm of spring wheat. A doubled haploid population of 773 lines was developed from the cross between moderately resistant Carberry and moderately susceptible AC Cadillac commercial cultivars. The population was evaluated along with parental controls for response to FHB infection in a corn spawn inoculated FHB nursery located near Morden MB. Continuous distributions of disease incidence (related to Type I resistance) and severity (related to Type II resistance) in the population indicated quantitative inheritance of both types of FHB resistance. Based on a linkage map that consisted of 2408 SNPs (Infinium iSelect 90k SNP wheat array) and four microsatellite markers, analysis revealed four significant FHB resistance QTL. Type I and II resistance mapped to the same chromosome regions. The level of phenotypic variation explained by Type I FHB resistance was 2.5% for a QTL on chromosome 3A, 5.7% for 5A, 1.4% for 2B, and 2.3% for 3B. The level of phenotypic variation explained by Type II FHB resistance was 2.3% for a QTL on chromosome 3A, 4.1% for 5A, 1.8% for 2B and 7.8% for 3B. The QTL on chromosome 5A appeared to be associated mainly with resistance to initial infection, while the QTL on 3B was more associated with resistance to fungal spread. The favourable alleles on chromosomes 5A and 3B derived from Carberry and on chromosomes 3A and 2B from AC Cadillac. These results indicate that the two types of FHB resistance were generally controlled by the same genomic regions in this population. The markers associated with the QTL could be used for marker-assisted selection to accelerate the development of resistant adapted wheat cultivars.

IMPACT OF WHEAT CULTIVAR EVEREST ON YIELD LOSS IN KANSAS FROM FUSARIUM HEAD BLIGHT DURING 2015

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ABSTRACT

Fusarium head blight (FHB) is a serious disease of small grains such as wheat. Significant losses can occur due to the blighting of many heads in the field. One of the best ways to manage FHB is by planting resistant cultivars. As a result of funding from the U.S. Wheat and Barley Scab Initiative, significant effort has been placed on developing cultivars adapted to Kansas with improved levels of FHB resistance. Because of this effort, the moderately-resistant cultivar Everest was released in 2009 and has gained popularity such that it is now the most-planted cultivar in Kansas and especially dominant in the eastern third of the state where FHB tends to occur. A significant Fusarium head blight epidemic occurred in Kansas during 2015. The goal of this project was to quantify the impact that the adoption of Everest had on the losses due to FHB during the 2014-15 wheat production season. Throughout the 2015 season, commercial wheat fields were visited and the average severity of FHB determined for each of the 9 crop-reporting districts in Kansas. Data on the percentage acres planted to various wheat cultivars for each district, cultivar FHB susceptibility ratings, and the actual bushels produced for each district were also collected. The above data were used to calculate losses due to FHB for each district. For the calculations, the loss for a susceptible cultivar in each district was estimated based upon the disease survey data. Then, it was assumed that losses for cultivars with intermediate susceptibility would be half of those for susceptible cultivars, and the losses for moderately-resistant cultivars would be half those of intermediate cultivars. Because the percentage acres planted to Everest in each district was known, loss estimates with and without Everest were calculated. In calculations without Everest, it was assumed that a susceptible cultivar took the place of Everest. Significant FHB occurred in all three eastern districts of Kansas. It was estimated that susceptible cultivars in those districts sustained 27.2% loss during 2015. Significant losses also occurred in the northcentral district where losses on susceptible cultivars were estimated to be 13.6%. FHB was a minor problem in the other five districts. Statewide losses of 11.69 million bushes were estimated for 2015. This represented a 3.4% loss when compared with the 334.4 million bushel production for the entire state. When Everest was replaced with a susceptible cultivar in the calculations, it was estimated that there would have been a loss of 16.58 million bushels or 4.8% loss. The cash grain price at harvest time in Kansas was about $5.25 per bushel. Therefore, the resistance level in Everest saved $25.7 million during 2015.

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QUANTITATIVE TRAIT LOCI ASSOCIATED WITH RESISTANCE TO FUSARIUM HEAD BLIGHT IN A CARBERRY X VESPER DERIVED WHEAT POPULATION

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum*, is a serious disease often inflicting losses through reduced grain yield and quality in wheat. Deployment of FHB resistant cultivars is an economical and environmentally friendly method of controlling the disease. This study was conducted to identify loci associated with FHB resistance in adapted Canada Western Red Spring (CWRS) wheat cultivars. A set of 180 doubled haploid lines developed from the cross of Carberry by Vesper at the Swift Current Research and Development Centre, AAFC, Canada were evaluated for FHB incidence and severity in nurseries near Morden, MB and Bratt's Lake, SK. Genotyping was done using the Infinium iSelect 90K wheat assay, in which 6211 polymorphic SNPs were mapped to 29 linkage groups. A set of 688 non-overlapping markers were used for QTL analysis. Four QTL with significant effects were detected on 1A, 2B, 4B and 6B chromosomes. Resistance alleles for the 1A and 4B QTL were contributed by Carberry and the 2B and 6B QTL were contributed by Vesper. Chromosome 1A and 4B QTL were associated with FHB incidence and severity, the 2B QTL with FHB incidence and the 6B QTL with FHB severity. Among the four QTL, the minimum phenotypic variation explained for FHB incidence was 6.2% and for FHB severity 5.6%. The 1A and 4B QTL seem to be more stable as they were detected in more than one location. This information will be valuable in maker assisted breeding for FHB resistance.

UTILIZING GENOMIC SELECTION TO ACCELERATE THE PACE OF DEVELOPING RESISTANT VARIETIES

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ABSTRACT

Fusarium Head Blight (FHB, Fusarium head scab) is a major disease caused by *F. graminearum* that infects wheat (*Triticum aestivum* L.) and other cereals. One major aspect for managing FHB in wheat is breeding for resistant varieties. However, evaluating FHB within a breeding program takes a large amount of resources. Marker assisted selection (MAS) has been effective for a few QTL, but most of the genes controlling resistance are not affected by traditional MAS. Genomic selection (GS) is a new form of MAS and can facilitate breeding for complex traits by estimating all marker effects simultaneously and predicting the genomic estimated breeding values (GEBVs). GS has the potential to increase the genetic gain per year by decreasing the time per cycle. The challenge remains now in implementing GS and identifying the model with the highest prediction accuracy for each trait. We evaluated the prediction accuracy of GS in a population of 640 soft winter wheat lines. The population was evaluated in inoculated FHB nurseries in multiple environments for incidence (INC), severity (SEV), index (IND), *Fusarium*-damaged kernel (FDK), kernel damage index (ISK), and deoxynivalenol concentration (DON). Across all traits we observed high entry-mean heritability (0.88 to 0.93) and trait correlations (0.63 to 0.98). Principal component and Fst analysis support a population stratification of 3 subgroups. Ten-fold cross validation prediction abilities ranged from 0.45 (INC) to 0.57 (SEV). Similar prediction accuracies were obtained within clusters but were much lower when data from one cluster was used to predict another. Eliminating the top 10-15% less predictable individuals increased prediction accuracy by up to 58%. Although accuracy was dependent on population size, accuracies similar to those obtained using elimination approach could be obtained with smaller sample sizes using two optimization approaches (coefficient of determination and predicted error variance). The results from this work will facilitate GS implementation and the identification of the best lines for selection and crossing for FHB resistance within this population.

MAPPING QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN CHINESE WHEAT LANDRACE HAIYANZHONG

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is a devastating disease in wheat (*Triticum aestivum L.*). FHB epidemics reduce not only grain yield, but also grain quality. Use of host resistance is one of the most effective strategies to minimize the disease damage. Haiyanzhong (HYZ) is a Chinese wheat landrace that shows a high level of resistance to FHB type II resistance. To map the quantitative trait loci (QTL) in HYZ and identify markers tightly linked to the QTL for FHB resistance, we genotyped 186 recombinant inbred lines (RILs) derived from a cross between HYZ and Wheaton, a susceptible cultivar, using simple sequence repeat (SSRs) and single-nucleotide polymorphisms (SNPs) derived from genotyping-by-sequencing (GBS). The population was phenotyped for percentage of symptomatic spikelets (PSSs) per spike in three greenhouse experiments using single-floret inoculation in spring and fall 2012 and spring 2013. A GBS library was constructed for 186 RILs and both parents using *PstI* and *MspI*. The library was then sequenced in an Ion Proton Sequencer. GBS data analysis was performed using UNEAK and independent reference pipeline of TASSEL. A total of 21,740 GBS-SNPs were called with 80% missing, but only 6232 showed 20% or less missing data, thus were used together with 132 SSRs to construct a linkage map for QTL mapping. A total of eight QTL were identified, and six of them were from HYZ and two from Wheaton. SNP *GBS3127* and SSR *Xbarc316* on the chromosome 5AS flanked a major QTL for FHB resistance at a 1.9-cM interval. Other SNPs linked to six minor QTL were identified on the chromosomes 6B, 2B (2), 3B, 4B and 4D. Ten GBS-SNPs tightly linked to the QTL on the chromosomes 5A, 6B and 2B-2 were validated using Kbioscience allele-specific polymorphism (KASP) assays in the mapping population. The ten KASP assays were also validated in a set 96 U.S elite winter wheat breeding lines and cultivars. Five of them, *GBS3127*, *GBS5669*, *GBS0158*, *GBS1852* and *GBS4305,* had the alleles different from these of HYZ in most of U.S. elite winter wheat lines, suggesting these SNPs are useful for transferring these QTL from HYZ into U.S. wheat through marker-assisted selection.

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.

ASSESSMENT OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN SPRING WHEAT LINES GROWN IN THE PACIFIC NORTHWEST AND CIMMYT

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ABSTRACT

Fusarium head blight is one of the destructive diseases of wheat in humid and semi-humid areas of the world. It has emerged in the Pacific Northwest (PNW) in recent years because of changing climate and rotation practice. The objectives of the present study were to characterize FHB resistance in spring wheat lines grown in PNW and CIMMYT and identify QTL associated with FHB resistance using SNPs and markers for two major dwarfing (*Rht1* and *Rht2*) and one for photo responsive (*PPD-D1*) genes. A total of 190 spring wheat cultivars and lines were evaluated in two field experiments in Minnesota, two field and one greenhouse experiments in Aberdeen, ID in 2015. One spring wheat line had disease severity less than 25% in all five data sets, four lines in four out of five data sets, and twenty-four lines in three out of five data sets in 190 lines evaluated. These lines have no Sumai 3 or related backgrounds and can be the starting resource to develop FHB resistant cultivar for the PNW areas. A subset of 134 lines was classified into four groups (*rht1Rht2*PS, *rht1Rht2*PI, *Rht1rht2*PS, *Rht1rht2*PI) based on marker alleles of the two dwarfing (*Rht1* and *Rht2*) and one photo responsive (PS, sensitive; PI, insensitive) genes. Two groups (*rht1rht2*PI, *Rht1Rht2*PS) were excluded in the analysis because of too few lines in each group. The results showed that the mean field incidence of lines in the groups of *rht1Rht2*PS and *Rht1rht2*PS was 10% lower than that of lines in the groups of *rht1Rht2*PI and *Rht1rht2*PI; this difference might be confounded by the differences in heading date of the lines evaluated. The mean field incidence and severity of lines with dwarfing allele at *RhtB1* locus (*rht1Rht2*PI and *rht1Rht2*PS) were 5 to 7% lower than that of lines with dwarfing allele at *RhtD1* locus (*Rht1rht2*PS and *Rht1rht2*PI). This suggests that FHB resistance can be manipulated through selection of the best combination of the three genes in adapted environments. Preliminary QTL associated with the five disease data sets are being identified and will be presented in the final poster.

CHARACTERIZATION OF FHB RESISTANCE IN SIX-ROW, WINTER BARLEY GERMPLASM

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ABSTRACT

Winter barley would provide a profitable cover crop to use in a double or relay cropping system with soybean in Minnesota. For this cropping system to work, improved winter barley varieties must be developed. In that vein, we have conducted several cycles of genomic selection for an index trait to improve winter barley for winter survival, heading date, plant height, grain yield, and malt extract. Fusarium head blight (FHB) resistance was not a component of this index trait but would be important to ensure that the developed winter barley lines were viable and profitable. The parent lines for the genomic selection breeding program were a combination of facultative and winter lines and a set of spring lines that contributed FHB resistance. The parent lines were genotyped with the Infinium iSelect single nucleotide polymorphism (SNP) assay markers, and the progeny from each cycle of selection were genotyped with a custom assay of 384 SNP markers. For this research, our objectives were to 1) quantify population structure in the genomic selection population using principal components analysis (PCA) of the genotypic marker data; 2) determine the amount of genetic variation present for FHB resistance and two related traits, heading date and plant height; and 3) identify quantitative trait loci (QTL) associated with FHB resistance, heading date, and plant height using association mapping. We evaluated lines from cycle 0 (parents) through cycle 2 of our genomic selection breeding program for FHB severity in three inoculated trials, for heading date in six trials, and for plant height in five trials. Based on a biplot of the first two principal components, we observed that while population structure was not present, the genetic variance of the population decreased over cycles of selection and the population shifted toward more similarity with the winter parent lines. All three traits assessed showed significant genetic variation, allowing us to conduct association mapping for each trait. We detected QTL for FHB resistance on chromosomes 1H and 7H. While the FHB resistance QTL on 7H coincides with a QTL for heading date, the 1H QTL was not associated with other traits. Pending further investigation of this QTL, it could prove useful in developing FHB resistant winter barley lines. Though this population exhibited genetic variation for FHB resistance, just one QTL was found indicating that the trait is quantitative in nature. Accordingly, we would plan to use genomic prediction to breed for this trait.

ACKNOWLEDGEMENTS AND DISCLAIMER

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MAPPING QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN HARD WINTER WHEAT OVERLAND

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ABSTRACT

Fusarium head blight (FHB) is a ravaging disease of small grain crops grown in humid and semi-humid areas of the world. FHB epidemics are sporadic in nature, but the affected crops face serious setback once epidemics occur. Among many approaches that are proposed to combat the disease, growing resistant cultivars is the most effective one to minimize disease damage. Overland is a hard winter wheat cultivar released from University of Nebraska, and shows moderate resistance to FHB. To dissect the quantitative trait loci (QTL) that control FHB resistance in Overland, 186 $F_{5:6}$ recombinant inbred lines (RILs) were developed by a cross between Overland and a highly susceptible cultivar Overley from Kansas. The RILs were inoculated by injecting a conidial suspension of *F. graminearum* (field isolate GZ 3639 native to Kansas) into a central spikelet in a spike in the greenhouses at Kansas State University, Manhattan KS. FHB resistance was measured as percentage of symptomatic spikelets (PSS) in an inoculated spike 12d after inoculation. The greenhouse experiments were conducted twice in fall 2014 and spring 2015. The RILs were also evaluated for FHB resistance in the FHB field nursery at Rocky Ford, Manhattan in 2015 using a grain spawn inoculation method. PSS was recorded 3 weeks after flowering. *Fusarium*-damaged kernels (FDK) was also scored for field harvested plants. Mean PSS in this population ranged from 25%-90%, while the mean FDK in the samples harvested from the field plots ranged from 30%-95%. The parents had significant difference in FDK, with 42.5% for Overland and 82.5% for Overley. In the field experiment, the correlation between mean PSS and FDK was 0.68. Both the parents and all the RILs were subjected to analysis of genotyping-by-sequencing (GBS) to discover SNPs tightly linked to QTL for FHB resistance in Overland. GBS was run in a Ion Proton sequencer in USDA Genotyping Lab at Manhattan, KS and SNPs were called using TASSEL pipeline as described by Poland *et al* 2012. A linkage map consisting of 3079 SNPs was constructed using JoinMap 4.1 and QTL analysis was done using ICIMapping software version2.3. Three QTL were detected from the field experiment and one from the greenhouse experiments which explained 15%, 10%, 8.5% and 8.22% of the phenotypic variation, respectively. Two QTL were detected for low FDK explaining 7% and 14% of the phenotypic variation. The QTL on 4D was significant for low PSS in both greenhouse and field experiments and low FDK in the field experiment. The experiments are being repeated in both the greenhouse and field experiments to further confirm these QTL. The results will help us to understand the resistance in Overland and to develop breeder-friendly markers for the QTL.

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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.

RESPONSE OF A COLLECTION OF WAXY (REDUCED AMYLOSE) WHEAT BREEDING LINES TO *FUSARIUM GRAMINEARUM*

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ABSTRACT

Loss of function mutations in the *Waxy* (*Wx*) gene encoding granule bound starch synthase I (GBSSI) that synthesizes amylose, result in starch granules containing mostly amylopectin. Wheat grain with this trait has increased usability for some foods due to the ability to modify starch composition and nutritional value in the end product. However, impaired GBSSI activity may alter grain and starch structure and, consequently, responses to pathogens. There are no published reports on response of *waxy* wheats to Fusarium head scab. A screen of colonization by *Fusarium graminearum* of *waxy* breeding lines and wild-type and *waxy* checks was conducted at Mead, NE, 2014. Grain was either surface disinfested before plating, or directly plated, onto medium semi-selective for *Fusarium* spp., indicating internal or both internal and superficial infections, respectively. Grains with fungal growth were enumerated for each line and grain treatment. Non-disinfested *waxy* grains (69.5%) were significantly less colonized as compared with wild-type (78.9%) ($P < 0.01$). Surface disinfested grains of both phenotypes had similar levels of infection (14.4% for wild-type *versus* 10.0% for *waxy*; $P = 0.07$). Fungal colonies growing onto the medium were transferred and morphologically identified as similar to *Fusarium graminearum*, *Fusarium* spp. or other fungi. Along with *F. graminearum*, *F. verticillioides, F. equiseti* and *F. acuminatum*, were common in wild-type grain, while the most commonly detected species in *waxy* grain was *F. proliferatum.* These preliminary results indicated that *waxy* wheats are not more susceptible to *F. graminearum* than wild-type. Analyses of mycotoxins such as deoxynivalenol will be needed to confirm whether these promising *waxy* lines in development are not more susceptible to *F. graminearum* than non-*waxy* lines.

GENETICS OF FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY: THE WAY FORWARD

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ABSTRACT

Fusarium head blight (FHB) is a perennial problem in many parts of the world, particularly in regions with environmental conditions suitable to disease development. Genetic resistance is considered one of the most cost-effective and environmentally friendly ways to control plant disease, but a global screening of 23,000+ barley accessions has so far yielded very few accessions with adequate levels of resistance and no truly immune accessions. Moreover, quantitative trait locus (QTL) mapping studies spanning two decades of intensive research have so far identified QTL with very small effects ($R^2 < 0.10$). In these previous mapping studies, the most significant QTL are associated with segregating agro-morphological traits, including row type, heading date, and cleistogamy. In an attempt to introduce potentially novel resistance alleles into adapted germplasm, wild barley accessions PI 466423 and W-365 and landrace Kutahya were crossed to Midwest cultivars Rasmusson or Quest. However, every major QTL detected in these three populations was actually contributed by the recurrent parent, which may be attributed to extensive efforts to breed for the distinct, but related, disease of kernel discoloration (KD) using Chevron as source of resistance. The largest effect QTL that we identified is located in chromosome 2H bin 4. This locus is likely novel for FHB resistance, but evidence suggest that we are detecting the photoperiod response gene *Ppd-H1*. Further, the allelic effect (α) varied between environments for this locus as well as other loci, so it may not be suitable for marker assisted selection (MAS). Biotechnological approaches could be used to introduce resistance in the absence of natural variation, but success in this area has also been limited. Techniques such as genomic selection (GS) offer a greater promise for improving upon presently-available FHB resistance. Ultimately, management of FHB will result from incremental improvements in genetic resistance, management of crop residues, and judicious use of fungicides.

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DEVELOPMENT OF ADVANCED CIMMYT WHEAT BREEDING LINES COMBINING *FHB1* AND *SR2*

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ABSTRACT

Fusarium head blight (FHB) and stem rust are two major wheat diseases worldwide and both are breeding targets of the breeding programs at CIMMYT. *Fhb1* confers consistently major effects on FHB resistance and deoxynivalenol (DON) content and played important roles in CIMMYT's early FHB resistance breeding activities. However, due to its tightly repulsive linkage with *Sr2* for adult plant stem rust resistance, *Fhb1* has been gradually lost due to the consistent selection of durable stem rust resistance. In order to get back *Fhb1*, germplasm with *Fhb1* and *Sr2* in coupling linkage needs to be utilized to meet the breeding requirements of both diseases. Using marker-assisted selection, four recombinant lines were developed in the background of the Australian cultivar Hartog (also known as Pavon) at CSIRO, Australia and introduced into the CIMMYT breeding program. The lines were crossed and backcrossed with seven recent CIMMYT bread wheat parents and one durum line. Pseudo-black chaff, a morphological trait tightly linked to *Sr2*, was used to retain *Sr2* during backcrossing. To identify the recombinants, the eight populations were genotyped with linked SNP and SSR markers from the *Fhb1* and *Sr2* region: wMAS000005 (based on csSr2), wMAS000008 (based on snp3BS-8), barc102, umn10, wms533, barc133 and wms493. In 2013, 264 F7 derived lines were screened for FHB resistance and 76 lines with good FHB resistance and *Fhb1- Sr2* haplotypes were selected for field and greenhouse evaluations in 2014. Based on both Type I and Type II FHB resistance and the haplotype data, 30 lines were selected for further testing. The 30 genetically diverse lines were derived from 16 different crosses and were evaluated in 2015 for FHB, yellow rust, Septoria tritici blotch, tan spot, spot blotch, and Stagonospora nodorum botch at CIMMYT, Mexico. They will also be tested for stem rust resistance in Kenya. Lines showing broad spectrum of resistance to tested diseases have been identified. Utilization of these lines in breeding programs has been initiated via marker-assisted backcrossing and will greatly facilitate the development of wheat cultivars with improved resistance to FHB and stem rust simultaneously.

FUSARIUM HEAD BLIGHT RESISTANCE LINES IDENTIFIED IN PRELIMINARY EVALUATION OF USDA-ARS BARLEY BREEDING MATERIALS IN IDAHO

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ABSTRACT

Fusarium head blight (FHB) disease is very harmful to barley yield, quality, and commercial value. With the climate change and more corn being planting, FHB infected plants have been sporadically identified in Idaho barley fields in more recent years. That is alarming for future production problems in a high quality barley production state. As the first step to deal with the potential disease, we screened 100 lines of our breeding materials in two screening nurseries of North Dakota State University in 2014. Preliminary results from two North Dakota locations in 2014 identified resistance lines when measuring infection rating and DON levels in seed. Comparing to the 2-row resistance check of Conlon, eight of 100 lines have lower DON content in all of four replications, while 21 lines have low DON than Conlon if the average DON level from four replications is used in the comparison. The 2015 Aberdeen nursery also showed a good level of infection. Early analyses of the results indicate that Aberdeen's elite breeding lines contain useful genetic resistance resources. This will greatly aid the breeding program in rapidly developing FHB-resistant cultivars.

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ENHANCING TOOLS FOR *FUSARIUM* RESISTANCE BREEDING IN CANADIAN WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) remains one of the most important diseases affecting wheat production worldwide. FHB damages multiple aspects of the wheat crop including grain yield, grade, and quality for end-use and propagation. The presence of mycotoxins, particularly deoxynivalenol (DON), will result in downgrading and at levels over 1 ppm can render the grain unsuitable for human and livestock consumption. In Canada, FHB associated crop losses in the 1990s exceeded $500 million in eastern Canada and the eastern Prairies where this disease is particularly prevalent. The use of resistant cultivars is central to integrated management approaches for control of FHB. The objective of this research is to enhance three components within the eastern Agriculture & Agri-Food (AAFC) winter wheat breeding program that facilitate the development of FHB resistant cultivars: (i) development and deployment of novel germplasm including wheat species to access untapped FHB resistance; (ii) establishment and maintenance of a robust FHB screening nursery and the highly trained personnel for phenotypic characterization of field symptoms and DON determination; and (iii) high-throughput molecular breeding strategies that enable the genotyping of promising germplasm including breeding lines and parental materials used in marker-assisted backcrossing.

ENHANCING FHB RESISTANCE IN DURUM WHEAT

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ABSTRACT

Durum wheat (*T. turgidum* L. var. *durum* Desf.), which is grown primarily in North Dakota, has been heavily impacted by Fusarium head blight (FHB). Thus, it is critical to identify means of defeating this disease or reducing its pathogenic effect to enhance durum wheat production. Whole genome association analysis of various Tunisian derived tetraploid sources of resistance revealed a significant region on 5BL (*Qfhs.ndsu-5BL*), which was further confirmed by traditional QTL analysis in a bi-parental population.

Further analysis using two additional Tunisian-derived advanced backcross populations, Tun 108/Lebsock//Lebsock and Tun 108/Ben//Ben, screened for FHB resistance revealed novel regions on 2BL (*Qfhb.ndsu-2BL*) and 5AL (*Qfhb.ndsu-5AL*). The 2BL region provides resistance to multiple FHB components including severity, incidence, mycotoxin production and frequency of damaged kernels while 5AL segment provides resistance to severity of infection. We have been developing KASPar SNP markers, for ease of introgression, and pyramiding these regions into a single cultivar to assist durum breeding programs in their effort to breed for more resistant cultivars.

Additionally, we treated six advanced durum breeding lines with 5-methyl-azacytedine that removes CG methylation. The resulting lines were advanced to the M_4 generation and tested for FHB resistance under greenhouse and field conditions. Twenty four lines were identified that show great promise having less than 20% severity as compared with 80-100% severity for parental lines and susceptible checks. We have further advanced these 24 lines, crossed the most promising to the parental cultivars to test the stability and inheritance of resistance, and are analyzing them further to determine what epigenetic changes are responsible for the enhanced FHB resistance. These could potentially be used as new sources of resistance for the breeding programs.

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INCORPORATION OF GENOMIC SELECTION INTO THE UNIVERSITY OF ILLINOIS' SOFT RED WINTER WHEAT BREEDING PROGRAM

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ABSTRACT

Breeding for resistance to Fusarium head blight (FHB), a destructive disease of wheat (*Triticum aestivum* L.), is challenging due to its quantitative inheritance and difficulties in obtaining high quality phenotypes. At the University of Illinois soft red winter wheat breeding program, phenotypic selection and early-generation enrichment by marker assisted selection (MAS) have been the main strategies for selecting individuals with higher levels of resistance. More recently, genomic selection (GS) models have been tested for predicting multiple traits associated with FHB resistance. GS is currently being implemented at two stages of the breeding program; for selection of parents and at the preliminary yield trial (PYT) stage.

Genotyping-by-sequencing (GBS) was used to identify a subset of ~ 20,000 informative SNPs across a panel of 273 diverse soft red winter wheat breeding lines. These SNPs were used to compare several MAS and GS model predictions for six FHB related traits (INC, SEV, FHBndx, FDK, ISKndx, and DON). Phenotypic data were obtained in a scab nursery in Urbana, IL in 2011, 2013, 2014, and best linear unbiased predictors (BLUPs) were obtained from a mixed model. SNP effects were estimated using ridge regression-BLUP in the R package PopVar. The analyses were performed using a four-fold cross validation approach. In all cases GS models outperformed the MAS models with prediction accuracies ranging from 0.58 (SEV) to 0.88 *Fusarium*-damaged kernels (FDK).

In a second stage, GS was applied to the PYT lines, consisting of 400 $F_{3:6}$ entries, using 6850 informative SNPs. These PYT lines were grown at four locations throughout Illinois in 2015 and two locations in 2014, including the scab nursery in Urbana. Yield (YLD) and test weight (TW) were obtained for each line. Genomic prediction accuracy for YLD and TW were 0.34 and 0.45 respectively. A combination of genotypic and phenotypic values are used to calculate genomic estimated breeding value (GEBV) predictions for all traits except DON. Typically, DON data from the current year are not available before the following cycle of planting, making GS a valuable tool for selection of resistant lines. GEBVs for DON are calculated from the training population (TP), which consists of those lines previously phenotyped for DON, including lines from the advanced yield trials (AYT) and previous PYTs. About 20% of the lines from PYT are selected to be in the AYT.

Initial results show the implementation of GS into the breeding program can be a useful tool for prediction of several traits and development of high yielding cultivars with FHB resistance. Incorporation of GS into earlier stages of the breeding program will continue as methods are developed to genotype an increasing number of lines while keeping costs low.

CHARACTERIZATION OF FHB RESISTANCE QTL IN SRW WHEAT CULTIVAR TRIBUTE

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ABSTRACT

Pyramiding genes from exotic and native sources would be an effective approach to enhance resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum*. The objectives of the study were to identify the FHB resistance QTL in the native soft red winter (SRW) wheat cultivar Tribute and develop diagnostic markers for use in marker-assisted breeding. A total of 114 double haploid (DH) lines, developed at NCSU, were evaluated for FHB incidence and FHB severity by cooperators in AR, KY, MD, NC, and VA during 2013 and 2014 (except MD). Grain samples from each location were visually assessed for *Fusarium*-damaged kernels (FDK) and analyzed for deoxynivalenol (DON) toxin content. The population was also evaluated for type II resistance to disease spread in the greenhouse at Virginia Tech. A set of SSR and 90K SNP markers were used to genotype the mapping population. Genotype-by-location interaction was significant for the population. Composite interval mapping identified five putative QTL on chromosomes 1A, 2A, 2D, 3BS, and 7B for FHB incidence, FHB severity, FDK, and DON content. Putative QTL for FHB resistance were detected on 1A, 2A, and 3BS, whereas putative QTL for FHB susceptibility were detected on 7B. Both FHB resistance and FHB susceptibility were associated with the QTL on 2D. The putative QTL for FHB on 2A and 2D were linked to loci governing heading date and flowering date across locations, whereas the putative QTL for FHB on 1A was linked to 1A.1R translocation. The variation explained by putative QTL on 1A, 2A, 2D, 3BS, and 7B was 8.2% to 27.8% (Additive = -1.9 to -11.3), 4.8% to 24.0% (Additive = -0-0.5 to -13.0), 9.1% to 38.8% (Additive = -10.8 to 14.4), 0.1% to 21.8% (Additive = -3.6 to -10.3), and 7.3% to 17.0% (Additive = 2.5 to 6.5), respectively. The diagnostic markers are IWB49926 (1A), IWB4499 (2A), Xgwm261 (2D), IWB7909 and Xbarc164 (3B), and IWB55522 and IWA1089 (7B). The diagnostic markers could be utilized in marker-assisted breeding in wheat breeding program.

FHB RESISTANCE AND AGRONOMIC PERFORMANCE IN GEORGIA SOFT RED WINTER WHEAT GERMPLASM

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ABSTRACT

Development of resistant wheat cultivars is the most efficient approach to control Fusarium head blight (FHB). Local broadly adapted cultivars of soft red winter wheat (SRWW) have been crossed with *Fhb1* derived lines, Truman, Neuse, and Jamestown to introduce FHB resistant QTL into adapted SRWW genetic backgrounds. Elite lines with FHB resistance derived from Truman/Bess, Neuse, MD08-27-E9 and Jamestown, were evaluated under Georgia's field conditions during 2015 for FHB resistance and agronomic performances. Several elite lines have been identified with good FHB resistance derived from Jamestown. GA061050-13ES17 (AGS 2020/Jamestown), GA051207-13ES11 (AGS 2000 / SC996284 // IN981359C1), GA08250-14ES7 (Jamestown/GA991336-6E9), and GA 071171-14ES8 (Jamestown/GA991371-6E12) had similar FHB ratings as Jamestown for incidence, index and ISK. GA061050-13ES17 has the QTL 1A from Neuse, and 2B and 3B QTL from Bess; GA051207-13ES11 has the QTL 1A and 6A from Neuse; GA08250-14ES7 has the QTL 1B and 6A from Jamestown; and GA 071171-14ES8 has the QTL 1B from Jamestown, and 5A from Ernie. Other elite lines with moderate levels of FHB resistance derived from Jamestown, Ernie, or Neuse were identified with high grain yield potential and will be further evaluated. Similarly, double haploid lines, NC 10014 (NC 06-198-96/NC 08-140) and NC 9337 (Jamestown/S8641) showed a high level of FHB resistance and high yield performance.

THE 2014-15 SOUTHERN UNIFORM SOFT RED WINTER WHEAT SCAB NURSERY

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ABSTRACT

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', 'Bess' and 'Jamestown'. Valuable data are provided on resistance to other important fungal and viral diseases, milling and baking quality and agronomic characteristics. In addition, genotypic analyses identify alleles present at numerous important loci. The nursery is the primary method to facilitate the sharing of the best resistant materials throughout the breeding community.

The 2014-15 nursery comprised 45 advanced generation breeding lines and four check cultivars, Ernie, Bess, Jamestown (partially resistant) and 'Coker 9835' (susceptible). Five U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State University, N.C. State Univ., and VA Tech.), and three private companies (Agripro-Coker, KWS, and Limagrain) submitted entries. The nursery was evaluated at 11 locations in AR, MO, KY, IL, VA, NC, GA, and LA for field, and/or greenhouse evaluations. In addition, three USDA-ARS laboratories conducted evaluations for Hessian fly resistance, milling and baking quality and marker genotypes.

The mean level of FHB resistance in the nursery was high. Between 87 and 93 percent of entries had significantly better means than the susceptible check for severity and FHB index. DON data are still being reported. Sources of resistance included Chinese and North American germplasms.

Copies of the full report will be available at the 2015 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

ACKNOWLEDGEMENT AND DISCLAIMER

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Table 1. Means across locations and genotypic content of regions associated with FHB resistance.

	Cultivar/ Designation	FHB Incidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	DON	RANK
1	ERNIE	58	11	36	28	19	16	34	29	36	18	9	23
2	COKER9835	86	49	68	49	55	49	57	49	61	49	13	42
3	BESS	55	8	25	7	15	10	27	15	36	18	6	7
4	JAMESTOWN	54	5	30	12	17	13	23	6	34	13	6	7
5	LA07085CW-P4	62	20	38	35	22	23	43	43	41	32	9	23
6	LANC8170-41-2	69	36	40	38	28	41	30	16	42	35	5	3
7	NC11-22289	50	1	23	5	13	4	26	14	26	2	5	3
8	AR06024-7-2	57	10	21	4	12	3	18	2	27	4	4	2
9	AR06037-17-2	78	47	34	22	26	37	31	18	41	32	10	30
10	AR06045-2-4	54	5	20	2	9	2	25	10	26	2	8	17
11	AR06045-16-4	59	12	24	6	13	4	24	7	31	6	8	17
12	AR06046-10-3	62	18	31	16	20	18	32	20	36	18	13	42
13	AR06061-11-1	61	15	26	8	17	13	20	3	31	6	6	7
14	LW08190C-57-3	64	22	30	12	20	18	33	24	34	13	5	3
15	ARGE08-1398	51	2	14	1	8	1	11	1	22	1	2	1
16	B12*1792	66	29	41	39	28	41	32	20	42	35	13	42
17	B12-2180NC#	78	47	33	20	25	31	25	10	41	32	9	23
18	GA 071171-14ES8	64	22	41	39	25	31	30	16	40	28	9	23
19	GA 071092-14ES11	67	32	36	28	22	23	34	29	34	13	11	38
20	GA071092-14ES13	66	29	39	37	25	31	35	33	37	21	11	38
21	GA 081129-14ES16	64	22	36	35	23	31	36	35	40	28	8	17
22	GA 08250-14ES7	62	18	31	16	20	18	32	20	32	12	9	23
23	GA 08250-14ES5	62	20	36	28	22	23	34	29	38	23	10	30
24	GA 071171-14ES19	67	32	35	26	24	30	31	18	37	21	11	38
25	GA 081562-14ES14	74	43	34	22	26	37	42	41	43	39	14	45
26	KWS 054	66	29	30	12	22	23	45	46	42	35	10	30
27	LA06146E-P4	59	12	32	18	19	16	42	41	44	41	11	38
28	LA08265C-50	71	39	42	42	35	46	33	24	48	45	7	11
29	LA09144C-6	74	43	51	48	36	47	43	43	47	44	16	47
30	LANC8248-1	76	45	49	47	40	48	25	10	48	45	8	17
31	ES13-1591	55	8	29	10	15	10	34	29	31	6	6	7
32	ES13-3423	52	3	36	28	14	7	40	40	34	13	9	23
33	ES12-3030	65	27	36	28	23	29	33	24	39	27	7	11
34	M11-2024#	65	27	37	34	27	39	24	7	40	28	10	30
35	M12-3301	61	15	34	22	16	12	33	24	31	6	8	17
36	M12-2036#	54	5	26	8	14	7	33	24	38	23	10	30
37	NC11-23084	67	32	42	42	22	23	35	33	38	23	10	30
38	NC12-23576	69	36	41	39	27	39	37	36	43	39	14	45
39	NC12-23219	64	22	33	20	25	31	24	7	38	23	7	11
40	NC12-20662	60	14	35	26	14	7	32	20	31	6	7	11
41	NC9305-7	64	22	30	12	20	18	20	3	34	13	7	11
42	VA11W-106	69	36	32	18	22	23	39	38	42	35	16	47
43	VA11W-313	71	39	42	42	29	43	47	47	45	43	10	30
44	VA12W-72	72	41	34	22	25	31	55	48	50	48	16	47
45	VA12W-54	72	41	44	45	30	44	44	45	49	47	9	23
46	VA12FHB-53	61	15	29	10	18	15	38	37	40	28	10	30
47	VA12FHB-4	68	35	36	28	21	22	25	10	31	6	7	11
48	VA13W-177	53	4	20	2	13	4	21	5	27	4	5	3
49	VA08MAS5-39-6-4	76	45	47	46	32	45	39	38	44	41	8	17
	Mean	64		34		22		33		38		9	
	LSD (0.05)	26		26		22		24		18		7	
	CV%	20.7		38.3		50.4		36.8		24.0		37.7	

Table 1 Cont.

	Cultivar/ Designation	Heading Date	RANK	Plant Height	RANK	Flour Yield %	RANK	Softness Equivalent %	RANK	Hessian Fly Biotype L	*Fhb1*	*Fhb Massey 3BL*	*Fhb 5A*	*Fhb 2DL-Wuhan1/W14*	*Bess 2B*	*Bess 3B*	*Jamestown 1B*	*Jamestown 6A*	*NC-Neuse 1A*	*NC-Neuse 6A*
1	ERNIE	129	12	32	16	67	30	55	36	0-15	no	3BL?	het	no	no	no	no	no	yes	yes
2	COKER9835	131	40	31	11	68	18	65	1	0-15	no	no	no	no	no	no	no	no	.	no
3	BESS	130	24	35	42	67	30	61	12	0-15	no	no	no	no	yes	yes	yes	no	.	no
4	JAMESTOWN	128	5	32	18	68	18	59	17	0-14	no	no	no	no	no	no	yes	yes	yes	no
5	LA07085CW-P4	128	5	33	28	69	13	63	7	0-17	no	no	no	no	no	no	no	no	.	no
6	LANC8170-41-2	130	24	31	12	66	41	50	48	11-0	Fhb1	no	no	no	no	no	yes	yes	yes	no
7	NC11-22289	128	5	35	43	66	41	52	45	0-17	no	no	no	no	no	no	yes	no	.	yes
8	AR06024-7-2	130	24	36	49	66	41	56	33	0-15	Fhb1 het	no	no	no	no	no	yes	no	yes	no
9	AR06037-17-2	132	48	30	2	68	18	59	17	0-18	no	no	no	no	no	no	no	no	.	no
10	AR06045-2-4	130	24	36	47	68	18	62	10	0-12	no	no	no	no	yes	yes	yes	no	no	no
11	AR06045-16-4	130	24	35	45	68	18	62	10	0-13	no	no	no	no	.	yes	yes	no	no	no
12	AR06046-10-3	130	24	33	26	68	18	58	23	0-14	no	no	no	no	no	yes	yes	no	no	no
13	AR06061-11-1	131	40	35	44	67	30	63	7	0-14	no	no	no	no	yes	yes	yes	no	yes	no
14	LW08190C-57-3	129	12	32	19	67	30	58	23	0-11	Fhb1	no	no	no	no	no	no	no	yes	no
15	ARGE08-1398	130	24	36	48	67	30	58	23	0-14	Fhb1?	no	no	no	no	no	yes	no	yes	no
16	B12*1792	129	12	33	29	68	18	59	17	16-1	no	no	no	no	no	no	no	no	yes	no
17	B12-2180NC#	130	24	30	3	67	30	60	16	16-0	no	no	no	no	no	no	no	no	no	no
18	GA 071171-14ES8	130	24	35	40	70	5	57	30	9-8	no	no	het	no	no	no	yes	no	.	no
19	GA 071092-14ES11	130	24	32	17	71	1	61	12	0-17	no	no	no	no	no	no	yes	no	no	no
20	GA071092-14ES13	130	24	33	25	71	1	64	2	0-16	no	no	no	no	no	no	yes	no	no	no
21	GA 081129-14ES16	129	12	31	5	70	5	53	41	0-16	no	no	no	no	no	no	yes	no	no	no
22	GA 08250-14ES7	131	40	32	13	70	5	63	7	0-16	no	no	no	no	no	no	yes	yes	no	no
23	GA 08250-14ES5	131	40	34	39	69	13	61	12	0-16	no	no	no	no	no	no	no	no	.	no
24	GA 071171-14ES19	130	24	34	36	70	5	56	33	0-18	no	no	no	no	no	no	yes	no	.	no
25	GA 081562-14ES14	134	49	32	20	71	1	56	33	0-14	no	no	yes	no	no	no	no	no	yes	yes
26	KWS 054	128	5	31	6	69	13	64	2	0-14	no	no	no	no	no	no	no	no	yes	no
27	LA06146E-P4	130	24	34	31	68	18	50	48	0-14	no	no	no	no	no	no	yes	yes	yes	no
28	LA08265C-50	129	12	35	41	68	18	58	23	0-16	no	no	no	no	no	no	yes	no	yes	no
29	LA09144C-6	131	40	34	38	70	5	57	30	0-14	no	no	no	no	no	no	no	yes	no	no
30	LANC8248-1	130	24	29	1	71	1	52	45	0-12	no	no	yes	no	no	no	no	no	yes	no
31	ES13-1591	127	2	34	33	69	13	61	12	0-16	no	no	no	no	no	no	no	no	yes	no
32	ES13-3423	129	12	33	22	65	47	58	23	0-21	no	no	yes	no	no	no	no	no	yes	yes
33	ES12-3030	129	12	34	34	66	41	53	41	0-15	no	no	no	no	no	no	yes	no	yes	no
34	M11-2024#	129	12	31	7	68	18	52	45	0-17	no	3BL	no	no	no	no	no	no	yes	no
35	M12-3301	130	24	34	35	69	13	64	2	0-19	no	no	no	no	no	no	yes	no	no	no
36	M12-2036#	129	12	34	37	70	5	64	2	0-18	no	no	no	no	no	no	no	no	yes	no
37	NC11-23084	131	40	33	23	70	5	55	36	0-18	no	no	yes	no	no	no	yes	no	yes	yes
38	NC12-23576	130	24	33	27	67	30	53	41	0-20	no	no	no	no	no	yes	yes	no	.	no
39	NC12-23219	129	12	33	30	65	47	55	36	0-18	no	no	yes	no	no	no	no	no	.	no
40	NC12-20662	126	1	31	8	66	41	53	41	0-16	no	no	no	no	no	no	no	yes	.	no
41	NC9305-7	131	40	34	32	67	30	58	23	0-17	no	no	no	no	no	yes	no	no	yes	no
42	VA11W-106	131	40	32	15	67	30	64	2	0-20	no	no	no	no	no	no	yes	no	.	no
43	VA11W-313	128	5	31	9	67	30	54	39	0-16	no	no	no	no	no	no	no	no	no	no
44	VA12W-72	128	5	33	24	65	47	57	30	17-1	no	3BL	no	no	no	no	no	no	no	no
45	VA12W-54	128	5	31	4	68	18	58	23	21-0	no	3BL	no	no	no	no	no	no	.	no
46	VA12FHB-53	129	12	32	14	66	41	59	17	0-17	Fhb1 het	no	no	no	no	no	no	no	yes	yes
47	VA12FHB-4	129	12	33	21	67	30	59	17	0-16	Fhb1 het	no	no	no	no	no	no	no	.	no
48	VA13W-177	127	2	35	46	68	18	54	39	0-17	no	no	no	no	no	no	yes	no	.	no
49	VA08MAS5-39-6-4	127	2	31	10	70	5	59	17	0-16	no	no	no	no	no	no	no	no	yes	no
	Mean	129		33		68		58												
	LSD (0.05)	3		2		.		.												
	CV%	1.1		3.6		.		.												

MOISTURE CONTENT OF GRAIN SAMPLES AFFECTS THE PERFORMANCE OF NEAR-INFRARED SPECTROSCOPIC CALIBRATION FOR ESTIMATION OF DON LEVELS IN WHEAT

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ABSTRACT

We have developed a near infrared (NIR) spectroscopic calibration for the single kernel near infrared spectrometer (SKNIR) for estimation of deoxynivalenol (DON) levels in single wheat kernels. This SKNIR calibration for DON estimation is helpful for nondestructive, rapid estimation of DON levels in small grain samples in that some Fusarium head blight resistance components in wheat germplasm can be evaluated by analyzing the single kernel DON distribution patterns among kernels in grain samples. Further improvement of the robustness and accuracy of DON predictions of the calibration requires the study of the effects of known sources of variation in grain samples on the performance of calibration. Moisture variations in samples of numerous materials have been shown to influence the NIR predictions of composition and quality factors of materials. Moreover, our previous studies, as well as independent studies by other workers with NIR absorption of DON, have shown that the NIR absorption bands of DON are positioned near the NIR absorption bands of water. Since grain samples for DON measurement may have differences in moisture levels at the time of analysis, a study was conducted to investigate the influence of grain sample moisture levels on the accuracy of NIR predictions of DON levels. DON levels of small bulk samples of manually sorted visually sound, scabby, and unsorted grain samples of two wheat cultivars were estimated at four different moisture levels varying from 9.4 -18.2%. NIR predicted single kernel DON levels of individual kernels and average kernel weight of sound and scabby kernels were used to estimate the DON levels of the bulk grain samples. After DON analysis by the SKNIR, DON levels of the grain samples were determined by the standard laboratory method using gas chromatography - mass spectrometry. At higher moisture levels NIR predicted DON levels of scabby kernels were considerably lower while most scabby kernels were predicted as having no DON at moisture contents above 15%. NIR predicted DON levels of scabby kernels increased as moisture contents of the samples decreased. These results showed that moisture content variations of grain samples influence the accuracy of DON predictions of the grain samples. Therefore, appropriate strategies should be followed to mitigate the effect of variation of moisture levels of grain samples to facilitate improvement of the calibration performance.

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QTL ANALYSIS OF FHB AND DON ACCUMULATION RESISTANCE IN THE TURKISH LINE CGN00483

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ABSTRACT

One of the limiting factors in the development of barley varieties with lower DON accumulation is the availability of resistance sources in the primary barley germplasm pool. The screening of 1550 landraces collected from several countries located in the centers of diversity of *Hordeum* from the Dutch Centre for Genetic Resources, identified a 2-rowed accession collected in Turkey, CGN00483, consistently showing lower DON levels than Conlon. A CGN00483 X Harrington cross was made and advanced to the F_7 generation representing a recombinant inbred line (RIL) population consisting 170 individuals. The RIL population was genotyped using an Ion Torrent 384 SNP PCR genotype-by-sequencing marker panel placing 131 markers on a skeletal genetic map. Utilizing the map and the preliminary DON data collected from two nursery locations on the F_4 generation progeny we identified two putative low DON accumulation QTL at ~ 4 cM on chromosome (ch.) 4H and 17-32 cM on ch. 7H. The major DON QTL on ch. 4H is novel suggesting that CGN00483 contains a new source of DON accumulation resistance that has not been utilized in breeding programs. Further, marker saturation and DON analysis from several FHB nursery site years is being conducted on the RILs to further characterize these QTL. CGN00483 has also been crossed with the cvs Conlon and ND-Genesis and the advanced breeding line 2ND27705 and a backcrossing and MAS scheme will be utilized to introduce the QTL into the elite two-rowed backgrounds. Barley breeders would like to see the levels of DON in released lines lowered and this resistance stacked with other resistance sources already present in 2-rowed lines with lower DON accumulation will be further investigated.

QUANTITATIVE TRAIT LOCI ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN AN ARGENTINE WHEAT LINE

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OBJECTIVES

To characterize quantitative trait loci (QTL) associated with Fusarium head blight resistance of an experimental spring wheat line (AR5) from Argentina.

INTRODUCTION

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)], is a destructive disease of wheat (*Triticum aestivum* L.) in Argentina and nearly all temperate wheat-producing regions worldwide. FHB causes both severe yield reductions and decreases grain quality (McMullen et al., 1997). Additionally, infected grains may contain significant levels of mycotoxins such as deoxynivalenol (DON), that can prevent its use for human consumption or feed (Goswami and Kistler, 2004).

The use of host resistance is the most economically and environmentally sound solution to this problem (Zhang et al., 2008). The Argentine spring wheat line AR5 possesses resistance to FHB in field tests and could carry novel genes for FHB resistance (Staltari et al., 2014).

The objective of this study was to characterize QTL associated with different types of resistance, in a wheat recombinant inbred line (RIL) population developed from the cross of AR5 with the highly susceptible Sonalika.

MATERIALS AND METHODS

Plant Material - A RIL wheat population was produced from the cross of AR5 and Sonalika. AR5 (Laj1409/Laj2231//Klat/Pel73001/3/Laj1409/Pel74142//Lr/Pel73001) is a hard red spring experimental wheat line developed by the National Agricultural Technology Institute (INTA Marcos Juarez, Córdoba Province, Argentina). Sonalika is an Indian early maturity spring wheat line highly susceptible to FHB (Zhang et al., 2008).

The initial cross was performed in the greenhouse in the winter of 2008. A 135 RIL population was generated by single-seed descent in the greenhouse through the F6 generation. Further seed increases were also conducted in the greenhouse.

Disease Evaluation and FHB Inoculum - The field trial was sown in November 1 2011 at the Lower Eastern Shore Research and Education Center (LESREC), UMD, Salisbury, MD. The experimental design was a randomized complete block with three replications for 135 RIL and parental lines. Each plot was a 1-m-long row and the sowing date was 300seeds m^{-2}. Inoculum was prepared from a mix of different isolates obtained from symptomatic spikes collected from commercial wheat fields at various Maryland locations in 2011. *Zea mays* L. kernels were

autoclaved and colonized by *F. graminearum* following Zhang et al. (2008). The nursery was inoculated by spreading Fusarium-colonized kernels at a 100 kg ha^{-1} rate 24 d prior mean heading date. To promote production of ascospores, the plots were mist-irrigated daily for 3 min with a 30-min recess between 2000 and 0800 hours. Misting was stopped 27 d post-heading (Fuentes et al., 2005; Zhang et al., 2008). The RIL population and parental lines were evaluated for incidence (Type I resistance) and severity (Type II resistance), 3 weeks after anthesis (Bonin and Kolb, 2009). As plants reached maturity, 15 spikes per plot were hand-harvested and threshed manually with a head thresher. Percentage of *Fusarium*-damaged kernels (FDK) of each plot was determined by counting 200 random seeds from each sample to estimate thousand kernel weight (TKW). A 15-g seed sample from each plot was analyzed for DON concentration at the University of Minnesota (Dr. Yanhong Dong, Department of Plant Pathology) in 2012 (Zhang et al., 2008). In order to improve homogeneity of variance and normal data distribution, incidence, severity, FDK and TKW variables were transformed. DON data was transformed using a log (x + 1) transformation where x represented DON (μg g^{-1}), remainder using arcsine (√x/100) where x represented incidence, severity, FDK and TKW variables in percent.

Molecular Marker Genotyping – The parental lines and the 135 RIL population were screened for single nucleotide polymorphism (SNP) using the 9k SNP chip (Cavanagh et al. 2013) at the USDA-ARS Small Grains Genotyping Lab in Fargo, ND.

Linkage Map Construction and Quantitative Trait Locus Analysis - A linkage map was constructed using GQMol 2007.0.0 (http://www.ufv.br/dbg/gqmol/gqmol.htm) (Cruz and Schuster, 2007). Markers were grouped using a logarithms of odds (LOD) value of 3.0 and distance <30 cM. 316 polymorphic markers distributed regularly throughout the genome were selected and all of them showed expected (1:1) Mendelian segregation. From them, 26 linkage groups were formed. Using the consensus map constructed by Cavanagh et al. (2013), the linkage groups were placed on wheat chromosomes. The QTL analyses were conducted using composite interval mapping (CIM) and permutation test with GQMol 2007.0.0 (Cruz and Schuster, 2007).

RESULTS AND DISCUSSION

Field FHB Evaluation - The RIL population showed a wide and continuous distribution for incidence, severity, FDK, TKW and DON values. Transgressive segregants within the RIL population were observed for all disease traits (Figure 1).

QTL Analysis and Detection - Significant QTL associated with FHB resistance components were detected using CIM (Figure 2). No major QTL associated with Type I and Type II resistance were detected. Two genomic regions on chromosomes 2D and 7A were identified as being associated with FDK resistance and TKW. Three QTL for resistance to DON accumulation were identified on chromosomes 3B, 4D and 7D. Sonalika contributed the resistance alleles for 7A QTL for TKW and AR5 contributed the resistance alleles for remainder of the QTL mentioned above (Table 1).

The major QTL on 3B accounted for 35% of the phenotypic variation in DON accumulation, was flanked by *wsnp_Ku_c12544_20235135* and *wsnp_RFL_Contig2177_1500201*. The QTL peak (82.19 cM) was located away from the region of the well-known QTL from Sumai 3 that is distally located on 3BS (Abate et al 2008).

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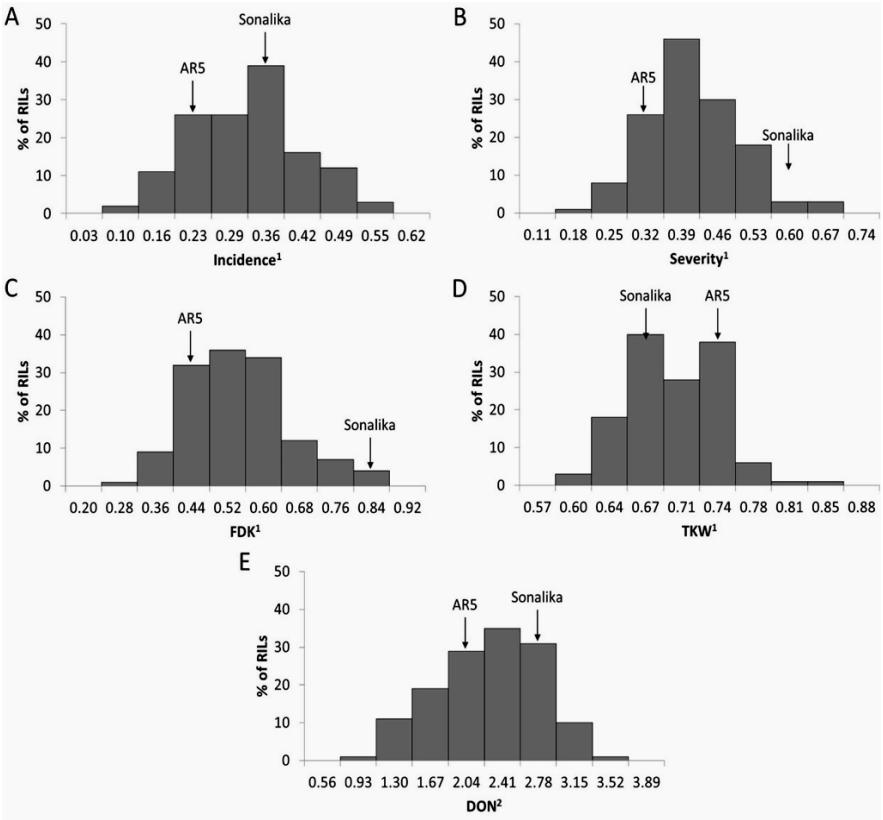

Figure 1. Frequency distributions of 135 wheat RILs developed from a cross between AR5 and Sonalika for (A) incidence, (B) severity, (C) FDK, (D) TKW and (E) DON content. Variables normalized using [1] arcsine ($\sqrt{x}/100$) transformation and [2] log (x + 1) transformation. Average values for the parental lines are indicated with arrows for each trait.

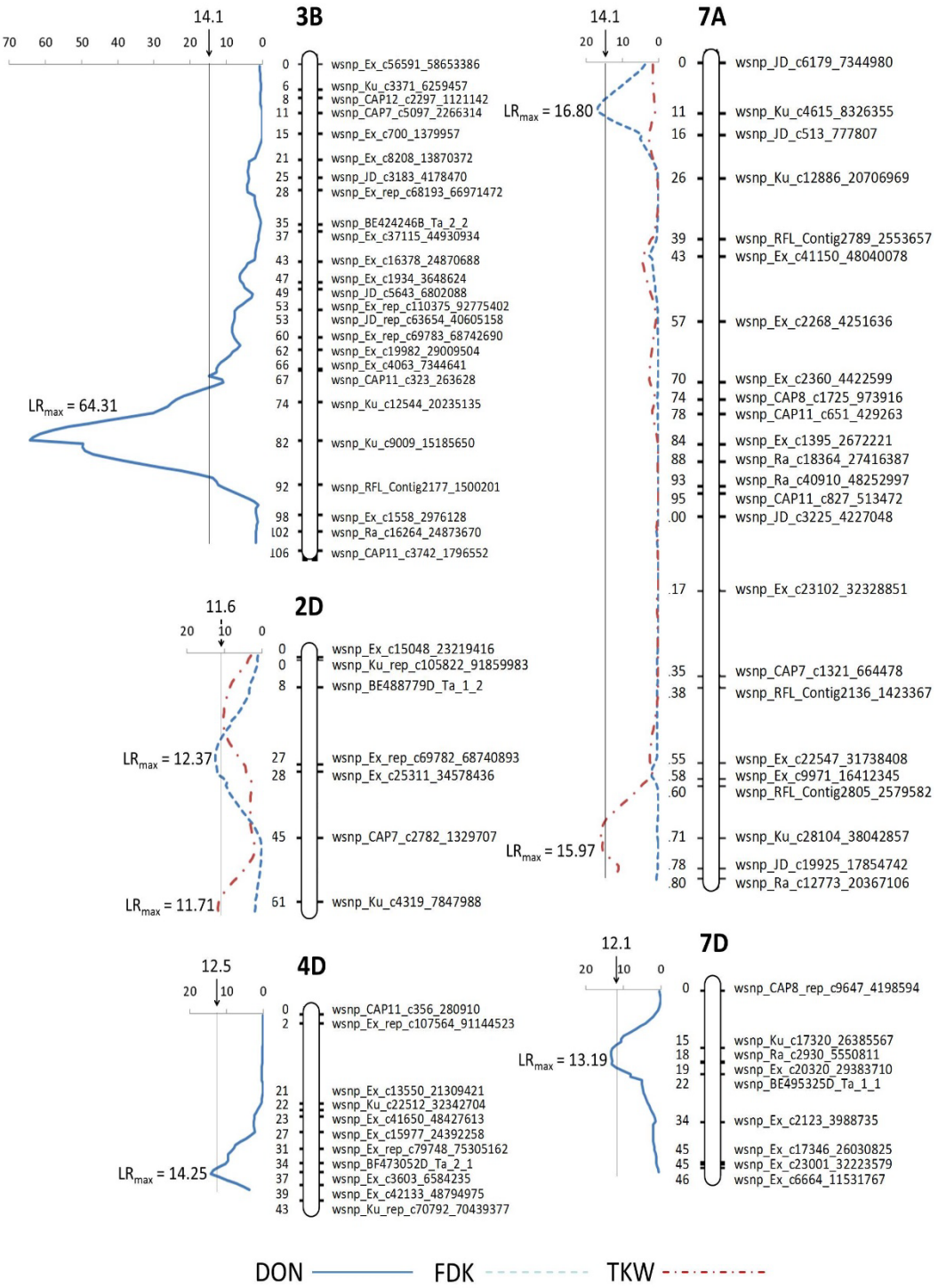

Figure 2. QTL associated with FDK, TWK and DON content in the cross AR5/Sonalika detected by composite interval analysis. The likelihood ratio (LR) scores were plotted against centimorgans on each chromosome. SNP marker locations on chromosomes are based on the Cavanagh et al. (2013) consensus map.

Table 1. Summary of quantitative trait loci (QTL) detected for Fusarium head blight resistance in a wheat RIL population developed from a cross between AR5 and Sonalika.

Trait	Chr[+]	Linked marker	Source	LR value	LOD value	R^2%[¤]
FDK	2D	*wsnp_Ex_c25311_34578436*	AR5	12.4	2.7	8.4
	7A	*wsnp_Ku_c4615_8326355*	AR5	16.8	3.6	10.1
TKW	2D	*wsnp_Ku_c4319_7847988*	AR5	11.7	2.5	10.0
	7A	*wsnp_Ku_c28104_38042857*	Sonalika	16	3.5	9.7
DON	3B	*wsnp_Ex_c700_1379957*	AR5	64.3	14	35.0
	4D	*wsnp_Ex_c42133_48794975*	AR5	14.2	3.1	9.7
	7D	*wsnp_Ex_c20320_29383710*	AR5	13.2	2.9	10.3

[+]Chromosomal location of marker.
[¤]Percent phenotypic variance.

CHARACTERIZATION OF NEW SYNTHETIC WHEAT GERM-PLASM FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

The wild and domesticated progenitors and related species of hexaploid bread wheat (*Triticum aestivum*) have been used as a genetic source for improving modern wheat cultivars for resistance to Fusarium head blight (FHB). In order to transfer useful genes from tetraploid wheat (*T. turgidum*) into bread wheat, we developed 153 synthetic hexaploid wheat (SHW) lines using durum wheat (*T.turgidum* subsp. *durum*) and other five tetraploid subspecies (*T turgidum* subsp. *carthlicum*, *dicoccum*, *polonicum*, *turgidum* and *turanicum*) in crosses with *Aegilops tauschii*. The goals of this project are to identify the SHW lines carrying the FHB resistance and to map putative novel FHB-resistant QTL in the FHB-resistant SHW lines. The evaluation experiments were performed using a randomized complete block design (RCBD) with three replications. The common wheat cultivars 'Sumai 3' and 'Grandin' were used in all the experiments as resistant and susceptible checks, respectively. At this stage of the project, 153 of the SHW lines and their 75 tetraploid wheat parents have been evaluated in one greenhouse season and in one field nursery season at two locations (Fargo and Prosper, ND). The statistical analyses of disease severity in the greenhouse and field nurseries showed a significant correlation among the three experiments. A number of SHW lines with high and moderate levels of FHB resistance have been identified. Among these SHW lines, 10, one, and five lines in the greenhouse and in Fargo and Prosper field nurseries, respectively, showed higher level of resistance than the resistant check Sumai 3, with three lines having a high level of resistance both in the greenhouse and in one of the field nurseries. Seventeen SHW lines showed significantly higher resistance than their tetraploid parents, suggesting that the D genome of *Ae. tauschii* may carry the resistance QTL or enhance the FHB resistance through genomic interactions in these lines. Thirteen SHW lines showed high resistance in line with their highly resistant tetraploid parent. The plant materials will be further evaluated in two more greenhouse seasons and in one field nursery season at two locations in order to validate the results. All the SHW lines and their tetraploid wheat parents are currently being genotyped using the Illumina wheat 9K-SNP array. All the phenotypic and genotypic data will be used for mapping of the QTL associated with the FHB resistance. The SHW lines with high levels of FHB resistance will be made available to the U.S. wheat breeding programs for developing adapted wheat germplasm and cultivars.

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CAN MULTIPLE RESISTANCE QTL IN COMBINATION WITH FUNGICIDE APPLICATIONS REDUCE FUSARIUM HEAD BLIGHT SEVERITY IN SPRING WHEAT?

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ABSTRACT

Genetic resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum*, is crucial to reduce the wheat grain yield and quality losses caused by this disease. The development of wheat cultivars with resistance to FHB is very difficult to achieve because of the laborious screening methods and the complex mechanisms of resistance, both of which are subject to environmental variability. DNA markers for FHB resistance QTL have been identified and may be used to speed the introgression of resistance genes into adapted germplasm. Previously, screened double haploid (DH) spring wheat lines derived from 4 way crosses showed promise in reducing FHB severity but more evaluation of this material is needed. Selection for resistance QTL and the use of fungicide (Prosaro®) are two different approaches when combined may present a better way of minimizing disease damage as well. Therefore, we conducted a field experiment to evaluate the effect of combining resistance QTL and fungicide application on FHB severity. FHB severity was significantly influenced by both resistant QTL and fungicide application. However, our results showed that the combination of resistant QTL provided reduction in severity without the presence of fungicide as well. Due to a lack of resources in regards to combining resistance QTL and fungicide application approaches, we hope that our finding can provide a better understanding of selection for resistant QTL and the interaction of plant host and pathogen.

VALIDATION OF FUSARIUM HEAD BLIGHT RESISTANCE QTL IN WHEAT USING DOUBLE HAPLOIDS DERIVED FROM FOUR-WAY CROSSES

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ABSTRACT

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is one of the most devastating plant diseases in the world. Specifically in wheat, FHB has become responsible for significant economic and health concerns worldwide due to mycotoxin accumulation in infected grain, as well as yield and quality losses. To date, sources of resistance conferring complete resistance to FHB have not been identified in wheat. Thus, extensive research efforts worldwide has focused on development and use of resistant cereal cultivars for the control of FHB. QTL for FHB resistance have been mapped to almost all wheat chromosomes when different mapping populations were investigated. In our research, we are using double haploid (DH) wheat lines derived from selected four-way crosses combining several sources of resistance to validate putative QTL (Xmc758. Gwm33, xbacr176, Xgm120, Xwmc317, Xwmc332, Xwmc522 and Xwmc296) that could minimize the threat of FHB including the reduction of mycotoxins to the producers, processors, and consumers of wheat. We used molecular techniques to validate DH lines and their corresponding parents. In this study, we report on our work on the DH derived lines screened for FHB in multiple Northern Plains location. Our findings will assist ongoing efforts aimed to develop resistant wheat varieties, minimize the impact of the disease, and provide resources that can possibly assist in the advancement of wheat germplasm research.

PREDICTING GENETIC VARIANCE IN BREEDING POPULATIONS: USING HISTORICAL BREEDING RECORDS TO EMPIRICALLY EVALUATE SIX PREDICTION METHODS

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ABSTRACT

Robust methods to predict genetic variance () of bi-parental breeding populations would facilitate greater gains per breeding cycle. To illustrate its utility, consider a crossing block of 100 elite parents – making all 4,950 pairwise crosses is impractical and evaluating all such crosses at a scale appropriate to quantify is impossible. Previously, metrics such as the phenotypic, genetic (measured as the proportion of non-matching markers), and kinship-based (estimated from genomewide markers) distances between parents have been tested for their ability to predict . In general there is little to no correlation between these metrics and . One explanation for this is the inability of such methods to explicitly model the segregation of associated genetic loci (i.e. QTL). Now, the commonplace use of genomewide markers and genomic selection (GS) in breeding programs, along with recent theoretical work in the area, has enabled the development of three additional methods that, at varying levels, explicitly model the segregation of QTL. The accuracies of six prediction models were evaluated using field-based estimates of from 40 bi-parental barley breeding populations. The accuracies of phenotypic distance, genetic distance, and kinship-based distances between the two parents were all low and non-significant. In contrast, the accuracies of the three methods that explicitly integrate the effects of associated genetic loci were all significant. The method that directly measures variation using the GEBVs of simulated bi-parental populations was the most accurate. The results indicate that predictions based on genome-wide markers may enable plant breeders to target specific parent combinations, or at the least winnow out low- predicted crosses.

A DIVERSE COLLECTION FOR USE IN GENOMIC SELECTION APPROACH TO DEVELOP FHB RESISTANCE AND LOWER DON ACCUMULATION IN TWO-ROW BARLEY

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ABSTRACT

Fusarium head blight (FHB) primarily incited by *Fusarium graminearum* Schwabe, has remained an economically destructive disease of barley (*Hordeum vulgare* L.) in the Canadian prairies since the mid-90's. As a result of infection, mycotoxins such as deoxynivalenol (DON) render grains unsuitable for use in animal feed or malting and brewing industries. In recent years, this disease has moved westward and become a more common occurrence in contaminated grains within the barley production regions of Saskatchewan and occasionally Alberta. Breeding FHB resistant cultivars has been a long-term goal of all western Canadian barley breeding programs. This objective has been facilitated through use of a large screening nursery at Brandon, MB, however progress has been impeded by numerous factors. Nonetheless, several cultivars have been released to date which demonstrate reduced DON accumulation. Given the significant time and resources that have been invested in developing such cultivars, it may be impractical to assume that reliance on only traditional breeding methods will be adequate to continue advancements. Further DON reductions are predicted to be more difficult to achieve given the low concentration at which the mycotoxin is found in the grain. Genomic selection has been proposed as an alternative method to improve precision of selection and hasten cultivar development. A substantially large (n=400) and diverse set of two-row barley genotypes was phenotyped in three environments in Manitoba (Brandon, Carberry and Carman) over two growing seasons (2014-15). FHB and DON data collected on these genotypes will be used in conjunction with genomic data to develop models for application of selection to breeding populations segregating for FHB resistance. Genomic selection will be evaluated as a method for enhancing FHB resistance in the two-row-malting barley breeding program at Agriculture and Agri-Food Canada's Brandon Research and Development Centre.

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN WINTER BARLEY CULTIVAR EVE

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ABSTRACT

Interest in winter barley (*Hordeum vulgare*) production for use in livestock rations, health foods, and malt products emphasizes the importance and need for development of elite cultivars having resistances to prevalent diseases. Fusarium head blight (FHB), caused by the pathogen *Fusarium graminearum* Schwabe, can result in severe yield and quality losses for barley producers via kernel damage and production of mycotoxins. The objectives of this study are to identify the FHB resistance QTL in the hulless winter barley cultivar Eve and to develop diagnostic markers for use in marker-assisted selection. Mapping populations, comprised of recombinant inbred lines (RIL), were derived from crosses of Eve to FHB susceptible parents (Eve/'Doyce' and Eve/VA07H-35WS) for use in mapping resistance to FHB. These RIL populations were evaluated for FHB incidence and FHB severity with the assistance from cooperators in KY, NC, VA and China during the 2014-2015 growing season. Genotype by location interaction was significant for FHB incidence, but not for FHB severity. In the Eve/Doyce (E/D) population a significant correlation for FHB incidence was observed between data from Lexington, KY and Mt. Holly, VA (r = 0.13817, $P = 0.045$). Significant correlations among locations in the Eve/VA07H-35WS (E/VA) population were not observed for FHB incidence. Significant correlations for FHB severity in the E/D population were observed between the Lexington and Mt. Holly locations (r = 0.21224, $P = 0.0019$) and between Lexington, KY and Kinston, NC (r = 0.17621, $P = 0.0103$). In the E/VA population, a significant correlation was observed between Lexington and Mt. Holly (r = 0.16072, $P = 0.0139$) for FHB severity. The populations will be genotyped with 9K SNP and second year phenotypic data will be collected in 2015-16 season. The FHB resistant QTL will be validated and diagnostic markers will be identified for use in marker-assisted selection.

DEVELOPMENT OF USER-FRIENDLY DNA MARKERS FOR FUSARIUM HEAD BLIGHT RESISTANCE QTL IN PI 277012

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ABSTRACT

The hexaploid wheat line PI 277012 exhibits a high level of Fusarium head blight (FHB) resistance in both greenhouse and field experiments. Previous QTL analysis with SSR markers and a doubled haploid (DH) population consisting of 130 lines from a cross between PI 277012 and 'Grandin' identified two major FHB resistance QTL located on chromosome arms 5AS and 5AL, respectively. The resistance QTL (*Qfhb.rwg-5A.1*) on 5AS peaked at marker *Xbarc40* between markers *Xcfa2104* and *Xgwm617*, while the resistance QTL (*Qfhb.rwg-5A.2*) on 5AL peaked at marker *Xcfd39* between *Xwmc470* and *Xbarc48*. To saturate the two resistance QTL regions with more DNA markers, the DH population was further genotyped using the wheat **9K** SNP iSelect Assay. A total of 2,877 polymorphic SNPs were identified between PI 277012 and 'Grandin', and mapped to the original genetic linkage map developed with SSR markers. To develop additional markers, sequences of the SNP markers flanking the two QTL regions were used to search homologous sequences of the *Brachypodium distachyon* (Bd) genome using blastN and two *B. distachyon* syntenic regions were identified, which contain 248 and 2500 genes, respectively. Then, these Bd genes were used to search for homologous genes in wheat chromosome 5A survey sequences, and 41 and 139 contigs were identified from the 5AS and 5AL sequences, respectively. From these contigs, 250 primer pairs were designed and used to amplify DNA sequences from genomes of PI 277012 and Grandin. Twelve cleaved amplified polymorphic (CAP) markers were developed from those homologous sequences showing polymorphism between PI 277012 and Grandin and further mapped to the resistance QTL regions using the DH population. Three of the CAP markers were located on 5AS while nine were on 5AL. These 12 CAP markers along with seven PCR-based markers converted from SNPs, three SSR markers and a gene specific marker closely linked to the resistance QTL were used to genotype a larger population consisting of 947 recombinant inbred lines (RILs) derived from the cross between PI 277012 and Grandin, which were phenotyped for FHB reaction in greenhouse and field for two seasons using the single floret point inoculation method in 2014 and 2015. The two QTL previously identified using the DH population were also detected using the RIL population. RILs containing the PI 277012 (resistant parental line) alleles at all three marker loci most closely linked to the resistance QTL on 5AS had 19.6% reduction in FHB severity, while RILs with the PI 277012 alleles at all three marker loci most closely linked to the resistance QTL on 5AL showed 46.2% reduction in FHB severity compared to RILs with the Grandin (susceptible parental line) alleles. Average disease severity was reduced by 51.5% when the PI 277012 alleles at all six marker loci in the 5AS and 5AL resistance QTL regions

were combined. These selected markers can be further used for marker-assisted selection of the FHB resistance from PI 277012 in wheat breeding programs.

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CYTOGENETIC DISSECTION OF A, B, AND D GENOME PROVIDES NEW INSIGHTS INTO FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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ABSTRACT

Durum wheat shares A and B genomes with common wheat, but lacks the D genome that common wheat has. It has been anticipated that the absence of D genome in durum wheat may influence expression of FHB resistance genes in the durum background. In the present study, we used 'Langdon' (LDN) durum-'Chinese Spring' (CS) D-genome disomic substitution lines (LDN-CS DSLs) that dissect CS D genome in LDN durum background by chromosome substitution to characterize the role of individual D-genome chromosomes and their A- and B-genome homoeologs on FHB resistance in durum. In addition, we developed the LDN-*Aegilops tauschii* 'RL5286' amphiploid that combines the entire D genome of RL5286 and LDN A and B genomes to determine whether addition of the entire D genome to durum affects FHB resistance. Also, we dissected the RL5286 D genome in the LDN durum background by producing LDN-RL5286 chromosome addition lines to determine the role of individual D-genome chromosomes on FHB resistance in durum. Both the amphiploid and addition lines had the same LDN durum background, making them ideal for characterizing the effect of D-genome chromosomes on FHB resistance. The reaction of LDN-CS DSLs to FHB indicated that the substitution of chromosome 5A by 5D enhanced resistance to FHB. This might result from absence of the Q gene on chromosome 5A that conditions spike structure. Also, we found that CS chromosome 6D might contain genes for FHB susceptibility and/or suppression of FHB resistance genes. LDN chromosomes 2B and possibly 6A and 6B might contain the genes that enhance FHB resistance. The LDN-RL5286 amphiploid exhibited higher susceptibility to FHB than LDN. However, the RL5286 chromosomes 1D and 5D were found to condition FHB resistance when they were individually or concurrently added to LDN. The FHB resistance genes on these two chromosomes seemed to act additively in LDN. The other RL5286 chromosomes (i.e. 2D, 3D, 4D, 6D, and possibly 7D) appeared to contain suppressors for the FHB resistance genes on chromosome 1D or 5D. Apparently, there are complex interactions among the genes on the RL5286 D-genome chromosomes as well as LDN A- and B-genome chromosomes for FHB resistance in durum background. Proper manipulation of the critical chromosomes may lead to the improvement of durum wheat for FHB resistance.

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