Proceedings of the 2013 National Fusarium Head Blight Forum




December 3-5, 2013
Hyatt Regency Milwaukee
Milwaukee, WI USA

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LOCAL GRAIN AND MALT: A RENAISSANCE BEING NEGATIVELY IMPACTED BY DON

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ABSTRACT

In 2013, a group of 8 micro-maltsers got together and formed the Craft Maltster's Guild. The mission of the guild is to promote and educate the general public about the tradition of craft malting in North America, provide educational opportunities to its members and to the general public, and to improve and uphold the highest quality and safety standards for Craft Malt.

The guild's members operate in parts of North America where *Fusarium* and DON levels in grain are a major concern. These areas include New England, North Carolina, Quebec, Michigan, and Oregon, Pennsylvania, Texas, Ney York and California. Within the next 5 years, dozens of craft maltsters will be opening up shop all over North America, trying to meet the consumer demand for more locally sourced ingredients.

In accordance with the guild's bylaws, "Craft Malt is defined as a finished malt product, produced from a variety of grains including but not limited to barley, wheat, rye, millet, oats, corn, spelt, and triticale. Craft Malt is in particular made using a majority (greater than 50% by weight) of locally grown grains as inputs, meaning grains grown within the region of the Craft Malthouse." This definition of Craft Malt was a unanimous decision made by all 8 founding members as a proud distinction of what our small but emerging industry represents. Coming from many backgrounds such as farming, engineering, and social work, we all started our operations to provide our local craft breweries with malt that comes from local farms. Our local malts are more expensive than commercial malts and in order to command that higher price, we are offering a product that craft brewers can market as "homegrown".

The challenges of operating a micro-malthouse are substantial. Malting Equipment is not easy to procure and the learning curve to manipulate grain into malt is steep. For all the challenges we face, the largest is finding a good reliable source of quality grains. As many of us know, "Good malt starts in the field". For many of us, finding the correct varieties to grow in our region is a huge hurdle. In New England we only consider growing barley and wheat varieties that have *Fusarium* resistance. It is the #1 reason why we reject an otherwise suitable lot of grain. We have seen DON levels over 8 ppm and many times these numbers discourage farmers from ever trying to grow grains again.

In New York, legislation was passed in 2012 requiring all breweries wanting to operate as a Farm Brewery to use 20% NY State grown ingredients in their beer. In 10 years the requirement for licensed Farm Breweries will be 90%. Within the 10 months since this legislation has been implemented, 15 Farm Brewery Licenses have been issued. This legislation was meant to spur a strong local economy; giving farmers a new crop to grow, craft brewers incentives such as being able to sell pints out of the brewery, and ultimately giving consumers some great locally grown beers to buy. In theory, laws such as these sound great but in actuality there was not enough malting barley grown this past year to support even 15 small breweries at 20%. Why? Three letters: DON. According to a Cornell Field Crops Specialist,

"50-75% of this year's malting barley crop had DON numbers over 1ppm. There are currently over 2000 acres of Winter Malting Barley planted in NY and over 1,000 acres of Spring Malting Barley is expected to go in the ground this spring. This may seem like insignificant acreage to many used to mid-west production however, it is significant in our region. With a huge market demand for local grains that is willing to pay a premium, it could equate to over $2.5 million for that 3,000 acres planted.

This is just one example of what is happening around our country with a renaissance in local grains and malt. Similar legislation to what Governor Cuomo passed in NY is being proposed in other states. Many states have seen the economic impact of local vineyards and wine trails in their state and want to see the same attention to locally grown, regionally distinct products coming from craft breweries as well. With all of the positive goodwill going into these emerging grain and malt industries, we cannot forget that all of it could be dampened out by the threat of DON. No matter how high the demand and how great the premium a crop may bring, if you are going to lose it 3 years out of 5 to DON, you are not going to continue grow it and take that risk. Corn is a much better bet. If funding is not put into researching resistant varieties and good cultural practices for growing DON free grains, this renaissance will never get off the ground.

SESSION 1:

VARIETY DEVELOPMENT AND HOST PLANT RESISTANCE

FHB RESISTANCE QTL MAPPING USING NATIVE SOURCE OF RESISTANCE AND SNP-GBS MARKERS

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ABSTRACT

Several wheat breeding programs across North America rely on exotic sources of resistance to FHB. Lack of adaptation and linkage drag are common problems associated with the exotic sources. DNA markers for FHB resistance, derived from adapted progenitors can increase our ability to select resistant lines with better agronomic traits. The winter wheat breeding program at the University of Illinois has identified several lines with improved levels of FHB resistance using native germplasm. One example is IL97-1828, which has been extensively used in the program, does not carry *Fhb-1*, and is one of our best breeding lines for FHB resistance. In order to map QTL associated with FHB resistance, a population of recombinant inbred lines was derived from a cross between IL97-1828 x Clark (susceptible) via SSD until the $F_{5:6}$ generation. Two hundred and five RILs were evaluated in two locations, Urbana-IL (2009 and 2010) and Wooster-OH (2010). Artificial inoculation and mist irrigation were applied in order to increase the inoculum pressure and favor the disease development. The Genotyping by Sequencing (GBS) protocol consisted of a two restriction-enzyme genome reduction, where a common cutter (*PstI*) and a rare cutter (*MspI*) were combined during the library preparation. Sequence data were obtained from an Illumina HiSeq2000 run. The data were analyzed with TASSEL UNEAK Pipeline and the program MapDisto was used for building a linkage map. The final version of the map was built using LOD = 3, r_{max} = 0.3 and 835 polymorphic markers: 671 "unique" SNP-GBS markers, in addition to 154 DArT and 10 SSR markers that have been previously used with this population. Single marker analysis, standard interval mapping and Haley-Knott regression were performed on R/QTL for all traits measured in the field, having heading date as a covariate in each analysis. Eight QTL were declared significant over multiple location/years: three were associated with FHB incidence (1B, 1D and 2B), three were found for severity (1B, 2B and 3B) and two were associated with FDK (2A and 3B). No major effect QTL were detected. The genetic distance between significant markers and QTL averaged 2.7 cM, ranging from 0 to 7.98cM. Fourteen extra QTL were found to be environment specific. The two-QTL scan procedure available in R/QTL revealed significant interactions between QTL for most traits. Next generation sequencing markers allowed us to map QTL that have gone undetected before, when only DArT and SSR markers have been used. This study also show that native sources of resistance like IL97-1828 can contribute to the development of wheat with improved FHB resistance.

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LIFELONG LEARNING: WHAT WE HAVE LEARNED BREEDING FOR SCAB TOLERANCE

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ABSTRACT

In the Great Plains, *Fusarium* head blight (FHB) caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.:Fr.) Petch] is a periodic disease which can be devastating due to disease losses and to the presence of its mycotoxin, deoxynivalenol (DON) that poses a significant threat to human health. Hard winter wheat is used roughly equally in domestic and export markets. Furthermore, bread wheat grain is increasingly used in whole wheat products. Hence our goal is to reduce the level of DON in the whole grain (as opposed to white flour) to the stricter European standard for DON in white flour. Of the two, disease losses or presence of DON, we have discovered lines (e.g. Harry) that have higher levels of DON at the same levels of disease as lower DON lines. Hence DON is the most critical measure of the lines' tolerance to FHB and its economic effects.

The Great Plains breeding programs have released lines with superior FHB tolerance (Lyman, Overland, Everest, Hitch, Art, T158, and Millennium). All of these lines are based upon native resistance. Building upon this native resistance, hundreds of crosses have been made to putative native resistance germplasm sources from other regions. Few of these crosses have led to new cultivars. Part of the low success rate may be due to the length of time it takes to release a new cultivar (12 years), but advanced experimental lines should be under testing by now. A more likely reason for the low success rate is that the germplasm was two diverse (hard to find and use the minor genes) in crosses and that our field assays were unable to separate small differences in segregating populations. Hence a new approach is needed. What has surprised us is that despite the success of *Fhb1* cultivars in the spring wheat region, relatively few have been released in the winter wheat region. A question was: does *Fhb1* or other major QTL have a negative effect on winter wheat or our selection process? In replicated studies in Nebraska using lines with and without *Fhb1* derived from a segregating population, little difference was found among the lines. This indicates that *Fhb1* does not have pleiotropic effects on agronomic performance. Furthermore a series of backcross derived lines for Wesley *Fhb1* have performed well in the field. The Wesley *Fhb1* lines have been used extensively as parents and their progeny are advancing through our selection process at a much higher rate than lines from unadapted native resistance sources. Furthermore, new backcrossing efforts are underway to put *Fhb1* into backgrounds like Overland that have native resistance in hopes the QTL and native resistance can be combined. In evaluating where we have been and how to go forward, we continue to believe in having outstanding field screens to identify native resistance and validate QTL resistance, coupling native resistance with proven FHB QTL such as *Fhb1* and newer QTL coming out of wild species (e.g. *Fhb3*) using molecular markers, and freely sharing the germplasm. The new QTL will be backcrossed in adapted germplasm to build new parent stocks. Hard winter wheat x hard spring wheat crosses and backcrosses to winter wheat

lines will continue to be used as we can recapture the needed winter adaptation relatively easily. In the future with the advent of hybrid wheat, the opportunity to study novel cytoplasms and floral biology may provide new ways of reducing FHB and DON in wheat.

Finally, we recognize that genetics can only take you so far and that a robust fungicide and crop management program is necessary to meet our goals of low DON whole grain flour.

UNIVERSITY/INDUSTRY COLLABORATIONS: POTENTIAL WAYS TO FURTHER SCAB RESISTANCE RESEARCH

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ABSTRACT

State and federal funding in the US for research into host plant resistance to scab is often limited due to budget constraints, changing budget priorities, and ever-increasing competition for state and federal funding. An excellent way to address this problem is through the formation of university/industry collaborations that leverage existing funding as well as the shared resources that are expected to be available through such collaborations. Potential research areas and types of collaborations as well as federal programs designed to foster collaboration will be discussed. Opportunities for collaborations exist across the breadth of research areas traditionally supported by the USWBSI, including educating and training the next-generation of geneticists, breeders, and pathologists to conduct scab research; identifying new host plant resistance genes; defining host genetic resistance mechanisms, host-pathogen interactions, and disease epidemiology; cloning host plant resistance genes, developing acceptable and safe transgenic approaches to host plant resistance; optimizing genetic and fungicide management practices; and limiting the production of grain toxins to safeguard the US food and feed supply. The former NIFA Plant Breeding and Education Program, a NSF Industry/University Cooperative Research Center (IUCRC) Program, and operational individual university/industry student fellowship and internship programs can serve as models to follow in furthering future scab research through collaboration. The establishment of the first IUCRC to focus on wheat genetic research was recently announced by Kansas State University and its partners. There are exciting possibilities to extend this type of IUCRC to develop collaborative projects and mitigate problems associated with scab all the way from cereal seed production, through feed and food production in the US.

ASSOCIATION OF THE EXTENT OF ANTHER EXTRUSION AFTER FLOWERING WITH FIELD RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

Inspired by results from Norwegian colleagues (Skinnes et al 2008, 2011, Lu et al. 2013) we assessed several wheat populations that have also been tested for field resistance to Fusarium head blight for the extent of anther retention. FHB resistance was measured in artificially spray inoculated field trials using established methods (Buerstmayr et al. 2003). The extent of anther retention was assessed by counting the number of florets with trapped anthers among 20 florets per lot 4-6 days after pollen shed and expressed on a relative scale.

Across all populations we measured so far we found the following pattern:

1) The extent of anther extrusion is highly heritable, broad sense heritability was generally $H > 0.80$; and 2) the extent of anther extrusion was significantly correlated with FHB severity, in the range of $r > 0.60$.

We also analysed one mapping population (Capo x Arina, 190 RILs). In this mapping population three highly significant FHB severity QTL were detected (LOD>3.5), two of which co-mapped with strong QTL for anther retention (LOD>7). These results confirm that the extent of anther retention has a pronounced influence on FHB severity under field conditions. QTL for anther retention/anther extrusion are likely at the same time passive FHB resistance QTL.

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EFFECTS OF *FHB1* ON RESISTANCE TO WHEAT FHB IN DIFFERENT HARD WINTER WHEATS

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease that significantly reduces grain yield and quality. *Fhb1*, a quantitative trait locus on 3BS from Sumai 3, has shown the largest effect on FHB resistance. Ning 7840 is a Chinese wheat line that carries *Fhb1* from Sumai 3. Because of so many undesired traits in the Chinese lines, direct use of Chinese lines as resistant parents in U.S. hard winter wheat (HWW) breeding has not been successful. To date, none of the U.S. HWW cultivars in the Great Plains has *Fhb1*. Transferring *Fhb1* into adapted U.S. HWW cultivars may facilitate utilization of *Fhb1* to improve FHB resistance of U.S. HWW. In this study, we first transferred *Fhb1* from Ning7840 to three locally adapted HWW cultivars, Overland, Jagger, and Overley, by marker-assisted backcross, and assessed the effect of *Fhb1* on FHB resistance in these U.S HWW backgrounds. Among the three recurrent parents, Overland is moderately resistant to moderately susceptible, thus may have some indigenous minor QTL for resistance, while Overley is highly susceptible and has no any resistance QTL. Jagger is in between. A total of 227 BC_3 and BC_4 families with *Fhb1* were selected from the three backcross populations for phenotyping and marker confirmation. Both markers *Xgwm533* and *Xumn10* were used to identify *Fhb1*. FHB resistance as measured by percentage of symptomatic spikelets (PSS) in an inoculated spike was evaluated in four greenhouse experiments (spring and fall of 2011 and 2012) and one field experiment (2012-2013). *Fusarium* damaged kernel (FDK) was also scored in the field experiment. Mean PSS was highly correlated with FDK ($r = 0.6038$). Mean FHB ratings were significantly different among the three populations. Overland-*Fhb1* lines had the lowest mean PSS (25.6%), ranging from 11.05 to 67.6%; Overley-*Fhb1* lines had the highest mean PSS (44.9%), ranging from 23.6 to 82.0%; and mean PSS of Jagger-*Fhb1* lines was in between (39.5%), ranging from 18.8 to 72.1%. The results indicated that the resistance levels of the recurrent parents had a large impact on the resistance of their progenies. *Fhb1* can significantly lower mean PSSs and FDK in the *Fhb1*-carrying lines selected from each population. On an average, these backcross-derived *Fhb1* lines had 7.1 to 45.9% reduction in PSS and 8.5 to 39.2% reduction in FDK compared with the lines without *Fhb1*. A combination of local minor resistance genes with *Fhb1* can significantly improve FHB resistance in U.S. HWW.

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MASS SELECTION WITH OPTICAL SORTERS FOR HEAD SCAB RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) or head scab, caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.:Fr.) Petch], is one of the most destructive diseases of wheat (*Triticum aestivum* L.) worldwide. Two important consequences of scab are a reduction in grain quality and the presence of mycotoxins. The most common mycotoxin is deoxynivalenol (DON). There is great interest among breeders in selecting for resistance to both of these traits and numerous strategies for scab resistance breeding are in use. We have tested mass selection during advancement of breeding populations using a USDA/ARS and National Manufacturing Seed Sorter System with color camera using calibrations that reflected visual differences between asymptomatic grain and grain showing FHB symptoms. In 2010, 20 bulk F_3 SRW wheat populations with scab resistant parents in their pedigrees were harvested by population from unreplicated plots naturally infected by a mild-moderate scab epidemic near Lexington, KY. Using this seed source the first cycle of selection was conducted by running bulk grain through the sorter and discarding rejected scabby grain. This process was repeated in 2011 using grain from C_1 plots that had conidial suspension applied at anthesis. In 2012, an additional cycle of selection was conducted using grain from unreplicated C_2 plots that were inoculated with grain spawn and sprayed with conidial suspension. In 2013, C_0, C_1, C_2 and C_3 selection cycles of the 20 populations, planted in a RCB experiment, in plots at Lexington and Princeton, KY and in the scab nursery at Lexington, KY, were evaluated for *Fusarium* damaged kernels (FDK) and DON concentration. Overall, no significant differences were seen between the cycles of selection. However, additional RCB studies comparing rows seeded with accepted and rejected grain separated by image-based, LED and SKNIR seed sorters were also grown in the 2013 Lexington scab nursery. Rows seeded with grain accepted by all three sorters showed reduced levels of FDK and DON compared to those seeded with rejected grain. Differences in FDK were significant ($P<0.005$) with the SKNIR sorter study. Significant differences were also seen for DON with the SKNIR ($P<0.05$) and LED ($P<0.1$) sorted material. Differences between the sorters could be due to dissimilar calibrations as well as the machines *per se*. The ability of seed sorters to improve genetic resistance to FHB is continuing to be investigated.

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MAPPING WHEAT FUSARIUM HEAD BLIGHT RESISTANCE QTL IN THE MD01W233-06-1/SS8641 DOUBLED HAPLOID MAPPING POPULATION

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ABSTRACT

Wheat breeding for Fusarium head blight (FHB) resistance relies on identifying and selecting for favorable quantitative trait loci (QTL). While major QTL have been identified in exotic germplasm (*Fhb1*), identifying QTL in US wheat varieties (US native resistance) is an important research area, as these are easily adaptable in breeding programs and can augment progress made with exotic material. The germplasm line MD01W233-06-1 was shown to be resistant in the 2007 and 2008 Southern and 2008 Northern Uniform Winter Wheat Scab Nurseries. It was then crossed with SS8641, a highly FHB-susceptible variety to create an F1-derived doubled haploid population with 124 lines. This population and the parents were evaluated in a single floret inoculation experiment in a greenhouse in College Park, MD in the winter of 2011 and in inoculated scab nursery field experiments in Salisbury, MD and Kinston, NC in both 2011 and 2012. Lines were phenotyped for heading date, FHB incidence (INC), FHB severity (SEV), percentage of *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) content. The population and parents were genotyped using a variety of molecular markers including: red coleoptile morphological marker, 29 SSR microsatellite markers, 23 KASP SNP markers, and 9K iSelect Beadchip Assay. After eliminating monomorphic and markers with segregation distortion, linkage analysis was performed using QTL ICIMapping v. 3.3 with 450 markers. Markers were anchored to wheat chromosomes based on data from Cavanagh et al. (2013), Somers et al. (2004), and Wilkinson et al. (2012). Linkage analysis was performed using a LOD threshold of 5.0 for linkage group construction, with the RECORD algorithm and SARF criterion for ordering and rippling, respectively. Markers mapped to 21 linkage groups, with at least 1 marker on each chromosome of the wheat genome. Phenotypic data was analyzed by location-year using PROC GLM of SAS to determine least square means for each line. QTL analysis was performed using the inclusive composite interval mapping method, with LOD threshold for significance determined by permutation test (1000 permutations, p=0.05). A total of 43 significant QTL were identified. Consistent resistance QTL were found on 2DS from 47- 64cM (2 DON and 1 FDK) and 2DL from 95-104cM (2 DON, 2INC, 2FDK, 1 SEV). The 2DS QTL were coincident with QTL for heading date, and correlation analysis showed that the resistance trait data were highly correlated with heading date, thus these resistance QTL may simply be due to disease escape. No heading date QTL mapped to the 2DL region with other resistance QTL. Additionally, several resistance QTL were identified from SS8641, where the MD01W233-06-1 alleles had a higher additive value. There were 3 such QTL regions on 3B from 56-57cM (2 DON), 75-78cM (2 FDK; 1 DON, SEV, and INC) and 93-113cM (3 SEV, 3 FDK, and 1 DON); on 1A at 129cM (2DON and 1 INC).

CONSTRUCTION OF DENSE LINKAGE MAPS "ON THE FLY" USING EARLY GENERATION PLANT BREEDING POPULATIONS TO FACILITATE MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE QTL

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ABSTRACT

Genetic linkage maps, consisting of marker loci ordered along chromosomes, provide the essential framework for identifying genomic regions contributing to Fusarium head blight (FHB) resistance and supporting molecular breeding efforts for varietal development. Linkage mapping has largely been confined to purpose-built mapping populations derived from a single cross. Construction of linkage maps with dense marker coverage further requires the integration of maps from several experimental populations, thus detracting from breeding efforts and delaying the application of new molecular tools. To facilitate marker assisted breeding efforts to pyramid FHB resistance QTL in our wheat breeding populations using the newly available wheat 9,000 SNP iSelect assay, as well as 26 SSR markers, we needed to construct a de novo linkage map. For this purpose, we applied multipoint linkage analysis of general pedigrees to develop a dense linkage map using our existing breeding populations consisting of 565 four-way F_1 plants from 28 four-way crosses. Linkage analysis was performed using the CRI-MAP version 2.504. A total of 3,880 loci were mapped, including 1,252 unique genetic bins. The estimated linkage maps covered a total genetic distance of 3,072 cM, with an average interval of 2.5 cM between genetic bins. Marker coverage was relatively poor for the D genome. Within the A and B genomes, 12 of the 14 linkage maps had rank-order correlations 0.97 to 0.99 with the locus positions in the consensus maps released for the 9,000 SNP assay. The high degree of concordance with the consensus map indicates that use of mapping algorithms for general pedigrees can be reliably adopted to develop dense linkage maps using existing segregating breeding populations. This strategy should allow researchers and breeders to develop dense linkage maps "on the fly" to accelerate marker assisted breeding efforts for host resistance to FHB.

MULTIPLE FUSARIUM HEAD BLIGHT RESISTANCE QTL PYRAMIDED ONTO ELITE SPRING WHEAT *FHB1* BACKGROUNDS USING A FAMILY-BASED MAPPING APPROACH

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ABSTRACT

Resistance to Fusarium head blight (FHB) in wheat is a complex trait, for which numerous QTL have been identified. While use of *Fhb1* for development of host resistance is commonplace in wheat breeding programs, smaller effect QTL have not been routinely exploited. Pyramiding novel sources of resistance with *Fhb*1 therefore presents an opportunity to enhance FHB resistance of elite wheat germplasm. It was demonstrated that family-based linkage analysis has potential for mapping FHB resistance QTL in early generation breeding populations. Therefore, we have applied a family-based mapping approach in our breeding populations to map and pyramid multiple FHB resistance QTL onto elite hard red spring wheat (HRSW) *Fhb1* backgrounds. Segregating F_1 populations were developed from 44 four-way crosses among 20 HRSW founder lines, providing a total of 826 four-way F_1 plants. Fifteen experimental lines from the SDSU spring wheat breeding program provided the elite *Fhb1* backgrounds. Founders conferring novel sources of resistance were 2 experimental lines (MN99112 and MN99126) from the UMN spring wheat breeding program, 2 lines from a cross between 'Wheaton' and Japanese landrace PI 81791 (Sapporo Haru Komugi Jugo), and Peruvian line PI 271127 (MULT 757). Founders and four-way F_1 plants were genotyped for 72 SSR markers and phenotyped by spray inoculation in the greenhouse for FHB severity. F_2 seed collected from each four-way F_1 plant was used to establish selfed progeny tests of the four-way F_1 plants over multiple sites. Family-based linkage analysis was conducted using the software package S.A.G.E to identify FHB resistance QTL segregating in the four-way F_1 populations. Linkage analysis detected a QTL on the short arm of chromosome 3B corresponding with *Fhb1* in the interval between *Xbarc133* and *Xgwm493*, which was most strongly associated with *Xbarc147*. A centromeric QTL was also identified on chromosome 3B in the interval between *Xwmc787* and *Xgwm108*, roughly corresponding to a QTL reported from PI 81791. A third QTL was mapped to chromosome 7B associated with *Xbarc176*, which corresponds to a QTL reported from PI 271127. These markers are being employed to select $F_{4:5}$ lines from these populations with desirable QTL combinations to move forward in the breeding program.

NATIVE FUSARIUM HEAD BLIGHT RESISTANCE FROM 'LYMAN', 'OVERLAND', 'ERNIE' AND 'FREEDOM' WHEAT CULTIVARS PYRAMIDED ONTO WESLEY-*FHB*1 BACKGROUNDS USING A FAMILY-BASED MAPPING APPROACH

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ABSTRACT

The primary strategy for development of host resistance to Fusarium head blight (FHB) in winter wheat has been the backcrossing of *Fhb1* into elite genetic backgrounds. However, there are numerous sources of native FHB resistance in winter wheat that could be combined with *Fhb1* to produce varieties with enhanced resistance. Therefore, we have adopted a family based mapping approach to map and pyramid multiple native sources of FHB resistance onto a Wesley *Fhb1* background. Segregating F_1 populations were developed from 28 four-way crosses among 10 winter wheat founder lines, providing a total of 565 four-way F_1 plants. Founders included two Wesley *Fhb1* backcross lines (WesFHB1-BC06 and WesFHB1-BC56) that provided the *Fhb*1 background for each cross. Founders conferring native sources of resistance were the hard winter wheat cultivars 'Lyman' and 'Overland', and the soft winter wheat cultivars 'Ernie' and 'Freedom.' Cultivars 'McGill', NE0645 and NI08708 were also used as founders. Founders and four-way F_1 plants were phenotyped in the greenhouse for FHB severity, and genotyped for 9,000 SNPs (Illumina iSelect Beadchip assay) and 26 SSR loci. F_2 seed collected from each four-way F_1 plant was used to establish selfed progeny tests of the four-way F_1 plants over multiple sites. A total of 39 F_2 lines have been selected based on phenotypic evaluations and used to derive approximately 500 $F_{2:3}$ lines, while 232 lines have been advanced as F_3 bulks for further evaluation. Family-based linkage analysis is currently being conducted to identify QTL and associated markers for FHB resistance segregating in these breeding populations. These markers will be used to select lines with desirable combinations of resistance QTL.

MOLECULAR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN GLENN, A HIGH QUALITY AND ADAPTED HARD RED SPRING WHEAT CULTIVAR

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ABSTRACT

Fusarium head blight (FHB) is a major disease that affects both wheat yield and quality in many wheat-growing regions, including the US Northern Plains. Therefore, developing wheat cultivars with high resistance to FHB is necessary. In the last few years, North Dakota State University's spring wheat breeding program has released several FHB-resistant cultivars including high yielding and quality 'Glenn'. Based on its pedigree, Glenn resistance is supposed to be from 'Sumai3'. However, molecular analysis showed that Glenn does not possess any molecular markers associated with Sumai3-*Fhb1* locus, including umn10. Therefore, this study aimed to decipher the genetics of FHB-resistance in Glenn. To achieve this goal, a (GM) RIL population was developed from a cross between Glenn and the moderately susceptible line MN00261-4. The RIL population and parents were evaluated for different FHB traits including incidence (INC), severity (SEV), and deoxynivalenol (DON); and some agronomic traits, including height (HT). The experiments were conducted under field and greenhouse (GH) conditions in ND, and MN using artificial inoculation. Additionally, the GM population was genotyped using Diversity Array Technology (DArT). A linkage map was generated and used to identify QTL associated with FHB traits. A total of 15 QTL for SEV, 15 for INC, 7 for DON, and 8 for HT were identified. One major QTL (5B) for SEV was detected in four environments. Six QTL (1B, 5B, 6B, 7A, and 7D) for SEV and one QTL for INC (1A) were detected in two environments. The study did not identify any consistent QTL on chromosome 3BS.

OBJECTIVES

1. Identify the genetic source of resistance in Glenn.
2. Determine if Glenn contains *Fhb1*.

INTRODUCTION

FHB disease in the upper Midwest region of the US (ND and MN) led to hundreds of millions of dollars in losses for farmers since 1993 (Nganje *et al.*, 2001). In year 2000, $160 million losses were recorded; reflecting the importance and need for releasing resistant varieties. Most famous markers/QTL used today in wheat breeding programs are those located on chromosomes 3BS (*Fhb1*), 5AS, and 6BS (*Fhb2*). The highest magnitude of a QTL identified to date is *Fhb1* in Sumai3. This QTL can reduce the disease on average, depending on the genetic background, by 20–25% (Pumphrey *et al.*, 2007). However, there is evidence that combining major QTLs can pyramid FHB resistance levels (Miedaner *et al.*, 2006). Recently, Liu *et al.*, (2008) identified another effective marker (*umn10*) with close linkage to the 3BS *Fhb1* locus. This new marker was widely used in many breeding programs including HRSW breeding program at NDSU, ND, USA.

Glenn, a 2005 NDSU release, developed using classical breeding methods, has very high quality, excellent agronomic performance and high levels of FHB resistance (Mergoum et al., 2006). Based on its pedigree, Glenn resistance is supposed to be from 'Sumai3'. However, molecular analysis showed that Glenn does not possess any molecular markers associated with Sumai3-*Fhb1* locus (including the Umn10).

MATERIALS AND METHODS

Population: A RIL population was developed (Glenn/MN00261-4 (GM) from a cross between Glenn and the moderately susceptible line MN00261-4. The population and parent were evaluated for FHB in Prosper and Carrington (ND), MN and GH. The field experiments were inoculated artificially using dispersed diseased-kernels method; and the spore-suspension inoculation method was used in GH. All experiments were laid out in randomized complete block design (RCBD) with four replicates in ND and with two replicates in MN.

Phenotyping and Genotyping: Data collected for heading dates (HD), plant HT, and FHB SEV, INC, and DON. The genomic DNA was extracted from lyophilized tissue of young leaves using Qiagen DNeasy Plant mini kit (Cat# 69106) with some modifications. For each genotype 30 µl of DNA (80 ng/µl) was sent to Triticarte Pty .Ltd (Canberra, Australia; http://www.triticate.com.au) for Diversity Array Technology (DArT) analysis (Akbari et al., 2006).

Map construction and QTL Analysis: all polymorphic DArT markers were converted into genotype codes according to the parental scores. Linkage maps for each chromosome were constructed using MAPMAKER v. 2.0 (Lander et al., 1987). Single-locus QTL analysis was carried out by composite interval mapping (CIM) using QTL CARTOGRAPHER v. 2.5 (Wang et al., 2007) to identify the main effect QTL for each genotype.

RESULTS AND DISCUSSION

QTL analysis for FHB SEV and INC: CIM identified a total of 16 QTL for FHB severity from 6 environments (Table 1). The 16 QTL were located on 10 different chromosomes, with chromosomes 2D, 4B, 6A, 6B, and 7A carrying 1 QTL each, chromosomes 1B, 2B, 3B, and 7D carrying 2 QTL each and chromosome 5B carrying 3 QTL. Among all these QTL, 1 QTL (located on 5B) was detected in 4 environments; 6 QTL (located on 1B, 5B, 6B, 7A, 7D) were detected in 2 environments each, while the remaining 9 QTL (located on 1B, 2B, 2D, 3B, 4B, 5B, 6A) could be detected in only 1 environment. The phenotypic variation (PV) explained by individual QTL ranged from 6.4-20.5%. Among the 16 QTL, 8 showed major effects on FHB severity (PV>10%), while the remaining 6 had minor effects (PV<10%). The positive alleles for increased resistance at eight loci each were contributed by both parental genotypes. A total of 15 QTL for FHB incidence located on 10 different chromosomes (1A, 1B, 2D, 3B, 4A, 4B, 5B, 6A, 7A and 7D). The number of QTL identified in individual environments ranged from 2 (Prosper 2011) to 7 (MN 2010). Only 1 QTL for incidence (located on 1A) could be detected in 2 environments, while remaining QTL were detected in only 1 environment. The PV explained by individual QTL ranged from 4.94-16.34% (Table 1). Three QTL showed major effects, while the remaining 12 had minor effects. The alleles from Glenn contributed towards increased resistance at 8 loci and alleles from MN contributing towards increased resistance at the remaining 7 loci.

QTL Analysis for DON and plant HT: CIM identified a total of 7 QTL for DON; 5 in Prosper-2010 and 2 in Prosper-2011. The QTL were located on 6 different chromosomes; 2 on 2B and 1 each on 1A, 3B, 4A, 5B, and 7B. PV explained by individual QTL ranged from 7.26 to 13.88%. Four QTL had major effect of DON, while 3 showed minor effects. QTL alleles for increased DON at 4 loci were contributed by MN, while Glenn contributed alleles for the remaining 3 loci. A total of 8 QTL for HT located on 7 chromosomes, 2 on 7A and 1 each on 1A, 1B, 2A, 2B, 5B and 6A. PV explained by individual QTL ranged from 5.35-18.65%. Three QTL had major effect of HT, while 5

showed minor effects. The alleles for increased HT were contributed by both parents as 4 loci each.

Genome A: The QTL we identifies on chromosomes 1A and 4A were associated with Type III (DON) FHB resistance while those located on 2A and 6A were associated with HT. QTL on 7A however, was associated with all above traits (Table 1). In previous studies using meta-QTL analysis (Mao et al., 2010) and 16 different FHB resistance sources including Sumai3, the QTL confirmed on 3A and 5A related to reduction in SEV was associated with HT allele *Rht*. Li et al., (2011) described a QTL on 1A, and subsequently in 2012 another QTL on 7A, both from 'Haiyanzhong' Chinese cultivar. However, a QTL on 7A was reported by Jayatilake et al. (2011) but from Sumai3 and for Type III resistance rather. In our study, the identified QTL on 7A was associated with INC (type I), and SEV (Type II), and HT, not with Type III (DON).

Genome B: Our study identified many QTL on B chromosomes (Table 1). These include 2B, 3B and 5B (DON); 1B, 2B, and 5B (HT); 1B, 3B, 4B, and 5B (INC); 1B, 2B, 3B, 4B, 5B, and 6B (SEV). Most QTL were confirmed to be from a source different from Sumai3. For instance, previous studies did not identify QTL on 1B that was associated with type SEV, INC and HT. The only report (Xu et al., (2001) that indicated a QTL on 1B was identified using a double haploid population of Sumai3, but was associated only with type II (SEV). Another study (Srinivasachary et al., 2008) identified 7 QTL including one on 1B in a Canadian hard red spring wheat cultivar derived from 'Frontana'. Likewise, we believe that the QTL on 5B we identified in our study is novel. The only 5B QTL identified previously (Tamburic-Ilincic et al., 2009) was for resistance to *Fusarium* seedling blight in 'Wuhan'/'Nyubai' population. Similarly, our study confirmed that the QTL on 7B was novel and associated with low DON. Though many studies has intensively studied QTL on 3B and 6B, our study has identified new QTL on 3B and 6B (Fig.1) different from than those well-known (*fhb1* and *fhb2*) derived from Sumai3 (Patricia et al., 2006; and 2007).

Genome D: We identified QTL in our study on 2D, 7D (Table 1) that were associated with Type I and II FHB resistance. Previously, an association between a QTL on 2D and a HT gene (*Rht8c*) from 'Aka Komugi', a gibberellic acid GA-sensitive (J. Gilbert. and S. Haber, 2013) was established. The locus (*Rht-D1*) was reported to explain 38% of the PV for FHB Type I resistance with no effect on Type II (Lu et al., 2011). This infers that our QTL on 2D different from that identified by Lu et al., (2011). Additionally, another QTL from Sumai3 identified on 2D and associated with reduced kernel weight was also reported (Suzuki et al, 2012). However, Glenn kernel weight is high confirming that 2D FHB resistance in Glenn is may not be based on Sumai3.

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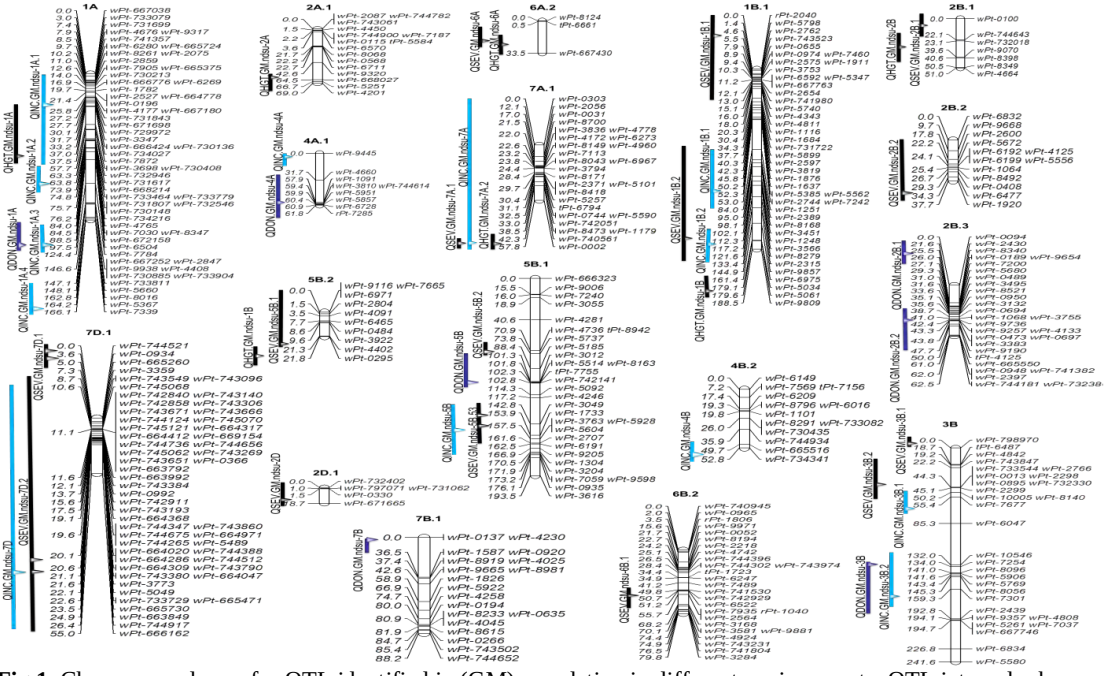

Fig.1. Chromosomal map for QTL identified in (GM) population in different environments. QTL intervals shown as (lines); QTL positions shown as (Triangles); QTL for SEV, INC, DON, and HT were shown in (Black, Blue, Brown, and Purple) respectively.

Table 1. Important QTL repeated in more than 2 environments for SEV, INC, DON and HT.

QTL	Chrom. (group)	Environments*	LOD	QTL effect	R^2 (%)	Other associated traits
FHB severity (SEV)						
QSEV.GM.ndsu-1B.2	1B.1	Pros-2010, MN-2010	3.21	3.44	10.26	INC
QSEV.GM.ndsu-2B.2	2B.2	Pros-2011	5.79	3.27	17.12	
QSEV.GM.ndsu-2D	2D.1	Pros-2012	3.7	-4.74	13.78	
QSEV.GM.ndsu-5B.2	5B.1	Carr-2011, Pros-2012	4.09	-4.47	12.09	DON
QSEV.GM.ndsu-5B.3	5B.1	GH-2011, Pros-2010, Pros-2012, Carr-2011	7.86	6.21	20.49	INC
QSEV.GM.ndsu-6B.1	6B.2	GH-2011, Pros-2011	4.29	-6.29	12.1	
QSEV.GM.ndsu-7A.1	7A.1	Carr-2011, MN-2010	2.6	-3.09	8.61	INC, HGT
QSEV.GM.ndsu-7D.1	7D.1	MN-2010,Pros-2010	2.86	7.23	10.44	
QSEV.GM.ndsu-7D.2	7D.1	Pros-2010, MN-2010	4.04	-8.27	12.39	INC
FHB incidence (INC)						
QINC.GM.ndsu-1A.1	1A	MN-2010	3.37	-4.1	9.03	HGT
QINC.GM.ndsu-3B.2	3B	MN-2010	2.04	-2.95	4.94	DON
QINC.GM.ndsu-7A	7A.1	MN-2010	2	-3.05	5.17	SEV-HGT
QINC.GM.ndsu-7D	7D.1	MN-2010	5.58	-16.59	16.34	SEV
QINC.GM.ndsu-1B.2	1B.1	Carr-2011	2.72	-1.56	10.12	Sev
QINC.GM.ndsu-4B	4B.2	Pros-2011	3.99	-1.22	12.82	
QINC.GM.ndsu-5B	5B.1	Pros-2011	2.3	0.84	6.9	SEV
QINC.GM.ndsu-1A.3	1A	Pros-2012	3.05	-3.7	8.32	DON
QINC.GM.ndsu-1B.1	1B.1	Pros-2012	3.27	3.65	8.9	Sev
QINC.GM.ndsu-3B.1	3B	Pros-2012	3.24	3.81	8.96	Sev
Deoxynivalenol (DON)						
QDON.GM.ndsu-1A	1A	Pros-2010	2.4	-0.09	10.19	INC
QDON.GM.ndsu-2B.2	2B.3	Pros-2010	4.52	-0.17	13.88	
QDON.GM.ndsu-5B	5B.1	Pros-2010	3.24	-0.1	12.61	SEV
QDON.GM.ndsu-7B	7B.1	Pros-2010	3.93	0.1	12.07	
QDON.GM.ndsu-3B	3B	Pros-2011	2.61	-0.34	9.06	INC
Plant height (HGT)						
QHGT.GM.ndsu-5B	5B.2	GH-2011	3.03	-1.55	8.02	SEV
QHGT.GM.ndsu-6A	6A.2	GH-2011	4.38	-1.87	13.31	SEV
QHGT.GM.ndsu-1A	1A	GH-2012	2.12	1.42	5.89	INC
QHGT.GM.ndsu-2A	2A.1	GH-2012	4.39	2.52	18.65	
QHGT.GM.ndsu-2B	2B.1	GH-2012	3.15	-1.98	11.84	SEV-DON
QHGT.GM.ndsu-7A.2	7A.1	GH-2012	3.03	1.79	9.65	INC-SEV

* QTL effect, indicating which parent contributes the positive or negative effect at which environment (Pros=Prosper, ND; Carr=Carrington, ND; GH=Greenhouse; MN= Minnesota State) ND; GH=Greenhouse; MN= Minnesota State)

ASSOCIATION MAPPING OF FHB RESISTANCE IN BARLEY UTILIZING HISTORIC NABSEN DATA AND GENOTYPE-BY-SEQUENCING

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ABSTRACT

Fusarium head blight (FHB) is one of the most devastating diseases of barley. The most insidious part of FHB infection is the production of harmful mycotoxins, with the most important being deoxynivalenol (DON). Since DON can cause many problems for end users DON assays have become a standard specification in grain purchasing. Thus, identifying loci conferring lower DON accumulation in barley is important for marker development and breeding for resistance. A total 441 lines of elite two-rowed and six-rowed barley from The North American Barley Scab Evaluation Nursery (NABSEN) were evaluated for DON content at four sites from 2002-2012. At each site the experimental design was a randomized complete block design with three blocks. DON levels were averaged across blocks for each line at each site and square root transformed to normalize the data. These values were then used as the response variable in a mixed effects model with sites treated as fixed effects and lines as random effects. Best Linear Unbiased Predictors (BLUPs) were estimated for each line using the lme4 package in R. To utilize the BLUPs to identify DON accumulation quantitative trait loci (QTL) by association mapping with JMP genomics program, we are genotyping the entire population of 441 lines using a PCR based genotype-by-sequencing method. All barley lines are being genotyped using a 384 single nucleotide polymorphism (SNP) marker panel containing known SNP markers that are evenly distributed across the barley genome. This information will potentially allow us to identify DON accumulation QTL in elite barley germplasm from the major barley breeding programs in the Northern Great Plains.

FHB RESISTANCE AND AGRONOMIC PERFORMANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

Local broadly adaptive cultivars have been crossed with *Fhb1* derived lines, Truman, and Jamestown to introduce FHB resistant QTL into adaptive genetic backgrounds. Elite lines with resistance from Truman, IN981359C1, or Jamestown, were evaluated in the field during 2013 for FHB resistance and agronomic performances. A double haploid line, GANC 8170, that was selected from the cross of NC-11458/Bess//SS 8641, showed a high level of FHB resistance which was similar to the resistant controls, Bess and Jamestown. The double haploid line also had low levels of resistance for FHB index, FDK, and ISK. The closely linked markers of the double haploid line were detected for the FHB resistant QTL, Fhb_ 5A_ Ning7840 and included important resistant genes for leaf and stripe rust (*Lr37/Yr17/Sr38*). In addition, an elite line, GA04151-11E26, that was evaluated in the 2013 Uniform Southern Wheat Nursery also showed a moderate level of FHB resistance and had very high grain yield. Several other lines with Jamestown, Truman, IN981359 and IN 97397 as source of resistance were identified with moderate levels for FHB index and ISK and high grain yield when compared to the checks "SS 8641" and "AGS 2035". These lines will be further evaluated for FHB and grain yield.

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MAPPING FHB RESISTANCE IN NATIVE SRW WHEAT CULTIVAR TRIBUTE

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ABSTRACT

Resistance to Fusarium head blight (FHB) has been deployed from native and exotic sources of wheat. Native sources of resistance are important because of their efficient utilization in breeding programs without yield drag. Since resistance to FHB is governed primarily by additive genetic effects, pyramiding different sources of resistance would be an effective approach to managing FHB through resistance breeding. The objectives of the study were to identify FHB resistance QTL in the native SRW cultivar Tribute and develop diagnostic markers for use by programs in marker-assisted breeding. A total of 115 double haploid (DH) lines were evaluated for FHB incidence and FHB severity by cooperators in AR, KY, MD, NC, and VA in 2013. Grain samples from VA have been analyzed for DON content. The population was genotyped using SSR markers. The genotype-by-location interaction was significant for the population. Correlation analyses of data among locations indicate that data from AR and MD are highly correlated as were data from VA and KY. Data from NC was correlated with that from all other locations. The results from single marker analysis indicate that SSR markers on chromosomes 2A (Xgwm47), 2D (Xgwm261), 3BSc (Xgwm78, Xgwm285, Xwmc418, Xwmc471), 5A (Xwmc795), 5D (Xwmc805, Xwmc443), and 7B (Xbarc95) were significantly associated with FHB incidence and severity across different locations. The SSR marker Xgwm47 on 2A also was significantly associated with DON content. The SSR marker Xgwm261 on 2D also is linked to *Rht8* and *Ppd-D1* and therefore use of the putative QTL on 2D may not be desirable in all programs. The putative QTL on 3BSc might be in a similar region as reported for Ernie, Wangshubai, and Nyu bai. The putative QTL on 2A, 5D, and 7B may be unique to Tribute. A second year of phenotypic data and 90K SNP genotypic data will be obtained and analyzed in the population to identify and validate QTL that can be used for marker-assisted breeding.

FUSARIUM HEAD BLIGHT RESISTANCE QTL IN THE KENYON X 86ISMN 2137 POPULATION

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ABSTRACT

Fusarium head blight (FHB) is one of the most damaging diseases of wheat in Canada. The genetic basis of FHB resistance in Asian spring wheats has been the focus of many genetic studies. FHB resistance in Canadian spring wheat germplasm has not been studied to date. The objective of this study was to identify QTL for FHB resistance in western Canadian spring wheat with the cross Kenyon x 86ISMN 2137. Kenyon is a Canada Western Red Spring (CWRS) variety with the pedigree Neepawa*5/ Buck Manantial. Neepawa was a major CWRS variety and a parent of many current varieties. Buck Manantial was the source of the leaf rust resistance gene *Lr16* in Kenyon. The wheat line 86ISMN 2137 is of unknown origin. A RIL population from this cross was tested in six environments over 2012 and 2013 for FHB visual rating index (VRI), plant height, and anthesis date. Simple and multiple interval mapping were used for QTL analysis. The strongest FHB resistance QTL were contributed by Kenyon on chromosomes 2D and 7D. 86ISMN 2137 contributed reduced plant height and increased FHB VRI on chromosome 2D at the map location of *Rht8*. So the 2D FHB resistance QTL is likely the pleiotropic effect of *Rht8*. Kenyon also contributed a putative FHB resistance QTL on chromosome 2A. 86ISMN 2137 contributed putative FHB resistance QTL on chromosomes 2B, 4A, and 4D. The minor FHB resistance QTL on 2B mapped near *Lr16*, with *Lr16* linked in coupling with FHB susceptibility. Kenyon was consistently more susceptible to FHB than Neepawa in these field tests, which supported this finding. The QTL identified in this study provide insight into FHB resistance QTL present in Canadian spring wheat varieties.

COMBATING FUSARIUM HEAD BLIGHT IN THE US SPRING WHEAT REGION: 'ELGIN-ND', A NEW HARD RED SPRING WHEAT CULTIVAR WITH HIGH LEVEL OF RESISTANCE

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OBJECTIVES

To develop new improved hard red spring wheat (HRSW) (*Triticum aestivum* L.) cultivars combining resistance to Fusarium Head Blight (FHB) disease [caused by *Fusarium graminearum* Schwabe (telomorph *Gibberella zeae* (Schwein.) Petch)] and superior grain yield and bread-making quality.

INTRODUCTION

Hard red spring wheat is the leading crop in the Central Northern US plains. In North Dakota (ND) in particular, HRSW is the major crop with about 6 million acres annually grown with an economic value surpassing one billion dollars. The development of superior HRSW cultivars is the key element to sustain future wheat productivity and maintain economic returns to ND growers and the wheat industry as well as the wheat export market. Scab or FHB has been for decades a major constraint for wheat worldwide and in ND, causing billions of dollars in losses in productivity and quality (McMullen et al., 1997; Nganje et al., 2004). Growing adapted genetically resistant HRSW cultivars is the best strategy for an efficient, economical, and safe control of FHB disease while protecting our environment. Recently developed superior NDSU HRSW cultivars (mainly 'Glenn' (PI 639273; Mergoum et al., 2006a); 'Faller' (PI 648350; Mergoum et al., 2008); and 'Barlow' (PI 658018; Mergoum et al., 2011)) with moderate FHB resistance are being grown on more than 50% of ND wheat acreages replacing susceptible cultivars or old very popular cultivars such as 'Alsen' (PI 615543; Frohberg et al., 2006), 1999; 'Steele-ND' (PI 634981; Mergoum et al., 2005b), 2004 and 'Howard', 2006 (PI 642367; Mergoum et al., 2006b) with the same levels of FHB resistance that dominated the spring wheat regions for years. Significant additional acreages in the neighboring states are grown to these cultivars as well. In MN for instance, Faller has been the leading cultivar since 2009 and 'Prosper' (PI 662387; Mergoum et al., 2012) was the leading in 2013 followed by Faller. The goal of the NDSU HRSW breeding program is to continue developing and releasing superior adapted cultivars to replace the susceptible cultivars grown in ND and the region.

MATERIAL AND METHODS

'Elgin-ND' was developed using a modified bulk breeding procedure. It was selected from the "Walworth/Reeder//ND721" cross made at NDSU between 'Reeder' (PI613586), the South Dakota State University (SDSU) cultivar 'Walworth' (PI630938), and the experimental line ND721. Reeder was released by NDSU in 1999 (PVP200000211) with excellent adaptation to Western ND and Eastern MT where drought is predominant. Walworth is also a HRSW cultivar but released by SDSU in 2002 (PVP#200200108) for it high yield. ND721, is an elite HRSW line developed by NDSU from the Grandin

'/3/IAS20*4/H567.71//'Amidon'/4/ND 674 cross. ND721 is an excellent combiner and possesses good agronomic characteristics, quality attributes and diseases resistance including FHB. Based on its pedigree, Elgin-ND resistance to FHB is not well known and could not be traced to 'Sumai 3' source.

Elgin-ND was selected from a bulk of one purified F_5 row-plot selected in 2002 winter nursery grown at Christchurch, NZ. Elgin-ND was first tested for agronomic and quality performance in preliminary (PYT), Intermediate (IYT), advanced (AYT) and elite yield trials (EYT) in up to four replicates at two to seven locations in ND from 2003 to 2008. From 2009 to 2012, Elgin-ND was tested as ND 818 at 22 location-years in the North Dakota Variety Trials (NDVT).

Screening of Elgin-ND for FHB was conducted from 2009 to 2011 in 8 FHB nurseries under field conditions. The FHB nursery was conducted at Prosper and Langdon, ND. The experiments were laid out in a RCBD with four replicates and inoculated with the FHB pathogen using the "Spray Inoculation Method" with overhead mist irrigation to enhance disease development. Entries were assigned to a hill plot consisting of eight to ten plants. Ten spikes from each hill were taken at random and evaluated for FHB disease incidence and severity (Stack and Frohberg, 1997).

The reaction type of Elgin-ND to the prevalent races of leaf rust (caused by *Puccinia triticina* Eriks.) was done on the basis of six field tests (RCBD, four replicates, and 1 m row-plot per replicate) and four greenhouse tests (RCBD, three replicates, and four plants per replicate) from 2009 to 2011. Similarly, Elgin was screened for major races of stem rust (caused by *Puccinia graminis* Per.:Pers. f. sp. *tritici* Eriks. & E. Henn) in 9 field and 3 greenhouse experiments. In the greenhouse experiments, Elgin-ND was specifically evaluated for resistance to the predominant stem rust pathotypes Pgt-TMLK, -QTHJ, -QFCQ, -RTQQ, -TPMK, -THTS, and –TCMJ; and leaf rust pathotypes MCDL and THBJ.

RESULTS

Elgin-ND was tested under experimental line ND 818 and was released because it combines very high yield and agronomic traits (Table 1), resistance to FHB and leaf diseases (Table 2), and excellent end-use quality (Table 1 and 3), particularly, grain protein (Table 1).

Based on 22 site-years of testing in the NDVT and AYT, grain yield of Elgin-ND (4001 kg ha^{-1}) was significantly higher than most released cultivars by NDSU, SDSU, the U of MN except for Barlow (3913 kg ha^{-1}), Faller (3957 kg ha^{-1}), and Prosper (3973 kg ha^{-1}). These three cultivars are all released by NDSU and are the leading cultivars in ND and MN. Elgin-ND is a semi-dwarf cultivar and tends to head later than most cultivars in general, the same as Faller and Prosper.

Under severe FHB disease pressure, the average disease severity (Stack and Frohberg, 2000) recorded on Elgin-ND from the field scab nursery (28%) was lower than the very susceptible check '2398' (73%) (Table 2). In the same trials, the average FHB severities recorded for Barlow, Faller, Glenn, and ND 2710 (PI 633976) were 25, 27, 25, and 13%, respectively. Glenn is an NDSU HRSW released cultivar with FHB resistance and was the leading cultivar in ND and the spring wheat region from 2007 to 2011. Similarly, Barlow (also an NDSU release) became the most popular cultivar in ND since 2012. Based on natural FHB inoculation, visually scabby kernels of Elgin-ND (0.6%) was low compared to the susceptible checks 'Velva' (2.4%) (Mergoum et al., 2013) (Table. 1).

The seedling and adult plant screening tests conducted under greenhouse and field conditions from 2009-2011 showed that Elgin-ND possesses high level of resistance to the predominant races of leaf and stem rusts. Under greenhouse tests, Elgin-ND was resistant to pathotypes MCDL and THBJ, the predominant race of leaf rust in the region (Table 2). However, Elgin-ND seems to be medium susceptible to the new race of leaf rust that has overcome the *Lr21* gene. Elgin-ND was

also highly resistant to stem rust pathotypes Pgt TPMK, TMLK, RTQQ, QFCQ, QTHJ, THTS, and TCMJ (Table 2).

Quality parameter including Falling number (FN), Flour extraction (FE), dough and baking parameters for Elgin-ND and major grown NDSU HRSW cultivars are reported in Table 3. Mean FN and FE, mixing time and tolerance of Elgin-ND over 20 location-years in NDVT from 2009-2011 were respectively, 406 s, 68.2 gkg^{-1}, 7.8 min, and 11 min compared to 357 s, 68.2 gkg^{-1}, 10 min, and 15 min registered for Glenn (our best quality check); and 388 s, 70.4 gkg^{-1}, 7 min, and 10 min for Barlow, the most popular cultivar in ND since 2012. Similarly, average Loaf volume and water absorption of Elgin-ND were respectively, 1005 ml and 66.3% compared to 1064 ml and 65.4%; 996 ml and 67.5% scored by Glenn and Barlow, respectively. Additionally, Elgin-ND has average kernel weight (28 g for the 1000 kernel weight) and with average grain volume weight (732 compared to 769 and 750 kg m^{-3} of Glenn and Barlow, respectively) (Table 1). Grain protein of Elgin-ND was high (154 g kg^{-1}), similar to Glenn and Barlow (Table 1).

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Table 1. Summary of agronomic data for Elgin-ND hard red spring wheat (HRSW) and check cultivars tested in the ND HRSW Variety Trials (2010-2012).

Variety	Days to Heading	Plant Height	Lodging†	FHB FDK	Leaf Diseases	1000 Kernel Weight	Grain Volume Weight	Grain Yield	Grain Protein
	Day	cm	1-9	%	%	g	kg m^{-3}	kg ha^{-1}	%
Advance	60.3	77.9		1.0	13.8	25.5	747	3546.9	14.8
Barlow	60.0	85.9	0.8	1.4	9.0	29.1	750	3912.6	15.4
Brick	56.4	86.2	1.3	0.2	30.6	29.3	756	3697.5	14.8
Briggs	59.0	84.6	1.4	0.7	10.0	29.4	746	3645.6	15.0
Dapps	55.4	88.9	1.7	0.3	8.9	30.3	734	3296.3	15.9
Elgin-ND	**62.1**	**88.8**	**0.5**	**0.6**	**10.7**	**27.8**	**732**	**4001.4**	**15.4**
Faller	63.1	83.1	0.4	0.2	10.9	29.5	726	3956.7	14.5
Forefront	57.8	88.9		0.4	18.8	28.2	753	3543.6	15.4
Glenn	59.6	88.0	0.2	0.0	13.0	29.5	769	3639.7	15.4
Norden	61.6	76.9	0.2	0.7	22.2	27.2	750	3779.3	15.0
Prosper	63.6	83.1	0.7	0.2	7.5	30.3	729	3972.9	14.7
RB07	60.4	78.2	1.0	0.1	14.6	27.0	735	3788.6	15.0
Reeder	60.8	83.4	0.2	1.8	6.4	28.0	733	3627.9	15.1
Select	58.2	84.9	0.9	0.8	37.1	28.9	749	3876.3	14.8
Steele-ND	61.1	85.5	1.5	1.2	17.7	28.9	744	3831.4	15.4
Velva	63.2	80.7	0.0	2.4	11.0	28.3	715	3759.9	14.9
LSD (0.05)	2.3	3.7	0.7	1.2	5.7	2.2	12	111.3	1.1
No of environment	22	22	5	3	4	22	22	22	21

† Lodging score: 1=completely erect to 9=completely flat at harvest.

Table 2. Diseases reactions of Elgin-ND and hard red spring wheat (HRSW) checks tested in the ND HRSW Variety Trials (2009-2011).

Genotype	FHB[†]	Leaf rust		Stem rust	
		Greenhouse[‡]	Field	Greenhouse[§]	Field
	%				
Elgin-ND	**28**	**R[¶]**	**tR/5R**	**R**	**R/MR**
Barlow	25	R	MR/MS	MR/R	5R
Faller	27	R	MR/MS	R	R
Glenn	25	-	R	R	R
2398	73	R	S	MR/R	5R
ND2710	13	R	R	-	-
No of environment	8	4	6	3	9

[†] FHB (Fusarium Head blight) severity as described by (Stack and Frohberg, 1997).
[‡] Greenhouse reactions for leaf rust races MCDL and THBJ.
[§] Greenhouse reactions for stem rust races Pgt TPMK, TMLK, RTQQ, QFCQ, QTHJ, THTS, and TCMJ.
[¶] R=resistant, MR=Moderate resistant, MS=Moderate susceptible, S=Susceptible, tR= trace/Resistant.

Table 3. Quality parameters of Elgin-ND and hard red spring wheat (HRSW) checks tested in 20 location-years in the ND HRSW Variety Trials (2010-2011).

Cultivar	Falling number	Flour Extraction	Mixing time	Mixing tolerance	Loaf volume	Water absorption
	sec	g kg^{-1}	min	min	ml	%
Elgin-ND	**406**	**68.2**	**7.8**	**11.8**	**1005**	**66.3**
Barlow	388	70.4	7.0	10.0	996	67.5
Faller	389	70.5	8.4	10.8	1005	63.9
Glenn	357	68.2	10.0	15.0	1064	65.4
Mott	373	68.8	7.0	11.0	978	64.3
Velva	404	67.4	6.8	10.6	959	64.8
Mean-Checks	382	69.1	7.8	11.5	1000	65.2
No of environment	20	20	20	20	20	20

MAPPING FUSARIUM HEAD BLIGHT RESISTANCE QTL IN A MID-ATLANTIC-ADAPTED BREEDING POPULATION

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ABSTRACT

Wheat host resistance to Fusarium head blight (FHB) is a quantitative trait historically mapped using biparental mapping populations. Utilizing breeding populations in quantitative trait loci (QTL) mapping will greatly minimize costs and time required for QTL of desired traits to be incorporated in commercial varieties. The goal of this project is to fine-map QTL of FHB resistance, particularly on chromosome 2D, from a tri-parental cross of the highly FHB-resistant Chinese spring wheat cultivar Ning7840 and soft red winter wheat cultivars McCormick and SS8641. At each generation of selfing, lines were selected based on flanking SSR markers of known resistance QTL from donor parent Ning7840 to target lines with recombination events near these QTL. Fifty-six of these selected lines were genotyped using the wheat 9K iSelect assay and additional SSR markers. Lines were phenotyped for FHB resistance in a greenhouse study in College Park, MD and two field trials, located in Salisbury, MD and Lexington, KY. Phenotypic data from the 2012-2013 season suggest that the putative QTL on chromosome 2D from donor parent Ning7840 has no significant effect on scab resistance in the absence of Fhb1. Further, there may be resistance contributed by local cultivars. To better elucidate these phenomena, the sample size for the 2013-2014 field trials was increased to explore resistance contributed by minor QTL in both the presence and absence of Fhb1. In total, 138 lines will be phenotyped in a greenhouse study in College Park, MD and three field locations, located in Upper Marlboro, MD; Lexington, KY; and Kinston, NC. Lines will be genotyped using the wheat 9K iSelect assay and additional KASP and SSR markers on chromosomes 2D and 3B for increased resolution.

THE 2012-13 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 2012-13 nursery comprised 58 advanced generation breeding lines and four check cultivars, 'Ernie', 'Bess', 'Jamestown' (partially resistant) and 'Coker 9835' (susceptible). Eight U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State University, Univ. of Maryland, N.C. State Univ., VA Tech., and USDA-ARS), and two private companies (Agripro-Coker, and Limagrain) submitted entries. The nursery was distributed to 11 U.S., one Romanian, and one Hungarian cooperator 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 based on established diagnostic markers.

The mean level of FHB resistance in the nursery was high. Between 60 and 84 percent of entries had significantly better means than the susceptible check for Severity, Index, FDK, ISK and DON. Sources of resistance included Chinese, South and North American.

Copies of the full report will be available at the 2013 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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Table 1. Genotypic Analyses of Regions Associated with FHB Resistance and Other Pertinent Loci.

	DESIGNATION	Rht-B1	Rht-D1	Rht8	Ppd-D1a insens	vrn-A1 short	vrn-B1 short	Lr34/Yr18	Lr37/Yr17/Sr38	Sr2	Sr36	Sr24/Lr24	Lr9	Fhb1	Fhb Massey 3BL	Fhb 5A ERNIE	Fhb 5A Ning7840	Fhb 2DL- Wuhan1W
1	ERNIE	b	a	no	no	yes	no	no	no	no	yes	no	no	no	yes	yes	no	no
2	COKER9835	a	b	no	yes	no	yes	no	no	no	yes	no	yes	no	no	no	no	no
3	BESS	het	het	no	het	no	no	no	no	no	no	no	no	no	no	no	no	no
4	JAMESTOWN	a	b	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
5	MD03W61-11-3	a	b	no	yes	no	no	no	no	no	no	no	no	yes	no	no	no	no
6	ARS07-1214	b	a	no	no	no	no	no	no	no	no	yes	no	no	no	no	no	no
7	ARS09-367	b	a	no	no	no	no	no	no	no	no	yes	no	no	no	no	no	no
8	ARS09-446	a	a	no	no	no	yes	no	no	no	yes	no	no	no	no	no	no	no
9	ARS09-643	a	b	no	yes	no	yes	no	no	no	no	no	no	no	no	no	no	no
10	LA05102C-1-2	b het	a	no	yes	het	no	no	no	no	yes?	no	no	no	no	no	no	het
11	LA05102C-8-8	a	a	no	yes	no	yes	no	no	no	yes	no	no	no	no	no	no	no
12	NC09-20986	b	a	no	no	no	no	no	yes	no	no	no	no	yes	no	no	no	no
13	AR00260-2-2	a	b	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
14	AR01044-1-1	b	a	no	yes	yes	yes	no	yes	no	no	yes	no	no	no	no	no	no
15	AR01110-3-1	b	a	no	yes	no	no	no	no	no	no	no	no	no	yes	no	no	no
16	AR01178-1-1	b	a	no	yes	het	no	no	no	no	no	no	no	no	no	no	no	no
17	ARGE05-1229-2-1	b	a	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
18	ARGE07-1339-10-5-8	b het	a	no	no	no	no	no	no	no	het	no	no	no	no	no	no	het
19	ARGE07-1374-17-5-4	a	b	no	yes	no	no	no		no	yes	no	no	no	no	no	no	no
20	ARGE07-1374-17-8-5	a	b	no	yes	no	no	no	no	no	yes	no	yes	no	no	no	no	no
21	ARS07-1073	a	b	no	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
22	ARS09-082	a	b	no	yes	no	yes	no	no	no	yes	no	yes	no	no	no	no	no
23	ARS09-228	a	a	Rht8c?	yes	no	no	no	no	no	yes	no	no	no	no	no	no	no
24	ARS09-745	a	b	no	yes	no	no	no	yes	no	no	no	no	no	no	no	no	no
25	GA04494-12ES33	a	b	no	yes	no	no	no	yes	no	no	no	no	no	no	yes	no	no
26	GA051477-12ES27	a	b	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
27	GA051477-12ES28	a	b	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
28	GA051477-12ES29	a	b	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no	no
29	GA051477-12ES32	a	b	no	yes	yes	het	no	no	no	no	no	no	no	no	yes	no	no
30	GANC8170-12DH7	a	b	no	yes	no	no	no	yes	no	no	no	no	no	no	no	yes	no
31	GANC8248-12DH1	a	b	no	yes	no	no	no	no	no	yes	no	no	no	no	no	no	no
32	GANCZ4-12DH21	a	b	no	yes	no	no	no		no	no	no	no	no	no	no	no	no
33	LA05079F-P05	b	a	no	no	no	het	no	no	no	no	no	no	no	no	no	no	no
34	LA06069E-P01	a	b	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
35	LA06149C-P7	a	b	no	no	yes	yes	no	no	no	no	no	no	no	no	no	no	no
36	LA07085CW-P4	a	b	no	yes	?	no	no	yes	no	het	no	no	no	no	no	no	no
37	LA07178C-44	a	b	no	no	yes	no	no	no	no	no	no	yes	no	no	no	no	no
38	LCS19227	a	b	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
39	LCS15963	b	a	no	no	no	no	no	no	no	no	no	yes	no	no	no	no	no
40	M10-1615	b	a	nd	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
41	M10-1659	a	a	no	no	yes	no	no	no	no	yes	no	yes	no	no	no	no	no
42	MD04W249-11-12	a	b	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
43	MD04W249-11-7	a	b	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
44	MD07W272-11-5	a	b	no	yes	yes	no	no	no	no	no	no	no	yes	no	no	no	no
45	MD08-26-H2-7-12-21	a	b	no	yes	no	no	no	yes	no	no	yes	no	yes	no	no	yes	yes
46	MD08-26-H2-7-12-9	a	b	no	yes	no	no	no	yes	no	no	yes	no	yes	no	no	yes	yes
47	MDNC8248-64	a	b	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
48	MH07-7474	b	a	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
49	NC09-20768	a	b	no	yes	no	no	no	no	no	yes	no	no	no	no	no	no	no
50	NC09-22352	b	a	Rht8c?	no	no	no	no	no	no	yes	no	no	no	no	no	no	no
51	NC10-25212	a	a	no	no	no	no	no	no	no	yes	no	yes	no	no	no	no	no
52	NC8170-4-3	a	b	no	yes	no	no	no	no	no	no	no	no	yes	no	no	no	no
53	NC8170-45-2	a	b	no	yes	no	no	no	no	no	no	no	no	yes	no	no	no	no
54	NC8840-19	a	a	no	yes	no	no	no	no	no	yes	no	no	yes	no	no	no	no
55	VA10W-112	a	b	no	yes	no	no	no	yes	no	no	no	no	no	no	no	no	no
56	VA10W-118	a	b	no	yes	no	no	no	yes	no	no	no	no	no	no	no	no	no
57	VA10W-119	a	b	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no
58	VA11W-FHB110	b	a	no	yes	yes	no	no	no	no	yes	no	yes	no	het	no	no	no
59	VA11WFHB57	het	a	no	no	yes	no	no	no	no	yes	no	yes	no	no	no	no	no
60	VA11W-FHB60	a	b	no	yes	no	yes	no	no	no	no	no	no	yes	no	no	no	no
61	VA11W-FHB61	a	b	no	yes	yes	no	no	no	no	no	no	no	yes	no	no	no	no
62	VA11W-FHB75	b	a	no	yes	yes	no	no	no	no	yes	no	no	yes	no	no	yes?	yes

	DESIGNATION	1RS	H9	H13	Sbm1	Tsn1	Glu-B1	Glu-D1	Glu-A1	Sucrose Synthase	Combined Colour
1	ERNIE	non-1RS	no		no	no	no	2+12	Ax1_or_null	yes	RR rr rr
2	COKER9835	non-1RS	no	no	yes	no	no	2+12	Ax2*	yes	rr RR RR
3	BESS	non-1RS	no	no	yes	yes	no	het	Ax1_or_null	no	RR rr RR
4	JAMESTOWN	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	RR RR RR
5	MD03W61-11-3	1RS:1BL	no	no	yes	no	no	5+10	Ax1_or_null	no	rr RR RR
6	ARS07-1214	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	no	RR rr rr
7	ARS09-367	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	no	RR rr rr
8	ARS09-446	non-1RS	no	no	no	no	no	5+10	Ax2*	yes	RR rr RR
9	ARS09-643	1RS:1AL	no	no	yes	no	Bx7oe_het	2+12	Ax2*	no	RR rr RR
10	LA05102C-1-2	1RS:1BL	no	no	yes	no	no	het	Ax2*	yes	RR RR RR
11	LA05102C-8-8	non-1RS	no	no	yes	no	no	2+12	Ax2*	yes	RR RR rr
12	NC09-20986	non-1RS	no	yes	yes	no	no	2+12	Ax2*	no	RR rr RR
13	AR00260-2-2	non-1RS	no	no	yes	no	no	5+10	Ax2*	no	RR RR RR
14	AR01044-1-1	non-1RS	no	no	yes	no	no	2+12	Ax1_or_null	yes	RR RR rr
15	AR01110-3-1	non-1RS	no	no	yes	no	no	5+10	Ax2*	no	RR rr rr
16	AR01178-1-1	non-1RS	no	no	no	no	Bx7oe	5+10	Ax1_or_null	no	RR rr RR
17	ARGE05-1229-2-1	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	no	RR Rr rr
18	ARGE07-1339-10-5-8	1RS:1AL	no	no	yes	yes	no	2+12	het	het	rr rr RR
19	ARGE07-1374-17-5-4	non-1RS	no	no	yes	no	no	2+12	Ax2*	yes	Rr rr RR
20	ARGE07-1374-17-8-5	non-1RS	no	no	yes	no	no	2+12	Ax1_or_null	yes	Rr rr RR
21	ARS07-1073	1RS:1AL	no	no	yes	no	no	2+12	Ax2*	no	RR RR RR
22	ARS09-082	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	yes	RR RR RR
23	ARS09-228	non-1RS	no		yes	no	no	5+10	Ax1_or_null	yes	RR rr rr
24	ARS09-745	non-1RS	no	no	yes	yes	no	2+12	Ax2*	no	RR rr rr
25	GA04494-12ES33	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	RR RR rr
26	GA051477-12ES27	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	RR rr RR
27	GA051477-12ES28	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	RR RR RR
28	GA051477-12ES29	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	RR rr RR
29	GA051477-12ES32	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	rr rr RR
30	GANC8170-12DH7	1RS:1BL	no	no	yes	no	no	5+10	Ax2*	no	RR RR RR
31	GANC8248-12DH1	non-1RS	no	no	yes	no	no	2+12	Ax1_or_null	yes	RR rr RR
32	GANCZ4-12DH21	1RS:1AL	no	no	yes	no	no	2+12	Ax1_or_null	no	RR RR RR
33	LA05079F-P05	non-1RS	no	no	yes	no	no	2+12	Ax1_or_null	no	RR RR RR
34	LA06069E-P01	non-1RS	no	no	yes	no	no	2+12	Ax1_or_null	no	RR rr RR
35	LA06149C-P7	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	RR RR RR
36	LA07085CW-P4	non-1RS	no	no	het	no	no	2+12	Ax2*	het	Rr RR RR
37	LA07178C-44	non-1RS	no	no	yes	no	no	2+12	het	no	RR rr RR
38	LCS19227	non-1RS	no	no	yes	no	no	het	Ax1_or_null	no	RR rr RR
39	LCS15963	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	no	RR RR rr
40	M10-1615	non-1RS	no		yes	no	no	2+12	Ax2*	no	RR rr rr
41	M10-1659	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	yes	RR RR rr
42	MD04W249-11-12	1RS:1BL	no	no	yes	no	no	2+12	Ax1_or_null	no	RR rr RR
43	MD04W249-11-7	1RS:1BL	no	no	yes	no	no	2+12	Ax1_or_null	no	RR rr RR
44	MD07W272-11-5	1RS:1BL	no	no	yes	no	no	het	Ax2*	no	RR rr RR
45	MD08-26-H2-7-12-21	non-1RS	no	no	yes	yes	no	2+12	Ax2*	no	RR RR RR
46	MD08-26-H2-7-12-9	non-1RS	no	no	yes	yes	no	2+12	Ax2*	no	RR RR RR
47	MDNC8248-64	1RS:1AL	no	no	yes	no	no	2+12	Ax2*	no	RR rr RR
48	MH07-7474	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	no	RR rr RR
49	NC09-20768	non-1RS	no	no	yes	no	no	2+12	Ax2*	yes	rr rr RR
50	NC09-22352	1RS:1BL	no	no	yes	yes	no	5+10	Ax1_or_null	yes	RR RR RR
51	NC10-25212	non-1RS	no	no	yes	no	no	5+10	Ax1_or_null	yes	RR rr rr
52	NC8170-4-3	non-1RS	no	no	yes	no	no	5+10	Ax2*	no	RR RR RR
53	NC8170-45-2	non-1RS	no	no	yes	no	no	5+10	Ax2*	no	RR RR RR
54	NC8840-19	non-1RS	no	no	yes	no	no	2+12	Ax1_or_null	yes	RR rr rr
55	VA10W-112	non-1RS	no	het	yes	yes	no	2+12	Ax2*	no	rr rr RR
56	VA10W-118	non-1RS	no	no	yes	yes	no	2+12	Ax2*	no	rr rr RR
57	VA10W-119	non-1RS	no	yes	yes	yes	no	2+12	Ax2*	no	RR rr rr
58	VA11W-FHB110	non-1RS	no	no	yes	no	no	2+12	het	yes	RR rr RR
59	VA11WFHB57	1RS:1BL	no	no	yes	no	no	5+10	Ax1_or_null	yes	RR rr RR
60	VA11W-FHB60	1RS:1BL	no	no	yes	no	no	2+12	Ax1_or_null	no	rr RR RR
61	VA11W-FHB61	non-1RS	no	no	yes	no	no	2+12	Ax2*	no	rr rr RR
62	VA11W-FHB75	1RS:1BL	no	no	yes	no	no	2+12	Ax1_or_null	yes	RR rr RR

PATTERNS OF SINGLE KERNEL DEOXYNIVALENOL LEVELS IN ARTIFICIALLY INOCULATED WHEAT SPIKES AS DETECTED BY NEAR INFRARED SPECTROSCOPY

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Abstract

Evaluation of single-kernel deoxynivalenol (DON) levels of artificially inoculated wheat spikes facilitates study of the magnitude and patterns of DON accumulation among kernels with reference to the point of inoculation. This can illustrate some modes of Fusarium Head Blight (FHB) resistance in wheat germplasm. Therefore, using FHB susceptible (S) and moderately resistant (MR) wheat cultivars, a study was conducted to estimate single-kernel DON levels in kernels extracted from artificially inoculated wheat spikes to investigate the patterns and magnitudes of DON accumulation.

Wheat spikes from cultivars 2137 (S), Overley (S), Everest (MR), and Heyne (MR) were used in this study. The 10^{th} spikelet from the basal end of each spike was inoculated with a spore suspension (10 µl of $1X10^5$/ml) of *F. graminearum* (isolate 3639) at the time of flowering and the inoculated spikelet was tagged. Non-inoculated spikes grown under the same conditions were used as controls. Spikes were harvested when the kernels were mature.

Six inoculated and six non-inoculated spikes from each cultivar were randomly selected to extract kernels for DON determination. Kernels in each spikelet were manually removed beginning from the basal end upwards. The kernels in each spikelet were separately placed in cells of numbered pill boxes. To identify the position of kernels in the spike in relation to the point of inoculation, the 10th spikelet with the inoculated floret was designated as spikelet 0; those above the central spikelet and progressing towards the distal end of the spike were assigned sequential positive integers; and those below and progressing towards the basal end of the spike were assigned sequential negative integers. A single kernel from the 2-3 kernels extracted from each spikelet was randomly selected and its DON level was determined by a Single Kernel Near-Infrared (SKNIR) instrument. The average DON level and the standard error for DON level in each spikelet position were calculated.

Kernels in inoculated spikelets (Spikelet 0) had very high levels of DON compared to other kernels in the spike. In susceptible cultivars 2137 and Overley, high levels of DON were also detected in kernels extracted from spikelets away from the inoculated spikelet. However, DON levels of kernels gradually decreased as the distance from the inoculated spikelet increased in both directions. Moderately-resistant cultivars Everest and Heyne reacted in a different manner in that DON accumulation was detected mostly in the kernels in inoculated spikelets or the one below it. Kernels in non-inoculated spikes had no detectable levels of DON. These results show that cultivars Everest and Heyne not only resist the spread of FHB infection compared to cultivars 2137 and Overley but that resistance is reflected in DON accumulation patterns.

The susceptible cultivar Overley accumulated significantly higher levels of DON in infected kernels compared to the other susceptible cultivar 2137 when grown and inoculated under similar environmental conditions. This may be due to a slightly higher resistance of 2137 for DON accumulation compared to Overley.

This NIR spectrometric method may be useful for the evaluation of modes of FHB resistance in wheat cultivars such as resistance to spread of infection within spike (Type II resistance) and resistance to toxin (DON) accumulation (Type III resistance).

ACKNOWLEDGEMENT AND DISCLAIMER

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QTL ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN THE NC-NEUSE X AGS 2000 RECOMBINANT INBRED POPULATION

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ABSTRACT

Breeding for resistance to Fusarium Head Blight is of major importance, as the disease can have serious negative impacts on wheat production in warm and humid regions of the world, including the state of North Carolina. Fusarium Head Blight can cause significant grain yield reduction, but also severely affect the grain quality due to accumulation of mycotoxins produced by the pathogen. The importance of finding native sources of resistance in U.S. soft red winter wheat lines has been emphasized in recent years. The North Carolina cultivar NC-Neuse is a moderately FHB resistant soft red winter wheat, released in 2003.

A population of 179 random F_5-derived recombinant inbred lines derived from a cross between 'NC-Neuse' and the FHB susceptible line 'AGS 2000' was evaluated for FHB resistance at one field location (3 reps) in the 2010-11 field season, at two field locations (2 reps/loc) in the 2011-12 season, and two field locations in the 2012-13 season (3 reps/loc). The FHB related traits evaluated included disease incidence (INC), severity (SEV), *Fusarium* damaged kernels (FDK), and accumulation of the mycotoxin deoxynivalenol (DON).

A linkage map developed prior to this QTL mapping study was updated with a total of 1839 polymorphic SSR, DArT and SNP markers across 27 linkage groups was utilized for mapping of QTL. QTL analysis for individual environments and across environments was conducted using Composite Interval Mapping (CIM) and then Multiple Interval Mapping (MIM) with WinQTLCart vs. 2.5. The critical LOD value to declare QTL significance was 3.2, based on 1000 permutations.

Preliminary results showed QTL associated with one or more FHB resistance traits on chromosomes 1A, 1B, 2A, 4A, 4B, 5B, and 6A. Their LOD score values ranged from 3.2 to 5.69 with R^2 values of 6.0% to 14.8%.

An update on pertinent results will be presented.

PROMISING FUSARIUM HEAD BLIGHT RESISTANCE IN DURUM WHEAT

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ABSTRACT

Cultivated tetraploid wheat, especially durum wheat (*Triticum durum*), is highly susceptible to the wide-spread disease Fusarium head blight (FHB). While many resistance QTL have been reported in hexaploid wheat (*Triticum aestivum*) the QTL identified to date in tetraploid wheat do not provide satisfactory FHB resistance. Consequently, several groups have introgressed resistance alleles from wild and cultivated relatives into durum. In this study, back-cross lines derived from crosses of *T. durum* and FHB resistance sources including *Triticum dicoccum*, *Triticum dicoccoides* and *Triticum aestivum* were used as resistant parental lines in several bi- and multi-parental crosses with *T. durum* cultivars. A large population was developed allowing the evaluation of FHB resistance derived from relatives in an agronomically acceptable durum background. This population was evaluated in 2012 and 2013 in disease nurseries at the site of the Institute for Biotechnology in Plant Production (BOKU, Austria) through artificial spray inoculation of *Fusarium culmorum* macroconidia at anthesis. The population showed a large spectrum of response for FHB resistance ranging from highly resistant to susceptible. FHB severity was significantly negatively correlated with plant height (r=-0.51 in 2012 and r=-0.46 in 2013) but variation for FHB resistance was observed among the short lines (≤ 80 cm height) with several resistant lines. Variation for flowering date was low and FHB resistant lines were present in all maturity groups. A subset of 475 lines will be analysed through both linkage and genome-wide association mapping. The lines will be genotyped in high-density at INRA Clermont-Ferrand (France) using the **GENTYANE** platform and phenotyped at two locations: Florimond-Desprez in Cappelle-en-Pévèle (France) and BOKU University in Tulln. We expect to unveil QTL linked with resistance and/or increased susceptibility and to evaluate the importance of epistatic interactions for FHB resistance in durum wheat.

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EFFECTS OF LATE-SEASON RAIN/SIMULATED RAIN AND GRAIN DRYING ON SELECTION FOR LOW DON CONCENTRATION IN WHEAT GRAIN

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ABSTRACT

Developing wheat cultivars with low concentrations of deoxynivalenol (DON) in grain is a high priority for breeding programs funded by the USWBSI, so it is important to understand the factors affecting DON concentration in grain. Some studies on the effects of late-season moisture found increases in DON while others found decreases due to leaching. The objectives of this study were to determine effects of late-season rain and misting on DON concentration in wheat spike tissues and to quantify the amount of DON leached from spikes. Field experiments were conducted on susceptible and moderately resistant wheat cultivars affected by FHB utilizing spike holders to catch water leaching through groups of 20 spikes, rain shelters to protect plots from rain and misting, and a rainfall simulator to apply simulated rain. Groups of spikes in spike holders were either not treated or treated with various amounts of rain/simulated rain. A sample of water that leached through the spikes was frozen at -80°C, lyophilized, and analyzed for DON. Spikes were dried in a grain dryer at 65°C, and samples of grain and chaff were analyzed for DON. A critical component of these experiments was having groups of spikes with similar levels of DON at the beginning of experiments, and methods were developed to make groups as similar as possible and to statistically test for similarity such that dissimilar groups could be eliminated to improve the accuracy of results. DON was detected in all water samples, indicating that leaching of DON is a common phenomenon. Similar percentages of DON leached from most spike samples that received a particular rain treatment, indicating that the amount leached is proportional to the amount in the sample. Chaff and scabby grain had the highest concentrations of DON and the greatest reductions in DON after rain treatments. DON concentrations in total grain were reduced with a large simulated rain event at soft dough stage and with large cumulative effects of rain and misting in unprotected plots compared to plots protected with rain shelters, indicating that late-season rain and misting reduces DON concentrations in grain. Drying wet spike samples in a grain dryer was found to degrade variable portions of DON among samples. Based on the findings of this study, several recommendations can be made to improve the accuracy of DON estimates in the inoculated and misted uniform nurseries used to evaluate breeding lines for FHB resistance. Stop mist treatments at the time DON production in grain slows considerably because misting after this time increases the probability for leaching and degradation of DON. Harvest naturally dried grain as soon as possible after entries first reach harvest dryness to avoid possible complications from leaching and degradation. If entries must be harvested at high moisture, dry as quickly as possible at moderate temperature to minimize degradation of DON. Although leaching and degradation of DON likely will continue to affect results of these screening nurseries, knowing that late-season rain and misting leaches DON and that DON is susceptible to degradation under wet conditions, especially at elevated temperatures, will allow these factors to be minimized or at least made as similar as possible across all entries in a test to improve the accuracy of the results. Evaluations done as well as possible across multiple environments should allow reliable estimates of DON concentration. For experiments requiring the most accurate estimates of DON levels in wet spikes, the spikes should be frozen at -80ºC as quickly as possible and then lyophilized for DON analysis.

CHARACTERIZATION OF WHEAT RILS DERIVED FROM HIGH YIELDING VARIETY WL711 AND DROUGHT RESISTANT VARIETY C306 UNDER WATER DEFICIT STRESS TREATMENT USING DROUGHT SUSCEPTIBILITY INDEX (DSI) AS SELECTION CRITERIA

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ABSTRACT

Climate change is projected to have significant impacts on temperature and precipitation profiles globally which will have profound effects on crop productivity. Breeding for drought resistance is a major aim of many wheat breeding programs both nationally and internationally and requires the identification of genetic material combining adaptation with higher yield. Grain number per unit area (GN) and grain weight usually expressed as 1000-grain weight (TGW) are the major determinants of grain yield. It is the reduction in both GN and TGW that accounts for grain yield reduction in wheat under water deficit stress environments. Eight RILs from the WL711/C306 wheat RIL population were identified as drought resistant germplasm based on drought susceptibility index (DSI) from field trials conducted at IARI, New Delhi from 2007-2013 under irrigated and water deficit stress environments. The parent cultivars have contrasting differences in morpho-physiological traits, yield potential and yield stability. WL711 is a semi-dwarf, medium flowering, high yielding and drought susceptible wheat cultivar while C306 is a tall, late flowering, medium yielding and drought resistant cultivar. The eight RILs identified in this study are desirable recombinants of this population having medium to late flowering, intermediate height, higher grain number combined with high grain weight, yield higher than C306, stability for yield and yield components. The range of grain yield, biomass, harvest index, grain number and 1000-grain weight of the selected RILs was 540.0-673.6 gm^{-2}, 1633-2375 gm^{-2}, 31.6-35.7 %, 14302-18286 m^{-2} and 33.5-38.0 g respectively. These RILs maintained better water relations, cooler canopies and tougher membranes under water deficit stress conditions like C306. Four of these RILs (R-7, R-61, R-19 and R-208) have been registered at National Bureau of Plant Genetic Resources (NBPGR) as drought resistant wheat germplasm and can be used as genetic material in breeding programs aimed at drought resistance in wheat.

MAPPING AND COMBINING GENES FOR FHB RESISTANCE IN WHEAT

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ABSTRACT

Single gene resistance to Fusarium head blight (FHB) in wheat provides only partial resistance and also the disease severity is highly influenced by environment. Consequently multiple genes are needed for effective resistance. Our hypothesis is that identifying DNA markers for Type I resistance will be very beneficial for selection, and combining Type I and Type II FHB resistance will be more effective than either type alone. The objectives of this project are to 1) characterize a RIL population from the cross INW0412 (Type I resistance)/992060 (susceptible) for frequency of initial infection and map QTLs for Type I resistance and 2) combine Type I resistance from cultivars Goldfield, INW0412 and Truman; and Type II resistance of *Fhb1 and Qfhs.pur-7EL* backcrossed into adapted soft winter wheat lines and quantify augmentation of FHB resistance. For objective 1, a population of 198 RILs and the two parents were characterized for FHB incidence in two replicates at Lafayette, IN in 2011 and 2013. A two-enzyme genotyping by sequencing (GBS) approach is in process to identify linked markers. The RIL Type I resistance field data and the GBS single nucleotide polymorphism (SNP) marker data will be used to construct a high-density genetic map to identify QTL contributing to Type I FHB resistance. For objective 2, BC $F_{1:2}$ lines have been genotyped with 7 simple sequence repeat (SSR) markers for *Fhb1*, *Qfhs.pur-7EL* and low incidence. $F_{2:4}$ lines were phenotyped in two replicated field tests for FHB incidence and severity at Lafayette, IN in 2013 for Type I and Type II resistance separately. By using phenotypic and SSR marker selection, lines with both Type I and II FHB resistance were identified, and it was confirmed that lines with multiple markers associated with FHB resistance provide more FHB resistance than lines with few or no markers. Those lines will be beneficial for the improvement of FHB resistance in wheat.

TRANSITIONING FROM PHENOTYPIC SELECTION TO GENOMIC SELECTION FOR LOWER DEOXYNIVALENOL IN BARLEY

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ABSTRACT

A major challenge in barley breeding is to simultaneously increase yield and resistance to FHB while maintaining malting quality. Historically this has been accomplished by phenotypic selection (PS) for disease resistance in early generations, followed by analysis of deoxynivalenol (DON) in harvested grain samples, then evaluation of yield in more advanced lines, and finally selective assessment for a suite of malting quality traits. Information on these traits is accumulated over several years of the breeding cycle and is costly. Genomic selection (GS) methodologies use genome-wide markers to estimate the breeding value of lines early in the breeding cycle at increasingly lower costs. While the theoretical basis and methodology of GS has been the subject of many studies, still in its infancy, GS has yet to be rigorously evaluated empirically. Given their classically quantitative nature of inheritance, FHB severity, DON concentration and grain yield are excellent candidates for improvement using GS. Accurate calculations of genomic estimated breeding values (GEBVs) for any trait, in theory, should minimize cycle-to-cycle noise caused by non-genetic factors and provide means to make more consistent gains. In 2010, the University of Minnesota began replacing traditional phenotypic field screening for FHB with genomic predictions of early generation breeding material. Using disease and yield trial data from 2005 to 2013 we have characterized changes in yield and DON levels over time. This time period includes years where phenotypic and genomic selection was implemented and provides an opportunity to assess the effect of transitioning to genomic selection. We have summarized DON and (grain yield) for 6 (7) years of PS breeding and 1 (2) cycles of GS breeding. DON levels decrease steadily over years of PS and the trend continues into the first cycle of GS. Grain yields are more sporadic over the years of PS and have decreased slightly in comparison to the aggregate PS value; however they do show a promising upward trend between cycles 1 and 2 of GS. Additional data from subsequent GS cycles is required for rigorous analysis, but the initial trends are thus far supportive of the utility of GS, which is further enhanced when the economic benefits of fewer plots per trial and fewer trials are considered.

EFFECTS OF DEOXYNIVALENOL ON THE WHEAT METABOLOME

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ABSTRACT

The trichothecene mycotoxin deoxynivalenol (DON) is a major virulence factor of the plant pathogenic fungus *Fusarium graminearum* and plays an important role in the Fusarium head blight (FBH) disease. The presented study was carried out to investigate the effect of DON on the metabolome of six wheat genotypes which showed a varying degree of FHB resistance. A gas chromatography – mass spectrometry based targeted metabolomics approach was chosen to identify substances differentially expressed according to treatment and/or wheat genotype.

Two parent wheat lines CM-82036 (resistant) and Remus (susceptible) as well as four near isogenic wheat lines (NILs) were treated with either (i) DON or (ii) water as a control. The NILs differed in the two major resistance QTLs against FHB, namely *Qfhs.ndsu-3BS* and *Qfhs.ifa-5A*. Treated plants were sampled after 0, 12, 24, 48 and 96 hours and immediately shock frozen in liquid nitrogen. Ground wheat ears were extracted with acidified aqueous methanol and purified by liquid/liquid extraction with chloroform. The methanol/water phase was analyzed using GC-MS after an automated two step derivatisation employing methoxyamine hydrochloride (MOX) and N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA). GC-MS chromatograms were deconvoluted and further processed with the tailor made MetaboliteDetector software. Currently, the method covers more than 130 polar analytes whereof the majority are primary metabolites.

In this contribution, we aim to identify the effects of DON on the metabolites determined by our GC-MS method. We will present detailed GC-MS results of this metabolomics experiment with the goal to identify wheat metabolites, which are closely linked to the tested resistance QTLs or inoculation with DON. Thereby we intend to gain deeper insights on fungal virulence and plant resistance towards FHB.

CHARACTERIZATION OF FHB RESISTANCE IN SRW ROANE AND JAMESTOWN NAM POPULATIONS

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ABSTRACT

Fusarium Head Blight (FHB), a pathogen of wheat (*Triticum aestivum* L.), frequently results in significant yield losses and accumulation of mycotoxins, such as deoxynivalenol and nivalenol in the grain. As a result, breeding programs have been working to identify resistance genes in wheat varieties known to be resistant to FHB. Some types of resistance that are particularly useful include: Type I (resistance to initial infection), Type II (resistance to pathogen spread in the spike), decrease in mycotoxin accumulation and number of *Fusarium* damaged kernels (FDK). The overall goal is to identify quantitative trait loci (QTLs) for the different resistance types and pyramid these into elite backgrounds. The objective of this study is to identify quantitative trait loci (QTL) for FHB resistance in the native soft red winter (SRW) wheat cultivars Roane and Jamestown. A total of 186 Pioneer 25R47/Jamestown (P47/JT) $F_{5:7}$ RILs, were evaluated for FHB incidence, severity, index, and DON concentration for two years in three environments (MD, NC, and VA). A set of 170 $F_{5:7}$ RILs derived from FG95195 x Jamestown (FG/JT) were evaluated for FHB incidence, severity, index, and concentrations of DON and NIV for two years in four environments (AR, LA, GA, and VA). Two Roane populations, including 33 $F_{4:7}$ RILs from Roane/Allegiance (R/A) and 18 $F_{4:7}$ RILs from Roane/KY93C-1238-17-1 (R/KY93), were grown in 5 locations (KY, MD, MO, NC, and VA) the first year and in 4 locations the second year (KY, MD, NC, and VA). The Roane populations were also evaluated for FHB incidence, severity, index, and DON concentration. Both public and proprietary single nucleotide polymorphism (SNP) markers were used to genotype 42 of the P47/JT RILs, 11 FG/JT RILs, 11 R/A RILs, and all 18 of the R/KY93 RILs at Monsanto. Bulk segregant analysis was used to select microsatellite markers (SSRs) putatively associated with FHB resistance. Linkage maps were constructed using Map Manager QTX, based on the consensus map provided by Monsanto. Windows Cartographer (WinQTLCart version 2.5) was used to identify possible QTLs. Three QTL were identified in P47/JT located on chromosomes 1B and 3B, associated with FHB severity, and on 7D, associated with FDK. In the FG/JT population, 8 QTLs were identified and almost all of these QTL were associated with FHB severity (1B, 2A, 2D, 4A, and 5D), while one was associated with incidence (2A). Of the 12 QTLs identified in the R/A population, four were associated with FHB severity (1A, 1D, 3A, 3B), six were associated with incidence (3A, 3D, 4B, 5A, 7A, and 7D) and one was associated with DON (7A). In the R/KY93 six QTLs were identified on

chromosomes 2A, 2B, 3A, 3B, and 4A. Of the chromosomes associated with FHB in R/KY93, FDK and DON were associated with 2A and 3B respectively, and severity was associated with chromosomes 2B, 3A, and 4A. The QTL on 1B was consistently associated with decreased FHB severity in both Jamestown mapping populations and should be useful to deploy via marker assisted selection.

DETERMINE THE AUGMENTATION EFFECT OF FHB RESISTANCE GENES IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused mainly by *Fusarium graminearum*, causes reduced yield and lowered grain quality. Lots of QTL conferring FHB resistance have been identified in the past few years. QTL pyramiding is one of the intuitive solutions to improve the FHB resistance. A backcross population with 2 major QTL for FHB was established in this study. The population was divided into 4 genotype groups based on the combination of the presence and absence of the 2 QTL. FHB resistance was evaluated by artificial inoculation of a mixture of 4 different local *Fusarium graminearum* isolates in 4 trials in both field and greenhouse in 2011 and 2012. The results in all the trials show no augmentation of FHB resistance in different genotype groups. It indicates that these two genes don't act in an additive manner, and thus provides some evidence that QTL pyramiding is not always an effective way to seek for more durable resistance for complex disease traits.

FINE MAPPING OF THE GENOMIC REGION HARBORING THE FUSARIUM HEAD BLIGHT RESISTANCE QTL *QFHS.NDSU-3AS* IN DURUM WHEAT

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ABSTRACT

The FHB resistance QTL *Qfhs.ndsu-3AS* was identified on chromosome 3A in the wild emmer wheat (*Triticum dicoccoides*) accession "Israel-A" (ISA). It was positioned to a 7.4 cM chromosomal interval in a mapping population of 83 recombinant inbred chromosome lines (RICLs) in a previous study. The RICLs were derived from the cross of Langdon (LDN) durum-ISA 3A substitution line [(LDN-ISA(3A)] with LDN. Five co-segregating wheat EST-derived STS markers (*Xwgc 1188*, *Xwgc716*, *Xwgc1143*, *Xwgc1204*, and *Xwgc501*) mapped 0.6 cM proximal to the SSR marker *Xgwm2* that closely linked to the QTL peak. In the present study, we attempted to increase map resolution of the QTL region and to position the QTL to a smaller chromosomal interval. The RICL#10, which was identified to contain *Qfhs.ndsu-3AS* in the shortest *T. dicoccoides* chromosome fragment among the 83 RICLs, was crossed with LDN to generate a large F_2 recombination population (n>1,800) within the chromosome region. To date, we have genotyped 372 individuals of this population with *Xgwm2* and other nine STS markers proximal to *Xgwm2*, including the five co-segregating STS markers near *Qfhs.ndsu-3AS*. As a result, *Xwgc716* and *Xwgc1188* co-segregated and mapped 0.8 cM proximal to *Xgwm2*. *Xwgc1143*, *Xwgc1204*, and *Xwgc501* also co-segregated and mapped 0.1 cM proximal to *Xwgc716* and *Xwgc1188*. The other four STS markers (*Xwgc1226*, *Xwgc510*, *Xwgc1296*, and *Xwgc1301*) mapped further proximal to the above markers in a higher resolution. Homozygous recombinant lines with smaller *T. dicoccoides* chromosome fragments have been selected using the molecular markers in the F_2 and F_3 generations. We have been evaluating the recombinant lines for FHB resistance in the greenhouse. Eventually, we expect to place *Qfhs.ndsu-3AS* to a smaller chromosomal interval on 3AS and to incorporate this FHB resistance QTL into durum background with minimal *T. dicoccoides* chromatin and reduced linkage drag.

SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

DETERMINATION OF *FUSARIUM GRAMINEARUM* CHEMOTYPES PREVALENT ON OAT, RYE HEADS, AND WHEAT ROOTS IN SOUTH DAKOTA

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INTRODUCTION

Fusarium graminearum Schwab (telomorph: *Gibberrella zeae* (Schwein.)) is the primary pathogen responsible for causing Fusarium head blight (FHB) or scab on barley, oat, rye, and wheat in the USA. In addition to FHB, the fungus has also been reported to be responsible for crown rot on wheat. The disease limits small grain production by impacting yield due to poor grain filling and reduced seed test weight. The fungus contaminates grains with mycotoxins (trichothecenes) during and after the infection process and further reduces monetary value. *Fusarium graminearum* mainly produce two types of trichothecenes; Deoxynivalenol (DON) and Nivalenol (NIV). DON producing isolates were further grouped into 3ADON and 15ADON based on where they were acetylated (Miller et al. 1991). Further, it was reported that 15ADON and 3ADON populations were most prevalent in the USA and Asia, respectively. Mycotoxins contaminated grains are of health risk to both humans and animals. The FDA has set 1ppm and 5-10 ppm DON as the maximum contamination limit in the food products and feed products suitable for human and animal consumption, respectively.

Knowledge of pathogenic variation in virulence and/or aggressiveness is important to the success of any program for disease resistance breeding and in the development of disease management strategies. Variation in the pathogen virulence and/or aggressiveness can be affected due to the broad host range, continuous selection pressure of host resistance, intensive use of fungicides with similar chemistry, and adverse environmental conditions. In recent years, a change in the *F. graminearum* population from 15ADON to 3ADON has been observed in the USA, especially in the northern Great Plains (Burlakoti et al. 2008; Gale, 2007), and in Canada (Ward et al. 2008). It has also been observed in some independent studies that the 3ADON population is more aggressive in disease development and mycotoxin production compared to the 15ADON population (Puri and Zhang, 2010; Ward et al. 2008; Ali et al. 2009). In South Dakota, wheat is the major small grain crop planted on around two millions acres in 2012. In addition to wheat, oat and rye are also grown in the wheat production area. All three crops are prone to FHB and could harbor diverse *F. graminearum* population in the state. In this study, we attempted to recover fungal isolates from FHB infected oat and rye heads, and wheat roots for chemotype characterization.

OBJECTIVES

1. Analyze *F. graminearum* isolates for their chemotypes recovered from oat, rye, and wheat roots in South Dakota

2. Determine if oat, rye, and wheat favors any one of the two 3ADON or 15ADON fungal population

MATERIALS AND METHODS

FHB Infected Wheat, Oat and Rye Samples and Recovery of Fusarium graminearum Isolates. One hundred and seventy-nine isolates were recovered from FHB infected heads of oat (n=37), rye (n=69)

and wheat (n=73). Oat and wheat root samples were collected from 11 and 21 commercial fields across the state in the 2012 and 2013 growing seasons, respectively. All 69 isolates from rye were recovered from 69 individual infected spikes collected from a single 4-acre field located at the SDSU Southeast Research Station near Beresford, South Dakota. To collect FHB infected oat head samples, twenty heads were randomly clipped from each field plot. Whereas, rye diseased spikes were collected from the plants located about 200 feet apart to increase the chances of capturing more diversity in the pathogen population. To recover *F. graminearum* isolates from wheat roots, 25-30 plants were randomly uprooted from each field plot. The roots were thoroughly washed under running tap water. Roots with crown portion were cut into small pieces 1- 2 cm long and then rinsed, and then surface disinfested with 5% bleach prior to fungal isolation. To obtain the fungal isolates from oat and rye, scabby grains were separated from the individual head of each sample and kept separate until plated. Ten scabby grains (tombstones) recovered from each head were plated on ½ PDA medium in 15 x 100 mm plastic petri dishes. Five scabby grains were placed on each plate. The plates were incubated under 12 hours of light light and 12 hours of dark for four days. The fungal colonies grown out of the plated grains were transferred individually onto new ½ PDA plates and grown for seven days. Similar methods were used to recover fungal isolates from wheat roots except roots segments were plated instead of scabby grains. The isolates identity was confirmed based on colony growth and spore morphology described by Leslie and Summerell (2006). In total, 351 isolates were recovered from the plated samples and stored in 15% glycerol at -80°C in the freezer.

DNA Extraction and PCR Assay. DNA of all 169 *F. graminearum* isolates was recovered by growing isolates individually for 2-3 days on cellophane membrane placed on ½ PDA. The fungal mycelia of the isolates were collected by scrapping the cellophane membrane surface using a flamed spatula. DNA was isolated from the collected mycelia by following the protocol developed by Liu et al. (2000). The isolates chemotypes were determined by using the tricothecenes specific 3CON, 3NA, 3D15A, and 3D3A primers (Starkey et al., 2007, Ward et al., 2002). PCR reaction was conducted in a S-1000 thermal cycler (BioRad, USA) using amplification steps of 94°C for 2 min, followed by 32 cycles of 94°C for 30s, 52°C for 30s and 72°C for 1 min with final extension of 72°C for 5 min. The PCR products were electrophoresed on 1.5% agrose gels and scored with reference to 100bp DNA ladder (New England Biolabs, USA). The PCR amplification produced bands of 243bp and 610bp corresponding to the 3ADON and 15ADON chemotypes, respectively (Figure 1).

RESULTS AND DISCUSSION

All plated scabby grains of oat and rye produced *F. graminearum*. In addition to *F. graminearum*, some of the plated grains also developed *F. sporotrichioides*, *F. acuminatum*, and *F. equiseti*. High-level recovery of *F. graminearun* from the plated samples indicates that this is still the major pathogen for FHB development on small grains in the region. The majority of the isolates from wheat roots (89%) and oat heads (91%) were grouped as 15ADON; whereas 3ADON isolates were recovered from both wheat (11%) and oat (9%) in low numbers (Table 1). All isolates recovered from rye produced 15ADON. Results indicate that the fungal population prevalent on wheat roots and oat harbor both chemotypes but 15ADON population is still the most prevalent chemotype within the sample collection area. The fungal population with 15ADON chemotype from rye could be expected because the isolates were collected from a single field plot. Recovery of 15ADON and 3ADON isolates from wheat roots samples could be expected as both chemotypes were recovered from wheat head samples of FHB resistant and susceptible cultivars in South Dakota (Ali et al. 2012). The results of this preliminary study also indicate lack of any specific trend of the fungal chemotypes prevalence on oat, rye and wheat. To obtain a complete inventory of *F. graminearum* population chemotypes and their trend of survival in the state, more isolates from all three hosts

collected from multiple locations in multiple years are under investigation. Occurrence of 3ADON population, low in intensity but more aggressive than 15ADON population in DON production and FHB development, warrant to use this in screening small grains germplasm for resistance to FHB in South Dakota.

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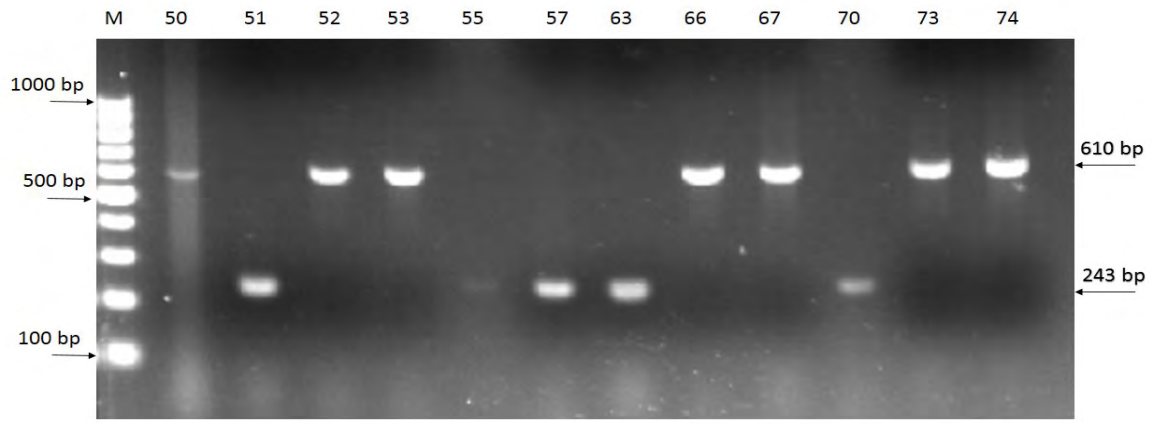

Figure 1. PCR amplification method (Ward et al. 2008). The bands at 610bp and 243bp amplified from the *F. graminearum* isolates (50 to 74) correspond to the 15ADON and 3ADON chemotypes, respectively. M represents the 100bp DNA marker.

Table 1. *Fusarium graminearum* chemotypes recovered from oat, rye and wheat in South Dakota.

Host plant	Isolates chemotyped	3ADON	15ADON
Oat[a]	37	3	34
Rye[a]	69	0	69
Wheat[b]	73	8	65

a = isolates were recovered from FHB infected heads; b = Isolates were recovered from roots

BIOPROSPECTING FOR DON DEGRADING ENZYMES AND MICROORGANISMS

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ABTRACT

New strategies are needed to mitigate deoxynivalenol (DON) contamination in wheat and barley. Here, we present preliminary data from a series of experiments to discover and employ unique DON degrading enzymes and microorganisms. Enzymes for DON transformation were identified using a systematic algorithm to identify possible enzyme-catalyzed reactions based on the functional groups present in DON and confirmed by molecular docking studies of DON-enzyme interactions. Nine candidate genes (five epoxide hydrolases and five cycloisomerases) were selected and cloned. These sequences will serve as templates for further engineering of enzymes to alter substrate specificity and enhance catalytic efficiency by combinatorial cloning. Environmental samples collected in Virginia in 2013 completely eliminated 100 ppm DON following growth in a minimal medium containing DON as a sole carbon source. Microorganisms from these environmental samples are in the process of being isolated and characterized. This research will offer new strategies for detoxifying DON in wheat and barley products.

ACKNOWLEDGEMENT AND DISCLAIMER

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QUANTITATIVE DETECTION OF TOXIGENIC *FUSARIUM* SPECIES AND TRICHOTHECENE GENOTYPES IN WHEAT FROM WESTERN CANADA IN 2011–12

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ABSTRACT

In 2011 and 2012, a total of 198 producer samples of Canada western red spring (CWRS), Canada western red winter (CWRW), Canada western soft white spring (CWSWS) and Canada western amber durum (CWAD) wheat from across the Canadian Prairies were analyzed for various *Fusarium* species and contamination by trichothecenes. Detection and quantification of toxigenic species, namely *Fusarium avenaceum*, *F. graminearum*, *F. poae* and *F. sporotrichioides*, was performed on DNA extracts from several grams of ground grain using validated real-time PCR assays and species-specific TaqMan probes. For the determination and quantification of deoxynivalenol (DON) genotypes, a qPCR assay was developed based on TRI8 (C-3 / C-15 esterase) of the *Fusarium* trichothecene gene cluster. The multiplex TaqMan assay allowed us to quantify 15-acetyldeoxynivalenol (15ADON) and 3-acetyldeoxynivalenol (3ADON) genotypes simultaneously. Validation of the assay was based on a number of *Fusarium* species including producers of trichothecenes type A and/or B.

The predominant species on wheat in 2011 and 2012 was *Fusarium graminearum*. Only in some areas of Alberta and south-western Saskatchewan, *F. avenaceum* was more frequently detected. *Fusarium poae* was detected in a number of wheat samples at trace level, but found in higher quantities only on CWAD and CWSWS from eastern Saskatchewan. Occasionally, *F. sporotrichioides* was detected on CWRS samples from south-western Manitoba and south-eastern Saskatchewan. DNA concentration of *F. graminearum* was highest on CWRW (2011) and on CWSWS (2012) grown in Manitoba and south-western Saskatchewan. In most of the wheat samples, DNA concentration of *F. graminearum* correlated with the percentage (by weight) of *Fusarium* damaged kernels (FDK) and concentration of deoxynivalenol (DON). In 2011, the frequency of the 3ADON genotype on CWRS and CWRW was above 50% in samples grown in Manitoba and eastern Saskatchewan. Only on CWAD grown in southern Alberta and Saskatchewan, the 15ADON genotype was more dominant. The 15ADON genotype was also predominant in CWSWS from Alberta. CWSWS samples from the other provinces showed mean frequencies of the 3ADON genotype ranging from 32% to 89% in 2012. There was no correlation between the concentration of DON and the predominant chemotype, as measured by DNA quantities of the two trichothecene genotypes, in naturally infected wheat samples.

FREQUENCIES OF 3-ADON AND 15-ADON *FUSARIUM GRAMINEARUM* FROM CORN STUBBLE, ATMOSPHERE, AND WHEAT HEADS IN THREE AGRICULTURAL REGIONS IN NEW YORK IN 2013

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ABSTRACT

In North America, Fusarium head blight (FHB) in cereals is caused predominantly by *Fusarium graminearum* of the 15-acetyl(A)deoxynivalenol(DON) trichothecene genotype. However, recent population shifts have been documented in some northern areas in the United States and in Canada where 15-ADON isolates are being displaced by 3-ADON isolates, apparently driven by adaptive fitness of the latter. Previous surveys of symptomatic wheat heads revealed a predominance of 15-ADON isolates in the principal wheat producing areas of central and western New York. Still lacking is any information on trichothecene genotypes obtained from saprophytic, airborne, and pathogenic phases of the life cycle of *F. graminearum*. We conducted intensive sampling of isolates from a) overwintered corn stubble, b) lower atmosphere, and c) symptomatic wheat heads in three diverse agricultural locations in 2013. These locations were: 1) Aurora, Cayuga County, central NY, Central Plain – mostly flat with widespread corn and wheat production; 2) Belmont, Allegany County, southwestern NY, Allegany Plateau – hilly, forested region with crops grown in valleys; and 3) Willsboro, Essex County, northeastern NY, Champlain Valley – broad valley with scattered farms between Adirondack Mountains and Lake Champlain. Approximately 100 *F. graminearum* monosporic isolates each from corn stubble, lower atmosphere, and wheat heads were collected within a 10 square mile area in each geographic location. Polymerase chain reaction (PCR) assays were used to identify B-trichothecene genotypes, 3-ADON, 15-ADON, or nivalenol (NIV), based on amplification of portions of *Tri3* and *Tri12* genes. Of a total of 882 isolates analyzed statewide, 23% were of the 3-ADON genotype and 77% were of the 15-ADON genotype. No NIV isolates were found. No significant differences were found in the trichothecene genotype frequency of *F. graminearum* among the three collection niches in any location (Belmont, χ^2=3.236, P=0.198; Aurora, χ^2=0.145, P=0.930; Willsboro, χ^2=3.662, P=0.160). Overall frequency of the 3-ADON isolates differed (χ^2=179; P<0.001) across the locations, being lowest at Aurora (6%), somewhat higher at Belmont (12%), and highest at Willsboro (50%). At least as viewed by the frequency of trichothecene genotypes, local populations of *F. graminearum* seem to not be structured by the three niches analyzed. The predominance of 15-ADON isolates in Aurora and Belmont is consistent with wider surveys of winter wheat in western New York in 2007 and again in 2011. The equivalence of 3-ADON and 15-ADON isolates in Willsboro also is not surprising as frequencies of 3-ADON exceeding 30% have been observed in other areas of eastern New York and in Vermont. We are currently investigating landscape ecology-related factors that may affect the population structure of the FHB pathogen in New York.

FUNCTIONAL ANALYSIS OF TRANSCRIPTION FACTORS IN THE CEREAL HEAD BLIGHT FUNGUS, *FUSARIUM GRAMINERAM*

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ABSTRACT

Fusarium graminearum is an important plant pathogen that causes head blight of major cereal crops. The fungus produces mycotoxins that are harmful to animal and human. During the last 5 years, we constructed a mutant library of 657 putative transcription factors (TFs) through homologous recombination in *F. graminearum*, providing a resource for understanding gene regulation in the fungus. By screening these mutants in 17 phenotypic categories, we constructed a dataset of over 11,000 phenotypes. This study provides new insight into understanding multiple phenotypes caused by single TFs as well as regulation of gene expression at the transcription level in *F. graminearum*. Furthermore, our TF mutant library will be a valuable resource for fungal studies through the distribution of mutants and easy access to our phenotypic and genetic data.

SAYING GOOD-BYE TO *GIBBERELLA ZEAE*

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ABSTRACT

The names of fungal species are governed by the International Code of Botanical Nomenclature. The Code includes rules for proposing names, describing species, and providing material for type specimens, amongst others. Until the recent International Botanical Congress in Melbourne, it was permissible for fungi to have two names – one for the asexual state and the other for the sexual state. This convention originated because the two different stages were sometimes observed independently and could not necessarily be connected unless one stage could be identified as originating from material of the other. This convention has out-lived its practical usefulness and at the Melbourne Congress the exception to the Botanical Code that allowed dual names for fungi was removed, resulting in "One name, one fungus". In species with an asexual stage in the genus *Fusarium* the alternatives are to retain the name "*Fusarium*" with the inclusion of some related groups that had been excluded, or to no longer use the *Fusarium* name and to split the genus up into a number of pieces in which only the sexual stage names, *e.g.*, *Gibberella*, would be used. A proposal was made in a Letter to the Editor in *Phytopathology* (Geiser *et al.*, 2013) earlier this year that the name "*Fusarium*" be retained and that the names for the sexual stages should no longer be used. Other than *Gibberella zeae*, most sexual stage names for species with asexual stages in the genus *Fusarium* are neither well known nor widely used. Thus, the recommended future usage for the name of the fungus that is the major cause of scab in the United States will be *Fusarium graminearum* rather than *Gibberella zeae*.

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INTERACTIONS OF *FUSARIUM GRAMINEARUM* WITH BARLEY AND WHEAT

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ABSTRACT

As the causal agent of head blight of wheat and barley, *Fusarium graminearum* leads to crop losses by damaging kernels and contaminating grain with the mycotoxin deoxynivalenol. We are studying the interaction of *Fusarium* with wheat and barley during fungal ingress of the surface of barley and progression through the wheat head following infection. We are particularly focused on surface interaction of *F. graminearum* with barley. Histological analysis of barley varieties has been done to identify changes in the process of fungal penetration. In both barley and wheat, we are examining how expression patterns from both fungus and host variety shift during disease progression and resistance response.

SESSION 3:

GENE DISCOVERY AND ENGINEERING RESISTANCE

TRICHOTHECENE EXPOSURE LEADS TO MITOCHONDRIAL ROS-MEDIATED CELL DEATH IN YEAST

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ABSTRACT

We had previously identified several yeast deletion mutants that conferred resistance to trichothecin (Tcin), a type B trichothecene and DON congener, which revealed a critical role for mitochondria in trichothecene-toxicity (1). Mitochondrial translation was directly inhibited prior to damage to mitochondrial membrane integrity and independent of cytosolic translation inhibition (2). To further explore the molecular mechanism of trichothecene toxicity we screened the yeast diploid knockout (YKO) library to identify deletion mutants that exhibited increased sensitivity to Tcin at concentrations that are sublethal to nonlethal (0.5-2μM) to the wild-type parental strain BY4743. We identified 121 deletion mutants which were disrupted in functions involved in cellular damage control from the toxic effects of trichothecenes, including DNA repair and response (15.7%), RNA degradation and stability (29.8%), ribosome biogenesis and protein degradation (10.7%). *Saccharomyces* Genome Database (SGD) phenotypic analysis revealed that a large fraction (42%) of the Tcin-sensitive mutants exhibited high sensitivity to oxidative stress. Oxidant-sensitive 2', 7'-dichlorofluorescin diacetate (DCFH-DA) staining of these mutants revealed that Tcin-induced ROS generation was up to 3-fold higher relative to BY4743. We observed a significant and dose-dependent increase in ROS levels (2-4 folds) in BY4743 treated with DON, T-2 and DAS and found a strong correlation between ROS generation and cell death. Moreover, treatment with antioxidants, such as ascorbic acid and vitamin E increased cell survival 3-4-fold in T-2 treated cells and 6-9-fold in Tcin treated cells, suggesting a direct role for ROS in trichothecene-mediated cell death. Trichothecenes failed to generate ROS in the petite strain lacking mitochondrial DNA (ρ^o) or when mitochondrial membrane potential (ψ_{mito}) was depolarized with the ionophore FCCP (carbonilcyanide p-triflouromethoxyphenylhydrazone), suggesting the mitochondrial origin of trichothecene-induced ROS.

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DOWN WITH DON: STRATEGIES FOR PRECISE TRANSGENE DELIVERY AND RNAI-BASED SUPPRESSION OF *FUSARIUM*

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ABSTRACT

Transgenic strategies can effectively supplement other methods for controlling Fusarium head blight (FHB). Impediments to deploying FHB-resistant transgenic barley include a long time-frame for creating and testing transgenes in barley, imprecise transgene insertions that lead to unstable gene expression, a poor understanding of exactly how to attack *Fusarium*, and negative public perceptions. Advances in *Fusarium* genetics have elucidated the genome sequence and genes critical to pathogenicity. Increasingly detailed knowledge of RNA interference (RNAi)-based gene regulation enables strategies to target specific *Fusarium* genes. The delivery of single copy transgenes using modified *Ds* transposable elements or by site-specific recombination (aka recombinase-mediated cassette exchange, RMCE) enables precise transgene insertion, stable and heritable transgene expression, and production of transgenic plants without bacterial genes or selectable markers. These characteristics should mitigate some of the public concern about transgenic crops. We will use *Ds*-mediated and RMCE for the delivery of transgenes encoding double-stranded RNA (dsRNA) capable of RNAi-based suppression of key *Fusarium* genes involved in virulence and mycotoxin production such as *Tri6*. These transgenes initially will be tested directly in *Fusarium*, to facilitate rapid efficacy assessments. Transgenes showing efficacy in *Fusarium* will be converted to plant transformation vectors and introduced into Conlon. Progress towards these objectives includes: 1) creation of near-isogenic Conlon lines expressing *Ac transposase*, for *Ds*-delivery; 2) design and progress in the construction of *Ds*-delivery backbone vectors; 3) design and construction of the TAG or recombination site platform vector for RMCE; and 4) the regeneration of fertile Conlon plants containing the TAG recombination site.

ACKNOWLEDGEMENT

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.

2013 NORTH DAKOTA TRANSGENIC BARLEY RESEARCH AND FHB NURSERY REPORT

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ABSTRACT

Research continues to develop and test new transgenic plants using genes provided by collaborators. As lines are developed in Golden Promise, they are crossed to Conlon for field testing. Transgenic lines developed in Conlon are being crossed to resistant lines developed by the breeding programs. Crosses between Conlon and five Golden Promise transgenic plants carrying different transgenes were begun in 2013. At least two of these lines will be ready for field tests in 2014. Crosses between Quest or ND20448 and two Conlon lines carrying either a tlp or a Tri12 transgene have continued, to determine whether the transgenes add to resistance from the breeding programs. Lines from this program should be ready for testing in 2014. The 2013 North Dakota transgenic field trials consisted of 15 barley lines, tested in three misted and three non-misted replicates. Plots were sown on May 29, 2013 in hill plots with 10 seed per hill spaced at 30 cm, and all plots were inoculated using the grain spawn method at heading. Lines included Conlon, Golden Promise, the resistant checks Quest and CI4196, four transgenic-null pairs derived from crosses between primary Golden Promise transgenics and Conlon, and one Golden Promise primary transgenic-null pair. FHB severity was evaluated approximately three weeks after anthesis, by counting the total and infected number of seed on ten randomly selected spikes per row. DON concentrations in the barley samples were determined by gas chromatography with electron capture detection using the method of Tacke and Casper. FHB and DON data were analyzed by SAS (SAS Institute, Cary, NC) with means adjusted for the nearest checks. Average FHB severity was 10% over all six replicates, 7% in the non-irrigated plots and 12% in the irrigated plots.

ACKNOWLEDGEMENTS

This material is based upon work supported by the US Department of Agriculture. This is a cooperative project with the US Wheat & Barley Scab Initiative. Thanks to Pat Gross, Megan Ramsett, and Cayley Steen for assistance with the field trials.

CHARACTERIZATION OF CEREAL GENES THAT ENHANCE DON/FHB RESISTANCE

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ABSTRACT

We focus on identifying biochemical pathways involved in the wheat response to the *Fusarium* virulence factor deoxynivalenol (DON). Using functional genomics techniques, DON-responsive transcripts were identified: these included transcripts encoding a basic leucine zipper transcription factor, a multidrug resistance protein ABC transporter, cytochrome P450s and novel proteins. These studies have also highlighted a novel, evolutionary divergent protein involved in the wheat response to DON. Transient expression and microscopy studies showed this protein fused to a fluorescent tag localised within punctate areas of the nucleus of wheat cells. Yeast two hybrid and bifluorescent complementation experiments showed that this protein interacts with SnRK1 (SNF1-Related Kinase 1). We are currently characterizing the functional significance of this interaction. The association of a gene with DON resistance does not mean it plays a direct role in resistance or that it can be used to improve DON/FHB resistance. We use virus-induced gene silencing to determine if there is a direct relationship between transcript levels and toxin resistance. Gene silencing of the ABC transporter in wheat led to both enhanced DON tolerance and reduced grain number. Thus, from a breeding perspective, it warrants further investigation in terms of its potential for FHB resistance breeding and its effect on seed number.

DISCOVERY AND REVALIDATION OF SCAB RESPONSIVE GENES IN WHEAT BY 2D-DIGE AND Q-PCR

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ABSTRACT

Fusarium head blight (FHB) or scab, is a disease of economic importance affecting small grain crops every year causing loss of billions of dollars in grain quality and quantity. In wheat, FHB affects the developing heads directly and has been regarded as a severe threat to U.S. and global food security. The molecular mechanisms that underscore the complex disease etiology, leading to the suppression of innate resistance in a susceptible line or keeping on maintaining the resistance levels in a resistant line of wheat, are not well understood. Given the complexity of breeding for FHB resistance/susceptibility, identification of molecular functional markers and the discovery of FHB responsive genes associated with FHB resistance/susceptibility is valuable knowledge in accelerating the efforts to breed FHB resistant wheat cultivars in the coming years. Thus the focus of this project was to seek comprehensive and fundamental knowledge with the discovery of FHB responsive genes in wheat upon *Fusarium* infections. The young heads of near isogenic lines (NILs), resistant (260-2) and susceptible (260-4), were challenged with *Fusarium* and the infected heads were subjected to 2D-DIGE analysis for the identification of *Fusarium* responsive wheat proteins expressed in the wheat head. The selected 80 protein spots displaying significantly differential expression on the gel were cut, trypsin digested and identified through MALDI-TOF mass spectrometry. Further, we have evaluated the effect of scab infection on wheat heads of a resistant NIL and a susceptible NIL at the molecular level. We have analyzed the significantly altered wheat proteins due to scab infection in resistant and susceptible lines respectively. Functional analysis of these altered proteins using the Gene Ontology (GO) suggest unique multiple molecular function, biological process and cellular component involved in the resistant and susceptible near isogenic lines of wheat. This study shows that, there are significant functional differences in the host (wheat) proteins that respond to the pathogen (scab) infection. Selected genes were further validated by an independent experiment (quantitative RT-PCR or QPCR). In this study, combined use of a proteomic platform, QPCR, and GO facilitated a better understanding of wheat-*Fusarium* interactions at cellular levels.

TRANSFER OF FUSARIUM HEAD BLIGHT RESISTANCE FROM *ELYMUS TSUKUSHIENSIS* TO WHEAT VIA A T1AL·1AS-1E^{TS}#1S TRANSLOCATION

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ABSTRACT

Elymus tsukushiensis Honda (2n=6x=42, S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}, syn. *Roegneria kamoji* C. Koch) is a perennial cross-pollinating hexaploid species native to China, Korea, and Japan. *E. tsukushiensis* is a distantly related wild relative of bread wheat and a source for resistance to Fusarium head blight (FHB). Previously, we reported the production and characterization of wheat-*E. tsukusiensis* chromosome addition lines and showed that the disomic addition having a group-1 *E. tsukushiensis* chromosome, 1E^{ts}#1, or a wheat-E. *tsukushiensis* translocation chromosome TW·1E^{ts}#1S added to the wheat genome, conferred resistance to FHB.

We used *ph1b*-induced homoeologous recombinantion to produce wheat- *E. tsukushiensis* recombinants. After screening 488 progenies of plants homozygous for *ph1b* and heterozygous for TW·1E^{ts}#1S one interstitial and one distal recombinant were identified. Further analyses revealed that the interstitial recombinant is highly rearranged, of noncompensating type, and, thus, agronomically undesirable. FISH analysis identified the distal recombinant chromosome as T1AL·1AS-1E^{ts}#1S, consisting of the long arm of wheat chromosome 1A, part of the short arm of 1A, and a distal segment derived from 1E^{ts}#1S. T1AL·1AS-1E^{ts}#1S confers type-2 resistance to FHB after point inoculation in a greenhouse test and may be used in cultivar improvement.

RNA SEQUENCING ANALYSES OF TWO BARLEY NEAR-ISOGENIC LINE PAIRS IDENTIFY GENES ASSOCIATED WITH RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Previous studies have identified several quantitative trait loci (QTL) for barley FHB resistance. Two of these QTL are located on chromosome 2H bin8 and 6H bin7. Near-isogenic line (NIL) pairs carrying resistant or susceptible alleles for each of the QTL were developed. The 2H bin8 resistant NIL (Gen1-001) and 6H bin7 resistant NIL (Gen2-036) carry the Chevron allele in the susceptible parent M69 and Lacey backgrounds, respectively. Disease severity, deoxynivalenol concentration and fungal mass were evaluated for each NIL pair. Barley spike samples were collected at 48 and 96 hours after *Fusarium* inoculation and their transcriptomes were analyzed by RNA-Seq. Comparative analyses revealed differential expression profiles within each NIL pair and between the two QTL. A large set of differentially expressed genes (1,247) were identified for the 2H bin8 NILs. When comparing transcriptome profiles of the 2hb8 NILs after *Fusarium* inoculation, Gene Ontology (GO) analyses suggest enrichment in the resistant line of genes encoding proteins with oxidoreductase, glycosyltransferase, cellulose synthase, peptidase and enzyme inhibitor activities. Two hundred and forty-seven differentially accumulated transcripts were identified in the Gen2-036/Lacey NILs after *Fusarium* inoculation. Transcripts induced in Gen2-036 encode proteins functioning in cell wall, ethylene signaling, gibberellin signaling, pathogenesis, proteolysis, protease inhibition, transport and ubiquitination. A common set of transcripts were up-regulated in both Gen1-001 and Gen2-036 when compared with the respective susceptible genotypes, suggesting that certain components were employed by both FHB QTL to confer resistance.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2013 FIELD NURSERY REPORT

Koeritz[1], E.J., Elakkad[1], A.M., Muehlbauer[2], G.J., Li[2], X., Dahleen[3], L.S., Abebe[4], T., Skadsen[5], R.W.and Dill-Macky[1*], R.

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ABSTRACT

The 2013 field screening nursery consisted of 22 wheat and 15 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 Minnesota (16 wheat lines + Bobwhite[*] and CB037[*]), and the USDA (10 barley lines + Conlon[*] and Golden Promise[*]). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivars Alsen, Rollag and Sumai 3 and the susceptible cultivar Wheaton. The barley checks were the moderately resistant cultivar Quest and the susceptible cultivars, Robust and Stander. Individual plots were 2.43 m long single rows. The trial was planted on June 3, 2013. All plots were inoculated twice. The first inoculation was applied at anthesis for wheat (July 16-Aug 2) and at head emergence (July 19-Aug 2) 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 30 *F. graminearum* isolates at a concentration of 100,000 macroconidia.ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml.L^{-1} as a wetting agent. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10 ml.sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on July 16 through August 12 to facilitate FHB development. FHB incidence and severity were assessed visually 21 d.a.i. for wheat and around 18 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 27 (barley) and August 21 (wheat). Fifty heads where 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 2013 the disease severities were generally a little higher than in the 2012 nursery. Mean FHB severities for the untransformed wheat checks, Bobwhite and CB037 were 27 and 17 %, respectively. Mean FHB severities for the other standard wheat checks, Alsen, Wheaton, Rollag and Sumai 3, were 11, 33, 7 and 3%, respectively. For barley, the untransformed check variety Conlon had a mean FHB severity of 15%. The untransformed check Golden Promise and one transformant in the Golden Promise background, were very late heading and did not produce seed. The barley standard checks, Quest, Robust and Stander had mean FHB severities of 2, 16 and 24%, respectively. For the wheat entries in the Bobwhite background, the FHB severity data indicated that resistance was significantly expressed ($P<0.05$) in all transformed lines compared to the untransformed Bobwhite check,

with all transformed lines similar to Sumai 3 in response. For the entries with a CB037 background, resistance (FHB severity) was significantly ($P<0.05$) expressed in one transformed line compared to the untransformed background. The FHB severities of all the barley entries in the Conlon background were statistically similar to the untransformed Conlon check, which ranked between Quest and Stander.

ACKNOWLEDGEMENTS AND DISCLAIMER

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MOLECULAR PATHOGENICITY OF WHEAT – *FUSARIUM GRAMINEARUM* INTERACTION

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ABSTRACT

Fusarium graminearum is responsible for Fusarium head blight (FHB) disease in wheat which makes it's end-use quality unsuitable for end-use quality of wheat. A global gene expression study was undertaken to analyze and identify the wheat disease reaction mechanism. The study was conducted using three genotypes of wheat, Japanese landrace Nobeokabouzu-komugi (Highly resistant), cv. Sumai 3 (Resistant) and cv. Gamenya (Susceptible). Florets were inoculated with 10 µl (10^5 conidia / ml) of *Fusarium graminearum* "H-3" strain during the flowering stage. Microarray analysis was carried out by extracting RNA from 3 DAI (days after inoculation) and 7 DAI and using wheat custom array 4x38k. The expression of defense-related genes were more up-regulated in highly resistant landrace Nobeokabouzu-komugi followed by cultivar Sumai 3 and finally by susceptible cultivar Gamenya. Comparing the genotypes, in highly resistant Nobeokabouzu-komugi the expression of up-regulated genes at 3 DAI was more than 7 DAI, while in resistant cultivar Sumai 3 the expression pattern was the opposite, this could be due to a different resistant mechanism. Moreover in susceptible cultivar Gamenya expression was minimal at both time points. The most highly expressed genes in resistant wheats were UDP-glycosyltransferase and Multidrug resistance associated genes, both are genes which encode proteins involved in detoxification process.

TRANSGENIC WHEAT CARRYING A BARLEY UDP-GLUCOSYLTRANSFERASE EXHIBITS HIGH LEVELS OF FUSARIUM HEAD BLIGHT RESISTANCE BY DETOXIFYING TRICHOTHECENES

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ABSTRACT

Fusarium head blight (FHB) is a worldwide disease of wheat and barley, mainly caused by *Fusarium graminearum*. During infection, the fungal pathogen produces trichothecene mycotoxins, such as deoxynivalenol (DON) and nivalenol (NIV), which increase fungal virulence. Moreover, grains contaminated with trichothecenes threaten the health of humans and animals that consume them. Previous work had identified a barley UDP-glucosyltransferase (*HvUGT13248*) gene that exhibited resistance to DON via the conversion to DON-3-O-glucoside (D3G) in transgenic yeast and *Arabidopsis*. We developed transgenic wheat lines constitutively overexpressing the *HvUGT13248* gene in the background of cultivar Bobwhite and CB037. We performed point-inoculation tests in the greenhouse for three seasons (2011 spring, 2011 fall and 2012 spring) and found that transgenic wheat exhibited significantly higher type II resistance compared with the untransformed parental lines. Moreover, in two field tests (2012 and 2013 summer), *HvUGT13248*-overexpressing wheat lines also showed significantly less disease symptoms compared to the untransformed controls. To assess the mechanism of resistance, we treated plants with DON and examined the concentration of DON and D3G from 1-21 days after inoculation. *HvUGT13248*-overexpressing wheat plants converted DON to D3G more rapidly to a higher extent than untransformed plants. We also extended our exploration of the function of *HvUGT13248* gene toward NIV, and found that *HvUGT13248*-overexpressing wheat lines exhibits a high level of type II resistance to a NIV-producing *Fusarium graminearum* strain.

AN *ARABIDOPSIS* NON-SPECIFIC LIPID TRANSFER PROTEIN PROVIDES ENHANCED RESISTANCE TO A TRICHOTHECENE MYCOTOXIN BY REDUCING OXIDATIVE STRESS

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum*, is one of the most important diseases of wheat and barley. Trichothecene mycotoxins, such as deoxynivalenol (DON), are virulence factors of *F. graminearum* and accumulate in the grain causing a serious threat to human and animal health. Current methods for control of FHB have had limited success in reducing disease levels and toxin contamination of small grains. We screened an activation tagged *Arabidopsis* population for resistance to trichothecin (Tcin) and identified two closely linked nonspecific lipid transfer protein (*nsLTP*) genes, which were overexpressed in a resistant line. Treatment of wild type Col-0 with DON or Tcin induced reactive oxygen species (ROS) in leaves as measured by a quantitative Amplex Red assay. Confocal microscopy with 2',7'-dichlorofluorescin diacetate (H2DCF-DA) was used to examine the effect of Tcin on ROS generation. ROS generation was observed in the cell wall/apoplast region of the leaves and clearly colocalized with the chloroplast, suggesting that potential damage to the chloroplast is a source of Tcin-induced ROS in the cell. Treatment of *Arabidopsis* leaves with 2 mM vitamin C, PABA, or vitamin E protected against the toxic effects of Tcin in detached leaf assays, providing further evidence that ROS plays a role in Tcin mediated tissue damage (also see Mohamed Anwar Bin-Umer's abstract/poster). In addition, we found a significant effect of light on Tcin toxicity. Incubation of detached leaves in the dark with Tcin provided the greatest protection from chlorosis and tissue death relative to 16 h light/8 h dark treatments. Previously we have shown that Arabidopsis lines overexpressing two different *nsLTPs* showed reduction in chlorosis and cell death after Tcin treatment and were able to germinate and form roots on medium containing Tcin. Overexpression of *nsLTP*s in *Arabidopsis* and yeast reduced oxidative stress upon trichothecene exposure. *AtLTP* overexpressing lines had significantly attenuated ROS levels upon exposure to Tcin relative to the non-transgenic control. These results demonstrate that ROS production, a component of which is derived from the chloroplast, contributes to the toxicity of trichothecenes in plants and overexpression of an *nsLTP* enhanced trichothecene resistance, possibly by reducing oxidative stress.

QTL-ASSOCIATED ALTERNATIVE SPLICING AND MAPPING OF DIFFERENTIALLY EXPRESSED GENES TO THE WHEAT GENE-OME

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ABSTRACT

Transcriptomic analyses of wheat near-isogenic lines (NILs) segregating for prominent resistance QTL have not yet yielded the causal genes for either *Fhb1* or *Qfhs.ifa-5A*. A main disadvantage of such experiments in wheat is the still unavailable genome of wheat. To date the most complete assembly of the *T. aestivum* gene space is described by the released wheat low-copy-number genome (LCG) assembly, which provides partial sequences of an estimated 94 k genes. In addition, the transcriptome of the close relative barley (*Hordeum vulgare* L.) may be used. However these gene models are either incomplete and lack detailed annotations or do not differentiate between homeoalleles. The IWGSC (International Wheat Genome Sequencing Consortium) has advanced far in the generation of a whole gene-ome map of wheat. We have used these preliminary data as reference for a recent RNA-seq experiment that captured the response of NILs to *Fusarium graminearum* (Kugler et al. 2013). Mapping transcripts that show differences in the *Fusarium* response between lines harboring the resistant or susceptible alleles of either *Fhb1* or *Qfhs.ifa-5A* highlights several transcripts onto the respective regions of the QTL on chromosomes 3BS and 5A. These genes will be selected for further analyses.

With the gene models at hand, we also observed alternative splicing between the reference genome of Chinese Spring, a set of four NILs segregating for both QTL in different combinations and CM-82036, the resistant QTL-donor. Analyses are still ongoing, prelimilary data on *Fhb1* related alternative splicing events will be presented.

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DON INDUCES GENES THAT INCREASE WHEAT SUSCEPTIBILITY TO FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

To characterize the effect of DON on the molecular defense response of wheat heads to *F. graminearum* infection, RNA profiling was performed using the Affymetrix GeneChip Wheat Genome Array, comparing the response of the FHB-susceptible wheat cultivar Roblin when inoculated with a wild type *F. graminearum* (DON+) strain and a related Tri5- knockout (DON-) strain. Several wheat genes that were specifically induced during infection with the DON+ *F. graminarum* strain were identified. This included two transcription factor genes, an ABC transporter, an AAA-type ATPase, aspartyl-tRNA synthase, gluthatione transferase, and two genes with unknown function. Additional experiments showed that most of those genes were induced directly by a treatment with DON. Further analysis of the DON-modulated genes across our expression profiles database showed a correlation between expression level of many of those genes and susceptibility of the genotypes to FHB. Silencing of one of the transcription factors, a homolog of AtNFXL1, and of the MRP-like ABC transporter in Roblin using a transient assay (VIGS) has showed reduction in infection spread and in head bleaching for both genes. These results support a contribution of DON to FHB-susceptibility in wheat via modulation of gene expression.

ENGINEERING MICROBIAL ELICITORS OF DEFENSE TO PROMOTE RESISTANCE AGAINST *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat and barley. We had previously shown that *Arabidopsis thaliana* provides an excellent model system to study plant defense mechanisms against *Fusarium graminearum*, the principal causative agent of FHB in wheat and barley (Makander et al., 2006, 2010). *F. graminearum* is capable of infecting the leaves and flowers of *Arabidopsis*. In *Arabidopsis*, microbe-derived molecules can stimulate pathogen-triggered immunity (PTI), leading to enhancement of resistance against *F. graminearum*, thus suggesting that the PTI mechanism could be targeted for enhancing resistance against *F. graminearum*. We have engineered one of these defense elicitors for expression in *Arabidopsis* and find that these plants exhibit high level of resistance to leaf and floral inoculation with *F. graminearum*. Transgenic wheat plants expressing the defense elicitor have been generated and will be evaluated for resistance to FHB.

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UDP-GLUCOSYLTRANSFERASES: RESISTANCE TO *FUSARIUM* MYCOTOXINS AND FORMATION OF MASKED MYCOTOXINS

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ABSTRACT

Maximum tolerated levels for the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) in food commodities have been enacted in the European Union. DON, a virulence factor of *F. graminearum*, is necessary for the spreading of the pathogen from the infection site to other spikelets in the wheat ear. The role of ZEN *in planta* is less clear, but both mycotoxins are inactivated *in planta* by formation of glucosides. We characterized candidate UDP-glucosyltransferases (UGTs) from barley, *Brachypodium*, Sorghum, rice and wheat by heterologous expression in yeast. Several genes encode enzymes with the ability to detoxify DON, and also NIV and other trichothecenes. Interestingly, one barley gene induced by DON had no DON-conjugating activity, but was able to convert ZEN to a mixture of the previously described ZEN-14-*O*-glucoside and the novel ZEN-16-*O*-glucoside. We functionally characterized a cluster of six BdUGT genes displaying the highest sequence similarity with the DON resistance conferring barley *HvUGT13248* (Schweiger *et al.* 2010). Surprisingly, the detoxification capability and substrate specificity towards different toxins was quite different for products of genes with high sequence similarity. Presumably the diversification of trichothecene structures allows the fungus to escape detoxification by individual UGTs. In turn gene amplification and generation of novel UGT variants by mutation allow the plants to regain resistance. The gene family of *Brachypodium* UDP-glycosyltransferases consists of 178 predicted genes (IPR 002213), 159 of which encode potentially functional proteins. Analysis of the current draft wheat genome sequence indicates that there is even a further expansion of the UGT gene family with estimated 748 genes (many of which are pseudogenes). Due to the lack of conservation of copy number and changes in substrates specificity caused by point mutations it is difficult to predict the relevant orthologous genes responsible for mycotoxin inactivation in different plant species. Functional testing of candidate genes in yeast is warranted.

Since improved detoxification by glycosylation seems to be an option for biotechnological control and is (unintentionally) increased also by breeders by deploying *Fhb1*, the formation of masked mycotoxins may become a relevant issue. The glucosides escape routine detection methods, but they can at least partly be reactivated by glucosidases of intestinal bacteria.

Due to lacking toxicological data the masked forms are not yet considered in a maximum tolerated sum value of mycotoxins. To explore the fate of the masked mycotoxins in animals and to determine toxicity equivalence values, larger amounts of the mycotoxin glucosides are needed for toxicological studies. We expressed a recoded rice UGT gene with a solubility enhancing tag in *E. coli*. The availability of affinity purified active enzyme allows efficient enzymatic production of DON-3-*O*-glucoside, and also of nivalenol-3-*O*-glucoside. Standards for the glucosides of 15-ADON, fusarenone X and HT-2 toxin are in preparation. These compounds should become valuable reference substances to study metabolism of mycotoxins in various plants. They are also a starting point to investigate the fate of glucosides, which are further metabolized *in planta*.

Recently a novel detoxification mechanism for DON was demonstrated using stable isotope labeled DON, the formation of glutathione adducts and processing products derived from it (Kluger *et al.*, 2012). Methylthio-DON (MT-DON) - which was first reported as S-methyl-DON by Kushalappa et al. at the 2010 National Fusarium Head Blight Forum - is proposed to be generated *in planta* from the DON-glutathione conjugate processing product DON-cysteine by cysteine-S-conjugate-beta-lyase and subsequent methylation of DON-SH. Methylthio-DON was chemically synthesized and used to test its toxicity. The MT-DON concentration required for 50% growth inhibition of a sensitive yeast bioindicator strain was about 9-fold higher than for DON. Tests of the ability to inhibit protein synthesis of wheat ribosomes (using a wheat germ *in vitro* translation system) showed that MT-DON is at least 12-fold less toxic than DON. This strongly indicates that addition of the much larger cysteine and glutathione substituents should also lead to reduced toxicity due to steric hindrance preventing interaction of the toxin-conjugate with the ribosomal target.

Conjugation with glutathione and glycosylation target different parts of DON, it should therefore be possible to combine increased detoxification capability of different cultivars by knowledge based plant breeding.

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TARGETING HOST DEFENSE AND SUSCEPTIBILITY MECHANISMS FOR ENGINEERING FHB RESISTANCE

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ABSTRACT

The interaction between *Arabidopsis thaliana* and *Fusarium graminearum* provides an excellent system to identify plant genes/mechanisms that govern resistance and susceptibility to *F. graminearum* (Makandar et al., 2006, 2010). We have targeted several of these genes/mechanisms for enhancing Fusarium head blight resistance in wheat. One of the defense mechanisms that has been successfully targeted for enhancing resistance against *F. graminearum* is systemic acquired resistance, which confers resistance against a broad-spectrum of pathogens. Host lipid metabolism genes involved in resistance and susceptibility have also been targeted for promoting disease resistance. A new approach under development involves utilizing a microbe-derived elicitor of plant defense for enhancing resistance against this fungus.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0200-3-003 and 59-0790-8-060. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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EXPRESSION QTL MAPPING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

To gain insights into the wheat response to Fusarium Head Blight (FHB) and uncover genes linked to known resistance QTL, we performed an expression quantitative trait loci (eQTL) study. eQTL studies allow for mapping of transcript abundances on the genetic map in order to infer genes that are involved in defense against FHB.

Our study captured expression profiles of 60k genes (Agilent wheat microarray) at two time points (30 and 50 hours) after inoculation with *Fusarium graminearum* spores from a population of 200 doubled haploid lines (CM-82036 x Remus). These segregate for the prominent resistance QTL *Fhb1* and *Qfhs. ifa-5A*. We found 400 genes at 30 hai and 5,000 genes at 50 hai differentially expressed (fold change >2, *adj.p*≤0.05) between the parental lines. We used a part of these to generate transcript derived markers (TDMs), which were used together with SSR and AFLP markers from a previous study (Buerstmayr et.al 2003) to improve the existing genetic map. Reanalysing the phenotypic data confirmed two major QTL (*Fhb1, Qfhs.ifa-5A*) and identified a novel QTL located on chromosome 6A. eQTL mapping for expression data revealed 14,994 and 13,116 significant eQTL at 30 and 50 hai, respectively. Distribution of these eQTL across the genetic map allowed us to identify eQTL that corresponded to phenotypic QTL (*Fhb1, Qfhs.ifa-5A* and 6A) and to hotspots. These 8 hotspots (which comprise between 350 to1900 genes) potentially encode for regulatory elements that govern the response to *F. graminearum*. To gain further insights into the activity of these hotspots and QTL we are currently working on GO enrichment analyses of the co-regulated genes. The results will be presented. Cis and trans-eQTL are defined based on distance of the eQTL from physical position of the respective gene. They are determined by plotting the physical position of eQTL against the genetic position of the TDMs. We mapped 1,500 eQTL, of which the majority (80 %) comprises trans-eQTL at either time points. Few cis-eQTL linked for instance to *Fhb1* are interesting candidates for further studies.

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SESSION 4:

FOOD SAFETY AND TOXICOLOGY

MYCOTOXIN CONTAMINATION OF CORN DISTILLERS' DRIED GRAINS WITH SOLUBLES FROM FORTY-SEVEN ETHANOL PLANTS IN THE U.S. IN 2011

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ABSTRACT

Several known fungal plant pathogens in the genus *Fusarium* produce dangerous mycotoxins that may contaminate corn (*Zea mays* L.) and small grains destined for fuel ethanol production and the resulting ethanol co-products known as distillers' dried grains with solubles (DDGS). Corn DDGS are a significant source of animal feed. Fuel ethanol production may concentrate mycotoxins in DDGS, posing a significant threat to the health of domestic animals. A recent survey of mycotoxins in corn DDGS reported that 12% of the samples (67 DDGS samples from 8 ethanol plants in the U.S.) contained mycotoxin levels that exceeded FDA advisory levels. In the present study, we used GC-MS to screen 141 DDGS samples collected in 2011 from 47 ethanol plants located in 12 states for the mycotoxins deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), and zearalenone (ZON). Mean DON levels ranged from <0.5 ppm (LOQ) to 15 ppm, mean 15-ADON levels ranged from <0.25 ppm (LOQ) to 3.9 ppm, and mean ZON levels ranged from <0.1 ppm (LOQ) to 1.0 ppm. None of the DDGS samples contained 3-ADON. DON levels were significantly correlated with levels of 15-ADON (R^2= 0.96, $P < 0.0001$) and ZON (R^2=0.93, $P < 0.0001$). Twenty five percent (35/141) of the samples contained a mean of 1 to 5 ppm DON, 3% (4/141) of the samples contained a mean greater than 5 ppm but less than 10 ppm DON, and 3% (4/141) of samples contained a mean of 10 or more ppm DON. DDGS lots contaminated with unacceptable levels of DON evaded detection prior to their commercial distribution and were consequently sold as feed products. These observations underscore the need for new and improved detection and mitigation strategies for mycotoxins in DDGS.

ACKNOWLEDGMENT 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.

HOW THE *FUSARIUM* TOXIN DON IS MADE AND DELIVERED TO PLANTS

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ABSTRACT

Several species of the fungus *Fusarium* colonize wheat and barley and produce toxic small molecules that contaminate grain, rendering it unsuitable for consumption. Among the most destructive of these species is *F. graminearum*, which causes Fusarium head blight disease and often infests the grain with harmful trichothecene mycotoxins such as deoxynivalenol (DON). Previous studies have shown that the fungus is remarkably adapted for producing DON by precisely regulating the genes for its synthesis in order to promote toxin accumulation in the host plants. We have now found, by labeling proteins for toxin synthesis with fluorescent proteins, that these proteins are directed to subcellular toxin factories; small vesicles called toxisomes that appear to serve as the staging area for the toxin biosynthetic assembly line. When cell culture conditions are changed in order to promote toxin biosynthesis, another pathway supplying precursor molecules for toxin synthesis may be shifted within the cell to toxisomes, streamlining the path to toxin synthesis. By making toxin in a confined vesicle within the cell, the fungus may protect itself from the inhibitory effects of its own toxin and may allow for an efficient way to deliver it to the plant. This work establishes that toxin synthesis requires a complex developmental event in the fungus which ultimately determines the outcome of plant infection and plant health.

TRICHOTHECENE CHEMOTYPES AND ZEARALENONE OF *FUSARIUM* ISOLATES FROM NATURAL CONTAMINATED WHEAT GRAINS FROM BRAZIL

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ABSTRACT

Wheat production in Brazil has developed and grown over the years. Aiming for safety and crop quality, we investigate the mycotoxins: deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV) and zearalenone (ZEA) profiles of 60 *Fusarium* isolates from wheat grains harvested from three states of Brazil: Rio Grande do Sul, Sao Paulo and Parana in 2011. Prior to chemical analyses, *Fusarium* spp. were identified through EF-1 partial gene sequence and all isolates studied are within Species Complex *Fusarium graminearum*. For toxin quantification, isolates were incubated for 10 days in YES medium (Himedia) at 25ºC. Toxins were extracted from mycelium plus medium, according to Zachariasova et al. 2010. HPLC system consisted of a Shimazdu model with UV (trichothecenes) and fluorescence (ZEA) detectors. Chromatographic separations were performed on a Phenomenex C-18 reversed-phase column (250x 4.6mm, 5 µm particle size). Mobile phase consisted of acetonitrile: water (70:30 v/v) at a flow rate of 0.5 mL min^{-1}. With an average recovery rate of 88%, detection limits of DON, 3-ADON, 15-ADON, NIV and ZEA were 83.3; 167.0; 167.0; 83.3 and 54.0 ug/L, respectively. We identified four different phylogenetic species: *F. graminearum senso stricto* (33%), *F. meridionale* (48%), *F. cortaderiae* (17.4%) and *F. astroamericanum* (1.6%). Our results showed a prevalence of DON (58%) and NIV (48%) followed by 15-ADON (29%), ZEA (26%) and 3-ADON (1,4%). Although DON was more frequent, mean NIV production was much higher (24,306 µg/kg) than DON (3,839 µg/kg). DON derivative 15-ADON was predominant over 3-ADON. We were surprised with the frequency found of ZEA and its mean production of 10,378 µg/kg. In addition, specie-toxin relations showed that 78% of *F. meridionale* produced NIV, 28% produced NIV and DON both, as to *F. graminearum*, production varied between DON, 15-ADON and NIV. *F. cortaderiae* produced just NIV and *F. astroamericanum* was the only isolate which produced 3-ADON. These results give us a bigger understanding of mycotoxins profiles of *Fusarium* isolated from wheat grain in Brazil. In the future, we intend to add isolates and analyze the wheat grains harvested for the same mycotoxins.

EFFECTS OF *LACTOBACILLUS RHAMNOSUS* VT1 CULTURE SUPERNATANT ON *FUSARIUM GRAMINEARUM* GROWTH AND MYCOTOXIN PRODUCTION IN CULTURE AND BARLEY MALT

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ABSTRACT

Cell free *Lactobacillus* culture supernatant (CFLCS) was demonstrated to inhibit *Fusarium graminearum* growth in glucose-yeast extract-peptone (GYEP) broth and homemade potato dextrose agar (HPDA).

The CFLCS at concentrations of 30%, 40% applied in rice culture inhibited the growth of *F. graminearum,* but increased mycotoxin concentrations in rice culture. At a concentration at 50% CFLCS, *F. graminearum* growth was inhibited completely and no mycotoxins were detected.

As replacements of steeping water during the malting process, the CFLCS concentrations of 30% and 50% significantly reduced the *F. graminearum* growth and mycotoxin accumulations in naturally infected barley. However, the germinative abilities of the barley samples were inhibited.

SESSION 5:

FHB MANAGEMENT

EFFECTS OF PRE- AND POST-ANTHESIS MOISTURE PATTERNS ON IND/DON RELATIONSHIPS AND FHB AND DON RISK PREDICTION

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ABSTRACT

The effects of various moisture patterns on relationships between Fusarium head blight (FHB) and deoxynivalenol (DON) and the risk of these responses exceeding critical thresholds were evaluated with a set of field and controlled environment experiments conducted in Wooster, Ohio. Moisture is critical for infection, colonization, FHB development, and grain contamination with DON. It remained unclear, however, how the distribution of moisture during the time windows pre- and post-anthesis affect these responses and relationships between them. Field experiments were conducted to quantify the effects of pre-anthesis rainfall patterns, and mist chamber experiments to quantify the effects of post-anthesis mist patterns, on FHB, DON, and fungal biomass (FBM). For both sets of experiments, four moisture (rainfall or mist) treatments, one continuous (mist or rain every day, but not all day) and three intermittent, were applied during the 7-8 days before or after anthesis, along with an untreated check. Intermittent treatments received similar duration (h) and amounts of moisture, but the alternation between wet and dry periods during the 7-8-day window varied among these treatments. FHB index (IND) was rated, and a grain sample from each treatment was analyzed for DON. Linear mixed model (LMM) covariance analyses were performed to model the IND/logDON relationship, as influenced by moisture treatments. In all cases, there was a significant positive linear relationship between IND and DON. Moisture treatment did not affect the slope of the IND/DON regression line, but the intercepts varied among treatments. Both under field and greenhouse conditions (pre- and post-anthesis), one particular intermittent moisture treatment (wet days at the beginning and end of the 8-day window, separated by dry days) consistently resulted in comparable or significantly higher logDON than the continuous moisture treatment. Generalized LMMs were then fitted to the data and probabilities of IND > 10% and DON > 2, 5 and 10 ppm were estimated. Nearly all pre-anthesis rainfall and post-anthesis mist treatments had similar probabilities of IND > 10%, which were significantly higher than the untreated check. Results from the controlled-environment (post-anthesis) studies showed that the moisture treatment with two days of mist at the beginning and end of the 8-day window had as high or higher probability of DON exceeding 2, 5, or 10 ppm as the continuous moisture control.

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EFFECTS OF LOCAL CORN DEBRIS MANAGEMENT ON FHB AND DON LEVELS IN SEVENTEEN U.S. WHEAT ENVIRONMENTS IN 2011 - 2013

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ABSTRACT

Reduction or elimination of within-field sources of inoculum of *Fusarium graminearum* is the basis for cultural control measures such as crop rotation sequences in which cereals follow non-cereal crops. The goal of this USWBSI research project is to provide realistic estimates of 'DON reduction' that can be expected from cultural controls that reduce within-field inoculum sources. We utilized moldboard plowing of corn debris as a proxy for planting after a non-cereal crop to compare directly with wheat planted no-till into corn debris in commercial-scale wheat fields planted following grain corn harvest in Illinois, Kentucky, Michigan, Missouri, Nebraska, New York, and Vermont. Following corn harvest, replicated wide (60 ft) strips were moldboard plowed or left non-plowed prior to sowing wheat over the entire field with a no-till drill. Wheat in each strip was monitored for FHB and sampled for laboratory quantification of head infection by *F. graminearum* and contamination of grain by DON. Results were collected over three years, 2011, 2012, and 2013, from winter wheat in three states (IL, NE, and NY) and over two years, 2011 and 2012, from winter wheat in three states (KY, MI, and MO) and from spring wheat in one state (VT).

In 2011, FHB symptoms at soft dough stage were low to moderate at every location except Missouri. Yet, at crop maturity, a high percentage of wheat heads was found to be infected by *F. graminearum* in all locations except Nebraska and Vermont. Measurable DON was found in grain from every environment and the levels were lowest in Vermont and highest in Kentucky and Nebraska. It is interesting that the Nebraska site showed the lowest disease index and lowest incidence of head infection, but the highest average toxin level. Moldboard plowing resulted in a significant decrease in FHB index in four environments (IL, MO, NY, MI), though the magnitude of the difference was large only in Missouri. In Nebraska, FHB index was significantly higher in the moldboard-plowed treatment in which the wheat crop matured earlier than in the no-till corn debris treatment. Moldboard plowing was associated with a small but significant decrease in recovery of *F. graminearum* from mature heads in three environments (IL, MI, NY). There was no significant effect of plowing on DON level in five environments (IL, KY, MO, NY, VT) and there were small but significant decreases in toxin in moldboard-plowed compared to no-till strips in two environments (MI and NE). An additional treatment of minimum tillage (chisel plow) was added in the Michigan experiment; DON levels in the minimum-till plots were intermediate between moldboard and no-till but not significantly different from no-till.

In 2012, a generally warm and dry cropping season across the experimental region, FHB symptoms at soft dough stage were not observed in four locations (KY, MI, NY, VT) and were observed at low levels at three locations (IL, MO, NE); plowing had no significant effect on FHB index in any location. At crop maturity, a moderate percentage of wheat heads (i.e., greater than 10%) was found to be infected by *F. graminearum* only in Missouri and Vermont; in both environments there was a significantly greater incidence of heads infected in no-till than in moldboard-plowed strips. DON was not detected in Nebraska, and was detected at low levels in all other states. Moldboard plowing resulted in a significant decrease in already low DON levels in New York and Vermont. A similar level of reduction in DON level was observed in wheat from moldboard-plowed strips in Michigan, but DON was assayed in small samples that were pooled from the replicate strips, so no statistical comparison could be made.

In 2013, at soft dough stage, FHB index exceeded 25% in IL but was less than 5% in NE and NY, yet DON levels exceeded 2 ppm in all three locations. Neither FHB Index nor DON differed significantly by tillage treatment in IL or NE. Plowing did not result in reduction of infection incidence of mature heads in any location. Plowing of corn debris resulted in significant reductions in FHB and DON in NY.

There is a strong trend in three years of data suggesting that inoculum from area atmospheric sources exerts a far greater effect than inoculum from in-field corn residue on the level of DON contamination, and this is especially true in years with severe epidemics. Yet, where economically and logistically feasible, avoiding cereal residues within wheat fields, through appropriate rotational sequence, will sometimes result in modest reductions in DON, especially when weather conditions are only moderately favorable for epidemics. Therefore, cultural practices that avoid corn and small grains debris within wheat and barley fields still have a valuable though incremental role to play in the integrated management of FHB and DON.

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LOCAL CORN DEBRIS MANAGEMENT: WHAT DOES IT CONTRIBUTE TO HEAD BLIGHT AND MYCOTOXIN REDUCTION?

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ABSTRACT

Wheat or barley crops planted directly into cereal debris (source of *Fusarium graminearum* spores) are at increased risk for head blight and mycotoxins, but atmospheric inoculum from spores released over a wider geographic region presents an even greater risk for local cereal crops. Cultural practices based on avoidance of cereal residues or practices that promote residue decomposition and decrease *Fusarium* survival could reduce atmospheric spore levels significantly if implemented over a wide production region. However, planting of wheat or barley following a non-cereal crop is not feasible for logistic reasons for many producers. Other producers need to weigh the disease and toxin suppression benefits of such cultural practices against the economic costs of those measures. Probably the worst-case scenario for inoculum exposure and Fusarium head blight risk is the no-till planting of a susceptible wheat or barley cultivar into overwintered corn stubble. A three year project covering 17 U.S. wheat environments was conducted with moldboard plowing of corn debris used as an experimental proxy for planting after a non-cereal crop to compare directly with wheat planted no-till into corn debris in commercial-scale wheat strips, and thereby produce some realistic estimates of the 'DON reduction' effect of debris management in individual fields. [See FHB Management Poster # 49 by Bergstrom, Cummings, Waxman, Bradley, Wegulo, Hazelrigg, Hershman, Nagelkirk, and Sweets.] Findings from this research along with other observations and results in the literature will be discussed with a goal to define a range of values that could be attached to cultural practices within an integrated FHB management program.

EFFECT OF CULTIVAR AND FUNGICIDE ON *FUSARIUM* MYCOTOXINS IN WHEAT STRAW

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ABSTRACT

Fusarium head blight is known for yield reduction and mycotoxin accumulation in wheat grains that are detrimental to both human and animal health. Fungicides and cultivars have been shown to have varying effects on toxin accumulation in grains, but little is known about their effects on mycotoxin accumulation in straw tissue. Wheat straw is commonly used as bedding material for livestock and can accumulate toxins in much higher levels than the associated grains from the same crop. Non-ruminants are especially affected by the toxins such as swine which can eat 2 to 4 kg of wheat straw bedding per day. Research trials conducted in 2013 in Illinois determined the levels of mycotoxins present in wheat straw samples (stems only) and if fungicides or cultivar had an effect on mycotoxin levels. Stem samples were collected immediately after harvest and were sent to the University of Minnesota mycotoxin testing laboratory.

Fungicide trials were conducted at 4 location in Illinois in 2011, 2012, and 2013 (Brownstown, Dixon Springs, Monmouth, and Urbana) to determine the effects of Priaxor® [in 2013] and Headline® [in 2011 and 2012] (fungicides containing pyraclostrobin; BASF Corp.), Caramba® (metconazole; BASF Corp.), Prosaro® (prothioconazole + tebuconazole; Bayer CropSciences), and Folicur® (tebuconazole; Bayer CropSciences) on mycotoxins in wheat straw. All locations were planted into corn stubble and were mist-irrigated. Priaxor or Headline was applied at Feekes growth stage (FGS) 9, while all other fungicides were applied at FGS 10.5.1. Mycotoxin concentration ranges for DON, 3ADON, 15ADON, NIV, and ZEA in wheat stems at these locations across all three years were 0.19-154.5 ppm, 0.0-10.1 ppm, 0.0-29.8 ppm, 0.0-5.2 ppm, and 0.0-2.5 ppm, respectively. When averaged over all location in 2011 and 2013, none of the fungicides decreased mycotoxin levels as compared to the non-treated control. However, pyraclostrobin-containing fungicides significantly ($P \leq 0.10$) increased DON, 15ADON, and 3ADON concentrations in wheat stems as compared to the non-treated control in 2011 and 2013.

Integrated management trials designed to evaluate cultivar (susceptible vs. moderately-resistant) × fungicide (Prosaro vs. non-treated) effects were conducted at Dixon Springs, Urbana, and Monmouth, IL. Two trials (mist-irrigated and non-irrigated) were conducted at Urbana each year. The Monmouth location was omitted for 2013. Ranges of DON, 3ADON, 15ADON, NIV, and ZEA concentrations in wheat stems from these trials from 2011 to 2013 were 0-45.3 ppm, 0-3.3 ppm, 0-15.7 ppm, 0-2.3 ppm, and 0-5.5 ppm, respectively. When averaged over all trials from 2011 to 2013, the susceptible cultivar (Pioneer 25R47) had significantly ($P \leq 0.10$) greater DON levels compared to the moderately resistant cultivar (BW5228). For the trials conducted in 2011 and 2013, 15ADON present in stem tissue of the untreated, susceptible cultivar was significantly higher than any of the other treatments. In 2012, no significant differences were observed among any of the treatments for mycotoxins levels in the wheat stems.

A mist-irrigated cultivar evaluation trial was conducted at Urbana in 2011 through 2013. Ranges of DON, 3ADON, 15ADON, NIV, and ZEA were 0.08-135.2 ppm, 0-20.0 ppm, 0-25.5 ppm, 0-1.9 ppm, and 0-0.98 ppm, respectively. Overall, significantly higher 3ADON was found in wheat stems in 2011 and 2013 across all cultivars, while 2012 recorded significantly higher NIV. In 2011 and 2013, significant differences were also found in the levels of DON, 3ADON, and 15ADON in stems among cultivars. In 2012, no significant differences in stem mycotoxin levels were observed among cultivars.

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UNIFORM TESTS OF BIOLOGICAL CONTROL AGENTS FOR MANAGEMENT OF FHB AND DON, 2013

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ABSTRACT

Fusarium Head Blight (FHB) caused by *Fusarium graminearium* (*Gibberella zeae*) is an economically important disease observed in wheat, barley and certain corn varieties. FHB can be controlled or reduced through the use of chemical and / or biological control agents (BCAs). Uniform biocontrol field plot trials were conducted at sites in New York, Nebraska, and South Dakota to analyze the efficacy of the commercially available *Bacillus* biocontrol product Taegro® with / without selected amendments. Prosaro® or Tebuconazole was sprayed at Feekes 10.51, while Taegro with / without Canola oil and nitrogen were applied late after the application of Prosaro or Tebuconazole. Biological control activity of Taegro was compared with an untreated control and a standard treatment of the fungicide Tebuconazole or Prosaro, with the use of additional amendments like Canola oil and a nitrogen amendment. Assessment of efficacy of the Taegro BCA was done by analyzing the wheat heads for FHB disease incidence, severity, index, DON, FDK, yield and grain test weight. In New York, severity of powdery mildew and fungal leaf blotches on flag leaves was also assayed.

For the one field site in Nebraska that provided data, the addition of Taegro with or without additional amendments did not result in any significant treatment effects compared to the fungicide alone.

In the Brookings, South Dakota field plot trial, no statistically significant (P=0.10) differences were observed for FHB disease incidence, severity, index, and grain test weight for any treatments in comparison to the untreated control. However, reduced FHB incidence, severity and index were observed for some treatments. Some treatments exhibited significant treatment differences for yield in comparison to the untreated control, particularly Taegro with Prosaro and the nutritional amendment to stimulate the BCA. The DON data for the South Dakota trial are not yet available as of November 2013.

In the New York field plot trial, all treatments resulted in significantly lower severity of powdery mildew and fungal leaf blotches on flag leaves than the non-treated control, with the exception of the late application of Taegro with canola oil and nitrogen. Overall, treatments that included Prosaro resulted in the best control of foliar diseases, and treatments including Tebuconazole resulted in better control of foliar diseases than any biocontrol alone treatments. FHB developed in all plots at moderately low levels, with significant differences among treatments for FHB incidence and FHB index. Prosaro application at flowering resulted in significant reductions in FHB incidence and index, but only resulted in modest reductions of FDK and DON which may be attributed to later infection after the fungicide applications. Though it resulted in significant reductions of FHB incidence and index, Tebuconazole application did not reduce FDK or DON. The combining of Prosaro or Tebuconazole with any of the biocontrols neither enhanced nor diminished the fungicide's ability to suppress FHB, FDK, or DON.

Taegro applications that were not combined with either fungicide resulted in no significant reduction of FDK or DON. Only treatments including Prosaro resulted in significantly lower FDK than the non-treated control. There were no statistically significant differences in DON or yield among any of the treatments. Only treatments including Prosaro and the treatment with Tebuconazole at flowering followed by Taegro resulted in higher test weights than the non-treated control.

Since Taegro is a commercially available *Bacillus* BCA product that is currently available to producers, further efforts to optimize its efficacy in the field using chemical amendments seem warranted. Some field sites and Taegro treatments in the 2013 uniform BCA trial indicated that Taegro in combination with fungicide can either reduce some measures of FHB and/or increase yield.

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PROGRESSION OF DON IN WHEAT INFECTED FROM 0 TO 13 DAYS AFTER MID-FLOWERING

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ABSTRACT

Previous research had indicated that physiological susceptibility of winter wheat to Fusarium head blight essentially ends between 7 and 10 days after mid-anthesis, but could vary according to both environment and host resistance genotype. The present study aimed to clearly establish when and how rapidly the window of wheat susceptibility to FHB infection closes. An inoculated, mist-irrigated field experiment was performed in Raleigh, NC. Timings were set up to explore the timeframe between 7 and 10 days after mid-anthesis. Plots of the susceptible cultivar P26R12 and the moderately resistant cultivar NC-Neuse, both soft red winter wheats, were inoculated at 0, 3, 5, 6, 7, 8, 9, 11, or 13 days after mid-anthesis (daa) with a suspension of 5 x 10^5 macroconidia/ml of *Fusarium graminearum*. A single inoculation-date treatment was applied to each plot. All cultivar inoculation-date treatments were replicated four times. Mist-irrigation was provided for 28 daa to ensure conditions were conducive for infection at all inoculation timings. From each plot, primary spikes were sampled at 14, 21, 28, 35, 42 days after inoculation (dai), and spikes were sampled at harvest ripeness in all plots, in order to assess the effect of infection timing on visual kernel damage, *Fusarium* infection of grain, and DON contamination**.** Growth stage at each inoculation date was determined by dissecting other sampled spikes, and temperature and rainfall were monitored using a local weather station.

For the 0- and 3-daa inoculations in both cultivars, DON was at extremely high levels in the 14- and 21-dai samples, tended to plateau in the subsequent 3 samples, and then declined as harvest approached. For inoculations at 5 daa and later, DON peaked on average at 35 daa (30-35 daa in the MR cultivar, 35-40 daa in the S cultivar), and declined in the subsequent 20 days.

For both cultivars, DON concentrations at harvest-ripeness showed a declining trend as inoculations became later after mid-anthesis, starting with the 5-daa inoculations. DON levels ≥2 ppm were obtained from the MR cultivar when inoculated at 0, 3, or 5 daa after mid-anthesis, and from the S cultivar when inoculated at those and also two later timings, 6 and 8 daa. Reinforcing earlier results, these findings indicate that the window of susceptibility to economically damaging scab epidemics may be longer in S cultivars than in MR cultivars. One implication is that cultivar susceptibility should be a factor in deciding how late after wheat flowering it will be cost-effective to apply a fungicide. In other words, a significant benefit is more likely to be seen from a late application to a susceptible cultivar than to a moderately resistant one.

EVALUATION OF INTEGRATED METHODS FOR MANAGING FHB AND DON IN WINTER WHEAT IN NEW YORK IN 2013

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OBJECTIVE

To evaluate the individual and interactive effects of moderately resistant cultivars and application of the fungicide Prosaro at initiation of flowering on wheat yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) under two cropping environments in New York.

INTRODUCTION

In response to the USWBSI goal to validate integrated management strategies for FHB and DON, the Disease Management RAC of USWBSI initiated a multi-state, multi-year, coordinated field study. In New York during 2013, we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with Prosaro fungicide in two different experimental environments.

MATERIALS AND METHODS

Both experiments were performed at the Musgrave Research Farm in Aurora, NY following cultural practices recommended for soft red winter wheat in the region. The four cultivars included were 'Pioneer 25R34' (moderately susceptible to FHB), 'Pioneer 25R46' (classified as moderately resistant to FHB), 'Otsego' (classified initially as moderately resistant to FHB), and 'Truman' (established as moderately resistant to FHB). The two experimental plots, both planted on October 10, 2012, were characterized by the planting of winter wheat no-till into 1) soybean residue and 2) corn residue in immediately adjacent parcels of land. Each experimental design was a split plot with four wheat cultivars as whole plots and inoculation or fungicide application treatments as subplots in four replicated blocks. Main plots were planted with a 10 ft wide commercial grain drill and were 20 ft long. Spray treatments applied at Feekes GS10.5.1 on 6/1/13 were 1) non-sprayed, non-inoculated 2) Prosaro® 6.5 fl oz/A & Induce 0.125%, non-inoculated 3) non-sprayed and inoculated with *F. graminearum*; and 4) Prosaro 6.5 fl oz/A & Induce 0.125% and inoculated with *F. graminearum*. Treatments 3 and 4 were inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) on the same day as the Prosaro application after the fungicide had dried and in early evening to provide a better environment for infection. Prosaro and *F. graminearum* applications were applied with a tractor-mounted sprayer with paired Twinjet nozzles mounted at an angle (30° from horizontal) forward and backward and calibrated to deliver at 20 gallons per A. FHB and foliar diseases were assessed at soft dough stages. Grain was harvested from a 4 ft wide x 20 ft long area in each subplot using an Almaco plot combine on 7/16/13. Grain moistures, plot yields, and test weights were recorded with the latter two adjusted for moisture at 13.5%. Analysis of DON content in grain was conducted in the USWBSI-supported mycotoxin laboratory of Dr. Dong. Means were calculated and subjected to Analysis of Variance. Fisher's protected LSD was calculated at P=0.05.

RESULTS AND DISCUSSION

The incidence of FHB over all plots in the two experiments ranged from 6 to 35%. The impact of supplemental inoculation with *F. graminearum* was determined by comparing the non-inoculated and inoculated treatments (combining non-sprayed and Prosaro treatments). Inoculation did not significantly affect yield, FHB index, or DON,

regardless of treatment or variety in the corn stubble environment, but had a modest effect on FHB incidence for the treatment means in the soybean stubble environment. There were no significant differences in cultivar response to inoculation for FHB index between the two environments. FHB and DON in 2013 are attributed primarily to natural rather than supplemental inoculum.

Under moderately low disease pressure, significant differences were detected in yield potential among the varieties with Pioneer 25R46 consistently yielding highest and Truman yielding lowest. Yield for each cultivar was significantly higher following soybean than following corn. This may be attributable to decreased FHB as well increased nitrogen following soybean.

When results of all the cultivars were combined, the overall impact of the Prosaro applications in both environments was to significantly decrease FHB incidence, index, DON, and foliar diseases, and to significantly increase yield and test weight. Prosaro application significantly reduced FDK only in the corn stubble environment where the disease pressure was highest.

All measures of Fusarium head blight were higher in the presence of corn stubble suggesting a dramatic within-plot increase in available spore inoculum from corn debris. The most striking observation was the average 7-8 fold increase in DON contamination levels in grain where wheat followed no-till after corn as compared to soybean. On the other hand, artificial inoculation at flowering with conidial suspensions had almost no significant effect on FHB parameters following either corn or soybean. The fairly late development of FHB symptoms is consistent with infections occurring during moist conditions after peak flowering and for which spores from within-plot corn debris may have contributed a greater portion than sprayed conidia or regional atmospheric inoculum. Otsego, regarded initially as moderately resistant to FHB, was significantly more susceptible than the other cultivars, thus should be designated as no better than moderately susceptible. Pioneer 25R46 showed reduced levels of FHB and DON and should probably be designated as moderately resistant along with the moderately resistant check cultivar Truman.

ACKNOWLEDGEMENT AND DISCLAIMER

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

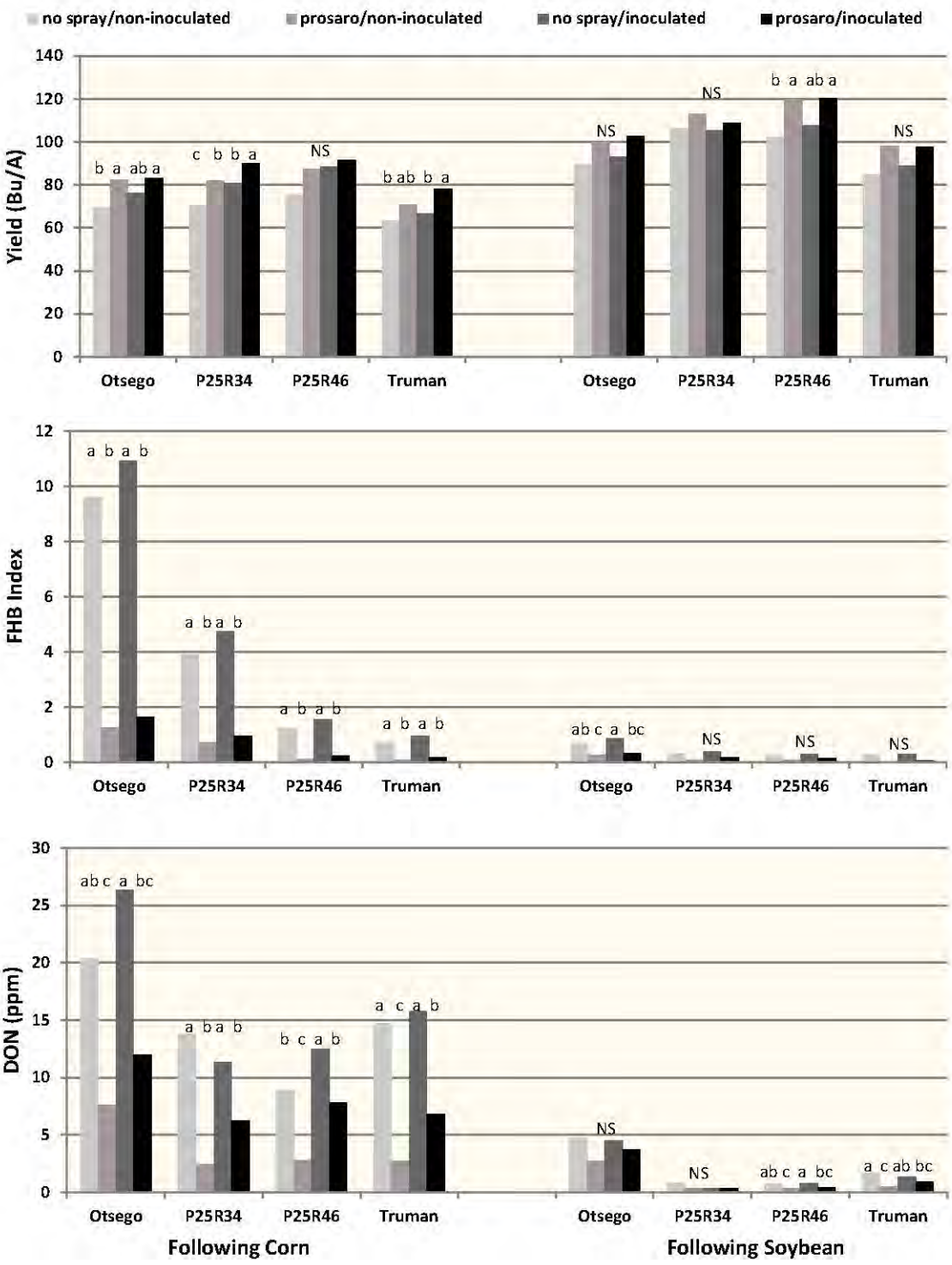

Figure 1. Effect of Prosaro® fungicide application and *F. graminearum* inoculation on yield, FHB index and DON contamination of four winter wheat cultivars in two different environments in Aurora, NY.

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

	Adjusted grain yield (bu/A) at 13.5% moisture		
Treatment:	After corn	After soybean	Average
Non-sprayed, non-inoculated	70.1 c	95.7 c	82.9
Prosaro, non-inoculated	80.1 ab	107.8 a	94.0
Non-sprayed, inoculated	79.1 b	98.9 bc	89.0
Prosaro, inoculated	85.8 a	107.5 ab	96.7
LSD (*P*=0.05)	5.99	8.81	
	Fusarium head blight index (%)		
Treatment:	After corn	After soybean	Average
Non-sprayed, non-inoculated	3.9 a	0.4 a	2.2
Prosaro, non-inoculated	0.5 b	0.1 b	0.3
Non-sprayed, inoculated	4.5 a	0.4 a	2.5
Prosaro, inoculated	0.7 b	0.2 b	0.5
LSD (*P*=0.05)	2.05	0.17	
	Contamination of grain by DON (ppm)		
Treatment:	After corn	After soybean	Average
Non-sprayed, non-inoculated	14.5 a	2.0 a	8.2
Prosaro, non-inoculated	3.9 c	1.0 b	2.4
Non-sprayed, inoculated	16.5 a	2.0 a	9.3
Prosaro, inoculated	8.2 b	1.5 ab	4.8
LSD (*P*=0.05)	3.87	0.53	

Table 2. Main effect of cultivar on grain yield, Fusarium head blight index, and deoxynivalenol contamination at Aurora, NY.

	Adjusted grain yield (bu/A) at 13.5% moisture		
Cultivar:	After corn	After soybean	Average
Otsego	77.8 b	96.4 b	87.1
Pioneer 25R34	80.7 ab	108.4 a	94.6
Pioneer 25R46	85.6 a	112.7 a	99.2
Truman	70.8 c	92.4 b	81.6
LSD (*P*=0.05)	6.60	7.46	
	Fusarium head blight index (%)		
Cultivar:	After corn	After soybean	Average
Otsego	5.8 a	0.5 a	3.2
Pioneer 25R34	2.6 b	0.2 b	1.4
Pioneer 25R46	0.8 c	0.2 b	0.5
Truman	0.5 c	0.1 b	0.3
LSD (*P*=0.05)	1.86	0.16	
	Contamination of grain by DON (ppm)		
Cultivar:	After corn	After soybean	Average
Otsego	16.6 a	3.9 a	10.3
Pioneer 25R34	8.4 b	0.9 bc	4.7
Pioneer 25R46	8.0 b	0. 6 c	4.3
Truman	10.0 b	1.1 b	5.6
LSD (*P*=0.05)	4.97	0.60	

EVALUATING THE EFFECTS OF QUINONE OUTSIDE INHIBITOR FUNGICIDES ON DON ACCUMULATION AFTER ADJUSTING FOR THE EFFECTS OF FHB AND *FUSARIUM GRAMINEARUM* BIOMASS IN SOFT RED WINTER WHEAT

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ABSTRACT

Quinone Outside Inhibitors (QoIs) are effective fungicides for foliar disease control in wheat, but are usually not recommended for Fusarium head blight (FHB) and deoxynivalenol (DON) management, as some members of this group have been reported to increase DON in the grain. However, reasons for such a response are largely unknown. In particular, in-planta evaluation of the effects of QoI fungicides on DON is complicated by the fact that DON contamination is often positively correlated with FHB symptom development and grain colonization with *F. graminearum*. Consequently, high DON levels in QoI-treated plots may be due in part to correspondingly high levels of FHB and/or grain colonization, resulting from the relatively poor efficacy of this group of fungicides against this disease. The confounding effect of FHB on DON response to QoI may lead to inconsistent results across studies. Field and greenhouse experiments were conducted to determine the effects two QoI active ingredients have on DON, after adjusting for the effects of FHB index (IND), *Fusarium* damaged kernel (FDK), and fungal biomass. QoI fungicide treatments consisted of 23.6% pyraclostrobin applied at Feekes growth stages 8-9 (H_8), 10 (H_10), and 10.5.1 (H_10.5.1) and 7% azoxystrobin at Feekes 8-9 (Q_8), 10 (Q_10) and 10.5.1 (Q_10.5.1). A demethylation inhibitor fungicide, 19% tebuconazole + 19% prothioconazole, applied at the same three growth stages (P_8, P_10, and P_10.5.1), plus an untreated check, were included as reference treatments. All plants were inoculated with a spore suspension of *F. graminearum* at anthesis, IND and FDK were rated, and a sample of grain assayed for DON and FBM. Linear mixed model covariance analyses were used to model IND/logDON and FDK/logDON relationships and compare mean logDON among treatments at fixed levels of IND or FDK. All fungicide AIs applied at Feekes 10.5.1 resulted in significantly lower arcIND than the check. P_10.5.1 consistently had the lowest levels of DON, IND, and FDK. Under field conditions, QoIs did not have a significant effect on FDK. QoIs at Feekes 8 (H_8 and Q_8) did not have an effect on IND or logDON. Both H_10.5.1 and Q_10.5.1, as well as Q_10 had significantly higher logDON than the check and the P_10.5.1 treatment, for a given level of disease, under field conditions, but not in the greenhouse. Among the QoI treatments, azoxystrobin applied at Feekes 10 consistently resulted in higher DON and FBM than the check under both field and greenhouse conditions, but this effect was not always statistically significant. These results suggest that both QoIs at Feekes 10.5.1 and 7% azoxystrobin at Feekes 10 have the potential to increase DON in wheat grain, however, both may be useful options for foliar disease management earlier in the growing season without affecting DON.

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This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-071. 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.

IMPACT OF PREDICTION TOOLS FOR FUSARIUM HEAD BLIGHT IN THE US, 2009-2013

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ABSTRACT

A multi-state effort to predict epidemics of Fusarium head blight (FHB) continued during the 2009-2013-growing seasons. This prediction effort includes web-based tools, which display daily estimates of disease risk for 30 states. Commentary developed by a disease specialist in each state covered by the tool is displayed along with the risk maps. Commentary is also distributed via an FHB Alert System that sends email and text messages to mobile devices. The prediction tools received over 20,883 visits (122,000 hits) during the 2013-growing season in the U.S. (April – August). Nearly all of the wheat disease specialists in the 30 states covered by the disease prediction system contributed commentary to the disease prediction effort. A total of 126 commentaries were submitted in 2013. The FHB Alert System sent commentary to just under 1,000 subscribers in 2013. Users of the FHB prediction models and the FHB Alert System were surveyed annually in 2009-2012. The survey results included input from 1,828 respondents and indicated that 64% of these users were either farmers or farm advisors. More than 70% of the users applied the information directly on their farm, or used it to make recommendations to others about disease management. In 2009-2012, 95% of the users considered the information to be of high or moderate value for their farm operations and businesses. A subset of questions targeting the influence of the information suggests that more than 90% of the users experienced moderate or great improvement in their awareness of the disease risk in their area. The results also showed that the information influenced disease management decisions directly for 35% of the respondents, and motivated another 28% to seek advice from others. The 2012 survey asked growers to estimate the monetary value of the information provided to their farm or business. This survey indicates that the average monetary value of the information provided by the prediction system was $17,000 per user. Combining this figure with use statistics suggests that annual impact of the FHB prediction model exceeds $170 million.

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EVALUATION OF SEQUENTIAL FUNGICIDE PROGRAM IN WHEAT AND BARLEY

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ABSTRACT

The objective of this study was to evaluate a sequential fungicide program on controlling Fusarium head blight (FHB) and deoxynivalenol (DON) contamination in hard red spring wheat and barley. Misted experiments, randomized complete block design with six replications, were established at Langdon Research Extension Center in summer 2013. Plot size was 15 x 5 feet. Plots were artificially inoculated with corn spawn inoculum at around boot stage. The fungicide treatments evaluated were 1) untreated, 2) Caramba® at Feekes 10.51, 3) Prosaro® at Feekes 10.51, 4) Headline® at Feekes 5 followed by Caramba at Feekes 10.51, 5) Priaxor® at Feekes 5 followed by Caramba at Feekes 10.51, 6) Tilt® at Feekes 5 followed by Prosaro at Feekes 10.51, and 7) Priaxor at Feekes 5 followed by Twinline® at Feekes 9 and Caramba at Feekes 10.51. For barley Feekes 10.51 application was applied at Feekes 10.5. Fungicide treatments were applied at the recommended rate with water volume of 10 GPA. In wheat, the FHB severity 28 days after treatment (DAT) was significantly lower in treatments 3, 6 and 7 compared to that of untreated. DON level was significantly lower than untreated in treatments 2, 3, 5, and 7. No significant treatment effect was observed for yield and test weight. In barley, All treatments resulted in significantly lower 21 DAT FHB severity compared to untreated. Only treatments 3, 4, and 5 resulted in significantly lower 28 DAT FHB severity compared to control. DON level was significantly lower than untreated only in treatment 6. Treatments were not significantly different for plump kernel percent and yield.

2013 FIELD PLOT TRIAL FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA USING *BACILLUS AMYLOLIQUEFACIENS* STRAINS

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ABSTRACT

Fusarium Head Blight (FHB) or Wheat Scab, caused by *Fusarium graminearium* is an economically important disease of wheat and barley. Yield losses can be controlled or reduced through the use of fungicides alone or in combination with biological control agents (BCAs). Field plot trials were conducted 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 surfactants) and/ or colloidal chitin were done on Briggs spring wheat heads at Feekes 10.51. No statistically significant treatment differences were observed for FHB incidence, severity and index. The combination of *Bacillus* 1BA, plant oil, colloidal chitin and Prosaro reduced the FHB incidence to 3.17%, which was less than the FHB incidence observed for Prosaro alone (5.91%) or for the untreated control (17.08%). The treatment combination of *Bacillus* strains 1BA, 1D3, plant oil, colloidal chitin and Prosaro reduced the FHB severity to 3.64%, which was less than the FHB severity observed for Prosaro alone (7.81%) or the untreated control (17.01%). The treatment of *Bacillus* strain 1BA with plant oil, colloidal chitin and Prosaro reduced the disease index to 0.6%, while the treatment of *Bacillus* strains 1BA and 1D3 with plant oil, colloidal chitin, and Prosaro reduced the disease index to 0.53%. The treatment of Prosaro alone reduced the FHB disease index to 0.77%, while for the untreated control it was observed to be 2.84%. Several treatments with the BCAs showed significant differences (P=0.05) for test weight and yield in comparison to the untreated control. The DON data are not yet available as of November 2013. This trial demonstrated that *Bacillus* strains 1BA or 1D3 in combination with Prosaro and/or colloidal chitin can reduce FHB in wheat, more than a single application of Prosaro.

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WIN EXPERIENCE WITH DON FORECASTING IN CANADA, EUROPE

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ABSTRACT

Weather INnovations Consulting, LP (WIN), based in Chatham, Ontario, Canada, provides turnkey online programs in climate and environmental monitoring and modelling, primarily for agriculture. WIN builds web-based agronomic solutions for growers that help them deal more proactively with the weather's influences on farming. WIN's website for Ontario grain growers, *WeatherCentral.ca*, offers site-specific weather forecasts, disease and insect risk advisories, growth stage models and includes tools targeted at *Fusarium*-related issues. The DONcast® model, for instance, predicts deoxynivalenol toxin concentration in wheat at harvest during the heading stage to assist growers with fungicide application decisions. DONcast® examines observed, forecasted and historical weather data, along with field-specific agronomic data, and updates predictions daily through to harvest time which assists growers in strategizing which fields with elevated DON potential should be harvested first.

WIN develops tools like DONcast® through research and development collaborations with industry, universities and other stakeholders. A variation of the DONcast® model has been calibrated for European conditions with support from Bayer CropScience. WIN's European modeling activity includes forecasts of several major *Fusarium* species which helps refine the prediction of mycotoxin contamination in wheat. Dr. Rishi Burlakoti, one of WIN's plant pathologists, is now concluding a collaborative Canadian chemotyping study with researchers from the University of Guelph and Agriculture and Agri-Food Canada that examined multiple strains of *Fusarium graminearum* from wheat, potato and corn with respect to geographic locations/years and weather variables.

Ian Nichols, WIN's founder and president, will present an overview of WIN's efforts in providing advisories for agricultural producers with respect to FHB.

FHB INTEGRATED MANAGEMENT: A 2013 UPDATE

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ABSTRACT

Experiments were established in fields previously planted with host or non-host crops of *F. graminearum* (*F.g*) to evaluate the integrated effects of fungicide and genetic resistance on FHB and DON in small grain crops under different environmental conditions. At least three commercial small grain cultivars, classified as susceptible (S), moderately susceptible (MS) or moderately resistant (MR), were planted in three to six replicate blocks in each trial. The standard experimental design was a randomized complete block, with a split-split-plot arrangement of cultivar (whole-plot), inoculation (sub-plot) and fungicide treatment (sub-sub-plot; UT, untreated and TR, treated). In some trials, a split-split plot design as used with cultivar as whole-plot and fungicide or fungicide x inoculation combination as sub-plot. In some trials established in *F.g* host crop residue, plots were not artificially inoculated. Prosaro® (6.5 fl. oz/A + NIS) was applied at anthesis, using CO_2 powered sprayers, equipped with Twinjet XR8002 or paired XR8001 nozzles, mounted at a 30 or 60^o angle, forward and/or backward. For trials with artificial inoculations, either *F. graminearum*-colonized corn kernels were spread on the soil surface of plots prior to anthesis or plots were spray-inoculated with a spore suspension of the fungus approximately 24 hours following fungicide treatments. FHB index (IND, plot severity) was assessed during the dough stages of grain development, and at harvest, grain samples were sent to a USWBSI-supported laboratory for mycotoxin analysis. At the time of this report, data were collected from 19 experiments, four from MD, three each from IL and SD, two each from NY and MO, one each from AR, OH, IN, NB and WI. Fifteen of the experiments were conducted with SRWW, two with HRWW and one each with HRSW and barley. Percent control ($[\bar{X}_{S\_UT} - \bar{X}_{MGNT\,COMBO}] / \bar{X}_{S\_UT}$)•100) was calculated as a measure of the efficacy of different cultivar resistance + fungicide management combinations (S_TR, MS_UT, MS_TR, MR_UT and MR_TR) against IND and DON relative to the susceptible, untreated check (S_UT). In six of the 19 experiments, mean index in the S_UT treatment combination was less than 2%. The highest level of disease were observed in IL, MO and NE. Averaged across experiments and grain classes, mean percent control in IND was 77% for MR_TR, 54% for MR_UT, 45% for MS_TR, 13% for MS_UT and 35% for S_TR. For DON, the corresponding percentages were 71, 52, 59, 47, and 26%, for MR_TR, MR_UT, MS_TR, MS_UT and S_TR, respectively.

The full report will be available by mid-January 2014 through the Scab Website (www.scabusa.org).

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IMPLICATIONS OF RAINFALL AT ANTHESIS FOR SCAB MANAGEMENT WITH FUNGICIDES

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ABSTRACT

Not only is rainfall during anthesis conducive to Fusarium head blight (FHB) development and deoxynivalenol (DON) accumulation, it also affects the implementation and efficacy of one of the most important FHB management strategies, fungicide treatment application. Rainfall simulators were used to study the effects of rainfall treatments applied at intervals ranging from 15 to 195 minutes after Prosaro® application on the efficacy of this fungicide against FHB index (IND) and DON and the presence of residue of tebuconazole, one of the active ingredients in Prosaro, on wheat spikes. In addition, field experiments were conducted in Ohio and Illinois to evaluate the efficacy of post-anthesis applications of Prosaro and Caramba® for FHB and DON management. In four of five simulated rainfall experiments, all fungicide-treated experimental units (EUs) had significantly lower mean IND and DON than the untreated check, regardless of rainfall treatment. EUs that received the earliest rains (15, 30 and 60 minutes after fungicide application) tended to have the highest mean IND and DON, but were generally not significantly different from EUs that received later rainfall (105 to 195 minutes after fungicide treatment) or EUs treated with the fungicide without being subjected to simulated rain. Tebuconazole residue on wheat spikes decreased exponentially over time, with the greatest rate of reduction occurring during the first eight days after application. When applied with the non-ionic surfactant Induce, Prosaro appeared to be very rainfast for the fixed set of rainfall characteristics evaluated in this study, and tebuconazole residue did not persist very long after application on wheat spikes. Results from experiments in which fungicide treatments were applied at anthesis or at 2, 3, 4, 5 or 6 days after anthesis showed that both anthesis and post-anthesis treatments resulted in significantly lower mean IND, FDK and DON than the untreated check. Mean IND and DON were either not significantly different between anthesis and post-anthesis treatments or were significantly lower for some post-anthesis treatments in experiments in which it rained or conditions were unseasonably cold during anthesis. Results were consistent for Prosaro and Caramba, and across cultivars. Mean percent control of IND and DON for post-anthesis treatments were 62 and 45%, respectively, compared to 57 and 39%, respectively, for anthesis treatments.

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2013 UNIFORM FUNGICIDE PERFORMANCE TRIALS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA IN HARD RED SPRING WHEAT

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for over twenty years. The objective of this study was to evaluate the effects of various fungicides and fungicide combinations at different application timings for the suppression of Fusarium head blight and other wheat diseases in hard red spring wheat. Two hard red spring wheat cultivars, 'Brick' and 'WB Mayville', were planted at three South Dakota locations (Groton, South Shore/Watertown and Volga). Studies at Groton and South Shore were conducted under ambient conditions. The Volga site was under ambient conditions until anthesis, after which mist irrigation was applied. Trial treatments included an untreated check and the following fungicides: Applied at Feekes growth stage 10.51: Prosaro® (6.5 fl oz/A), Caramba® (14 fl oz/A), Tebucon® (4 fl oz/A), Tebucon (4 fl oz/A) + Caramba (10 fl oz/A) and Tebucon (4 fl oz/A) + Thymol (10 g/A); Applied at 3-7 days after Feekes growth stage 10.51: Prosaro (6.5 fl oz/A) and Caramba (14 fl oz/A). All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. The experimental design was a split plot in randomized complete block with six replications. Main plots were the two cultivars and subplots were the fungicide treatments. Wheat plots at the Volga location were inoculated by spreading *Fusarium graminearum* (isolate Fg4) infected corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied from 5:00 pm until 10:00 pm each day for two weeks following anthesis. Other sites had natural inoculum from corn stalk residue and no misting. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, FHB field severity, and FHB disease index (FHB incidence x severity). Samples were collected for *Fusarium* damaged kernels (FDK), deoxynivalenol (DON), grain yield and test weight.

No significant effect of treatments for FHB incidence, FHB severity and FHB disease index were found at the South Shore/Watertown location but four treatments (Prosaro applied at Feekes 10.51, Tebucon applied at Feekes 10.51, Tebucon + Caramba applied at Feekes 10.51 and Caramba applied at 3-7 days after Feekes 10.51) were significant in reducing FDK levels. At the Groton location, there were three treatments significant in reducing FHB incidence (Tebucon applied at Feekes 10.51, Tebucon + Caramba applied at Feekes 10.51 and Caramba applied at 3-7 days after Feekes 10.51. Also at the Groton location, all of the treatments reduced FHB disease index. At the Volga location, all treatments except for the Tebucon, significantly reduced FHB incidence. Only Caramba applied at Feekes 10.51 and Caramba applied at 3-7 day after Feekes 10.51 reduced FHB severity at Volga location. At the Volga location, all of the treatments except the Tebucon treatment were significant at reducing the FHB disease index and all of the treatments except Tebucon and Tebucon + Thymol were significant in reducing FDK.

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PRELIMINARY ECONOMIC ANALYSIS OF FHB MANAGEMENT STRATEGIES

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ABSTRACT

The use of moderately resistant cultivars integrated with Prosaro® or Caramba® application and cultural practices has been very effective at reducing Fusarium head blight (FHB), deoxynivalenol (DON), as well as grain yield and quality losses caused by this disease. However, under highly favorable weather conditions, even when the best management options are implemented, yield and quality (test weight of grain, *Fusarium* damaged kernel [FDK] and DON contamination) losses still occur, leading to price discounts or dockage. But not using a fungicide when it is most warranted may result in even larger reductions in yield and quality. On the other hand, when FHB is low, losses may be minimal; consequently, applying a fungicide may reduce a grower's income because the application cost may not be recouped. There is therefore a need for information regarding the economic value of implementing FHB management strategies that could be used to help growers make more informed management decisions. Different combinations of cultivar FHB reaction (MR = moderately resistant and S = susceptible), Prosaro fungicide treatment (TR = treated and UT = untreated) and grain harvesting strategy (C1 = default and C2 = modified to remove scabby grain) were combined to derive 8 different FHB management programs: P1 = MR+TR+C2, P2 = MR+UT+C2, P3 = S+TR+C2, P4 = S+UT+C2, P5 = MR+TR+C1, P6 = MR+UT+C1, P7 = S+TR+C1, and P8 = S+UT+C1. P8 (without any management intervention) served as the reference program. Relative to P8, the greatest percent reduction in FDK and DON and increase in yield was observed for programs that included a Prosaro fungicide treatment (P1, P3, P5 and P7). The greatest percent increase in test weight relative to P8 was observed when C2 was integrated with MR and TR. The effects of different management programs on IND/yield and IND/test weight relationships was evaluated through linear mixed model covariance analysis, showing that programs affected the height but not the slopes of the IND/yield and IND/test weight regression lines. Predicted yield, test weight, FDK, and DON for P8 for a range of index values (5, 10 and 20%); percent FDK and DON reduction and percent yield and test weight increase for the four best management programs (P1, P3, P5 and P7) relative to P8; a range of estimated grain prices and price discounts; and a range of fungicide application costs were used to conduct a cost/benefit analysis of each of the management programs. Preliminary results showed that under moderate to high IND levels (10 and 20%), management programs that include an MR cultivar and Prosaro application (P1 and P5) consistently had lower grain price discounts (US$/MT of grain) and higher gross cash income (US$/ha) than other management programs. The use of modified combine configuration (C2) was most beneficial for lowering price discounts when either a MR cultivar or the Prosaro treatment was not implemented, IND levels were high (10 and 20%), and estimated grain prices were high (US$ 197 and 276/MT).

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