Proceedings of the 2010 National Fusarium Head Blight Forum

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D. Ellis and D. Van Sanford

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- David Hansen, Minnesota Ag. Experiment Station - Hand holding Barley spikes of the new variety Quest with improved FHB resistance. Quest was released by the Minnesota Agricultural Experiment Station in 2010.
- Scott Heisel, American Malting Barley Association - Barley being loaded into germination compartment in malthouse.
- Gary Hanning, Busch Agricultural Resources - Anheuser Busch brewery in Fort Collins, Co. with Busch Agricultural Resources barley research plots in the foreground.

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PLENARY SESSION

Chairperson: Dave Van Sanford

INDUSTRY NEEDS FOR EARLY WARNING AND INTEGRATED MANAGEMENT SYSTEMS FOR HARMFUL MYCOTOXINS

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ABSTRACT

Many grains are susceptible to infection by various microorganisms that produce harmful mycotoxins. These mycotoxins present a food safety risk and are carefully controlled by food companies and governmental agencies. This control comes at a cost to the food manufacturers all along the value stream and ultimately the cost is passed to consumers. This presentation will discuss the impact of mycotoxins on the food industry through a series of case studies. Also, we will present a case for better testing to provide early warning and better management of crops when these infections are present.

SESSION 1:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Co-Chairpersons: Steve Scofield and Jyoti Shah

CHARACTERIZATION OF WHEAT CYTOCHROME P450S UP-REGULATED AS AN EARLY RESPONSE TO THE *FUSARIUM* MYCOTOXIN DEOXYNIVALENOL

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ABSTRACT

Using transcript-profiling studies, we identified two cytochrome P450 (CYP) transcripts (CYP1724 and CYP840) up-regulated in wheat spikelets as an early response to DON treatment (5 mg ml^{-1}; 4 h post-treatment). This toxin-induced accumulation was found to be associated with the DON tolerance of cultivar CM82036 contributed by the quantitative trait locus (QTL) *Fhb1* (chromosome 3BS). Using real time RT-PCR analysis, the temporal accumulation (1 – 4 h) of these transcripts in roots of DON-treated (20 mg ml^{-1}) wheat seedlings (cultivar CM82036) was determined. In roots of DON treated seedlings, the CYPs were induced within 1 h and their levels reached a maximum at 3.5 h post-DON treatment. In seedlings of cultivar CM82036, both the CYP transcripts were induced 1.7 fold in salicylic acid-treated roots, while only CYP840 transcripts were 3.2 times more abundant in jasmonic acid-treated roots at 4 h post-treatment as compared to control roots ($P < 0.001$). The CYPs expression in coleoptiles of seedlings of cultivar CM82036 (DON resistant) and cultivar Remus (DON susceptible) whose roots were treated with 20 μg ml^{-1} of DON for 24 h in light and dark conditions was analyzed. Although no detectable levels of CYP 840 transcripts were found in coleoptiles of both cultivars, CYP1724 transcripts were induced in coleoptiles both by DON and light in both genotypes. While CYP1724 expression levels did not differ among genotypes under dark, its expression levels were 2.37 times higher in coleoptiles of DON-treated seedlings of cultivar CM82036 than of cultivar Remus when incubated in light, suggesting a light dependant DON tolerance in wheat. Further characterization of the CYPs is being carried out using heterologous expression systems.

IDENTIFICATION OF A DIRECT ROLE FOR MITOCHONDRIA IN TRICHOTHECENE RESISTANCE

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ABSTRACT

Trichothecenes produced by various species of *Fusarium* are increasingly contaminating cereal crops worldwide. *Fusarium graminearum* causes Fusarium head blight (FHB) in both wheat and barley resulting in reduced plant yield and contamination of the grains with trichothecenes, in particular DON. Improving FHB resistance, hence, remains a high priority in wheat and barley breeding programs throughout the world. Identifying the molecular mechanisms underlying trichothecene toxicity is therefore vital to understanding *Fusarium* pathology and engineering FHB resistance.

We have previously shown that mitochondria are critical for trichothecin (Tcin) toxicity in yeast. Sensitivity to Tcin increased when yeast cells were grown in non-fermentable media, which requires functional mitochondria, while cells devoid of mitochondria (ρ^0) showed increased resistance to Tcin. Over 60% of gene deletions that conferred resistance to Tcin were associated with mitochondrial function in our genome wide screening of the yeast deletion library. Moreover, mitochondrial translation was shown to be inhibited by Tcin in the wild type but not in the resistant mutants. To determine if Tcin has a direct effect on mitochondria, we examined translation in isolated yeast mitochondria treated with Tcin. Furthermore, we employed flow cytometry to assess functionality of the yeast mitochondria when treated with trichothecenes.

A 60% inhibition in translation was observed in isolated yeast mitochondria treated for 10min with 4μM Tcin, solubilized in 50% ethanol, when compared to mitochondria treated with 50% ethanol. This inhibition increased to 78% at 8μM Tcin suggesting a direct inhibition of mitochondrial translation by Tcin. Flow cytometric analyses of Tcin-treated yeast cells stained for mitochondrial membrane potential, ROS generation and cell death also suggest a role for mitochondria in Tcin-induced cell death. Peak shifts in the median fluorescence intensities of Tcin-treated cells indicate that Tcin triggers ROS generation resulting in hyperpolarization of the mitochondrial membrane which eventually leads to cell death.

SEQUENCING AND PRELIMINARY ANALYSIS OF CHROMOSOME 2H BIN 10 PREDICTED GENES

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OBJECTIVES

Map and sequence barley chromosome 2H bin 10 region in order to identify candidate genes for Fusarium head blight resistance.

INTRODUCTION

Fusarium Head Blight (FHB) is a serious disease of barley and wheat that has been difficult to control due to lack of available Mendelian resistance genes. Nevertheless, numerous quantitative trait loci (QTL) conferring variable degrees of resistance have been identified. One strong, recurring FHB resistance QTL was identified on chromosome 2H bin 10 (Canci et al., 2004; Dahleen et al., 2003; de la Pena et al., 1999; Hori et al., 2005; Horsley et al., 2006; Ma et al., 2000; Mesfin et al., 2003). The chromosome 2H bin 10 QTL has been ascribed to the 2-rowed head type present in one of the resistant parents CIho 4196 (Zhu et al., 1999). This idea has been discredited by the development of a 6-rowed CIho 4196 mutant that has consistently shown FHB resistance comparable to the parent CIho 4196 (Boyd et al., 2008) and selection of 6-rowed recombinants from crosses of CIho 4196 by susceptible cultivars that have maintained FHB resistance (Kleinhofs et al., unpublished; Horsley et al., unpublished). The chromosome 2H bin 10 region from MWG865 to MWG503 has been the focus of our research to develop a saturated genetic and physical map. Recently we have narrowed this region from BF265762A to ctg15522. This region covers approximately 3.5 cM and is saturated with 15 BAC contigs containing a minimal tiling path of 31 BAC clones. Sequencing and preliminary analysis of these BAC clones is reported.

MATERIALS AND METHODS

BAC DNA isolation and sequencing - BAC DNA was isolated using the NucleoBond BAC 100 kit (Clonetech, Mountain View, CA, USA) per manufacturers instructions. The quality and quantity of the individual BAC DNA was evaluated by agarose gel separation and using nanodrop technology. Individiual BAC clones were quantified and adjusted to ca.1ug/ul and pooled for construction of the libraries. The 454 libraries were prepared by fragmenting ca. 10 ug pooled BAC plasmid DNA using a nebulizer. The fragmented DNA was run on a 1% agarose gel and size selected for fragments of 400-600 bp by isolating the appropriate band from the gel. The gel slice was extracted using the Qiagen (Valencia, CA, USA) gel elution kit (to isolate and purify the fragmented DNA. The purified fragments were evaluated for size and quantity using a BioAnalyzer genechip. Fragmented DNA was ligated to the provided adaptors and purified using oligotex beads. An additional size and quantity verification was run using a BioAnalyzer genechip. Libraries were evaluated for concentration by titration according to the manufacturer's protocols and sequenced on three regions of a four-region gasket. Sequencing was performed using the Genome Sequencer FLX titanium series protocol (Roche 454 Life Sciences).

Sequence analysis - Sequences were assembled by the Genome Sequencer FLX system software. Contigs from the analysis were screened using the BLASTx function at the NCBI website to eliminate *E. coli* contamination. The remaining contigs were analyzed in Softberry FGENESH (linux1.softberry.com) for gene prediction. Predicted genes were then screened using the BLASTx function to sort out putative

retro elements and to assign putative function to other genes.

Southern probe development and mapping to BAC clones - Predicted genes were screened against the *Hordeum vulgare* database of NCBI using the BLASTn function limited to "EST others" to find ESTs in our library. Those ESTs were hybridized to BAC filters containing the BACs sequenced in the appropriate region in order to connect the sequenced contig with the correct BAC.

Primer development and re-mapping to individual BAC clones - When no EST was available from a contig, primers were designed and amplified using a touchdown PCR protocol performed on the twelve BACs of the region to localize the marker to the BAC(s) within the region. Touchdown PCR was as follows: 94 C for 5 min, then 10 cycles of 95 C for 1 min, 70 C for 30 sec decreasing by 1 C every cycle, and 72 C for 1.5 min. Then followed 25 cycles of 95 C for 30 sec, 55 C for 30 sec, and 72 C for 1.5 min and a final 72 C for 5 min. When more than two amplicons amplified with a single primer set from multiple BACs, the amplicon was sequenced and compared to the original contig to determine the correct BAC combination.

RESULTS

The 16 minimum tiling BAC contigs containing 36 clones identified for chromosome 2H bin 10 and a small part of the bin 9 region were divided into three groups of 12 each. Each group of 12 was sequenced in bulk at Washington State University using the 454 Life Sciences methodology. The sequence was delivered in computer assembled gene contigs, which were analyzed. Gene finder program Softberry (linux1.softberry.com) identified 129 putative genes of which 23 were previously known and mapped by us, not including the *Rrn5S1* gene. Based on recombinant analyses, we expect that the BF265762A to ctg15522 segment should contain the FHB resistance gene(s). We previously estimated this region to cover approximately 3,814 kb, not including the approximately 1,250 kb 5S RNA gene locus *Rrn5S1* present in this interval (Boyd et al., submitted).

Here we report more detailed analysis of Region 1 extending from BF265762A to BI948584 (Fig. 1). This approximately 1.2 cM region is covered by 6 BAC contigs represented by 12 minimum tiling BAC clones with a total minimum size of 1.39 Mb. Sequence analysis identified 50 putative genes, ten of which have been previously identified and mapped (Fig. 1). Gene numbers 30 and 32 come from the same sequence contig and identify the same EST, thus probably represent a recent duplication. Detailed characterization of the putative genes is shown in Table 1. Although some of the genes have putative functions assigned, for the most part they represent hypothetical proteins or have no significant homology (S value 80 or higher) in the NCBI database.

DISCUSSION

DNA sequencing using the newer sequencing technologies has become relatively easy and inexpensive. Taking advantage of the 454 sequencing technology available, we sequenced 36 BAC clones in 3 groups of 12 from the chromosome 2H bin 10 region presumed to contain the FHB resistance gene(s). Sequence analysis, however, is still a time consuming and hands-on process. Here we report the preliminary sequence analysis of Region 1 BAC clones, which includes the genomic region from marker BF265762A to BI948584. Although we had fairly saturated this region with markers, the number of putative genes identified exceeded our expectations, thus clearly illustrating the validity of the sequencing approach for gene discovery. Of the 53 putative genes identified by database searches only 22 have a putative function and several of these are listed as hypothetical proteins. Twenty-nine of the putative genes have "no significant similarity" in the NCBI database, defined as an S value below 80. It is quite probable that some of the putative genes are not real, but this is expected to be a relatively small portion of the total and might be offset by some genes that were missed by the gene finder program.

In summary, we have identified a large number of putative genes that reside in the chromosome 2H bin 10 region. Analysis of these putative genes will

facilitate completion of the BAC clone contigs to completely cover the chromosome 2H bin 10 region and lead to the identification of putative FHB resistance genes.

ACKNOWLEDGEMENT AND DISCLAIMER

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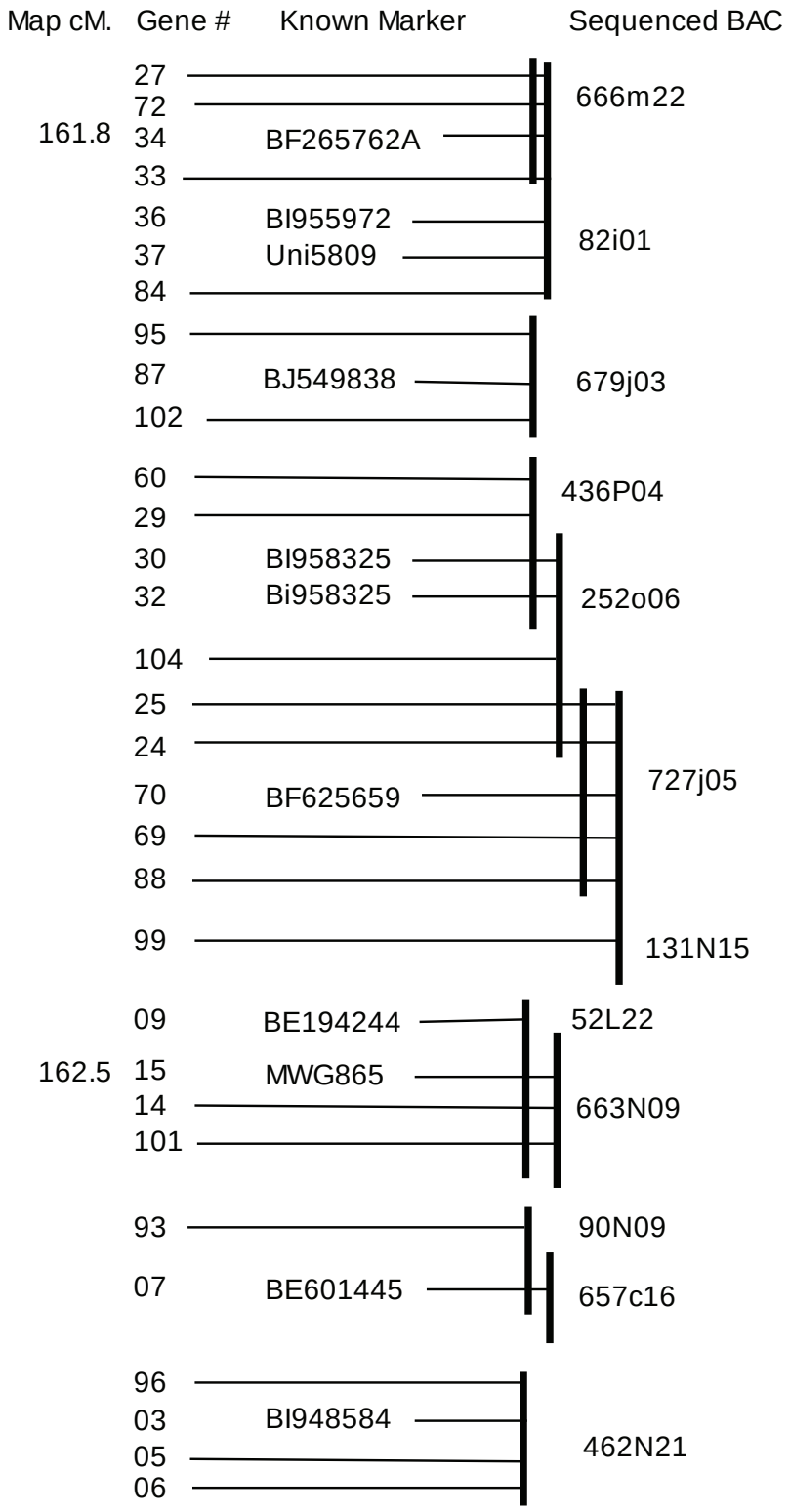

Fig. 1. Physical map location of predicted genes from chromosome 2H bin 10 Region 1. Known markers – previously mapped markers arranged in order from proximal to distal region. The unknown genes are arranged in a probable, but not confirmed, order based on their relationship with the known markers. Two cM values from mapped markers are indicated to facilitate orientation of the physical map. Gene # - is an arbitrary number assigned to predicted genes by the computer program used for analysis of the sequence. Sequenced BAC clones are identified. The predicted genes from Region 1 that are not yet assigned to specific BAC clones (see Table 1) are not included in this Figure.

Table 1. Chromosome 2H bin 10 Region 1 predicted gene BAC location, barley EST homology and putative function. Genes below #46 have not been localized to specific BAC clones at this time. Sequence contig number (seq. ctg#) refers to the contig assembled by the sequencing program software and an arbitrary number. Blastn hits on barley ESTs were scored as positive if the S value exceeded 80.

Region 1						
Gene#	seq ctg#	Known marker	Seq. BAC(s)	Blastn	Blastx	Score
72	12		82i01, 666M22	no hits	No significant similarity	
27	1732		82i01, 666M22	no hits	No significant similarity	
34	1366	BF265762A	82i01, 666M22	BF266945, BF265762	Os04g0542800 (Oryza sativa, Japonica)	540
33	1366			no hits	No significant similarity	
36	45	BI955972	82i01	BJ472255, BI955972	hypothetical protein OsJ_15640 (Oryza sativa, Japonica)	618
37	45	Uni5809		AJ462415	hypothetical protein SORBIDRAFT_06g024060 (Sorghum bicolor)	493
84	1415		82i01	no hits	No significant similarity	
95	1461		679j03	no hits	No significant similarity	
87	1470	BJ549838	679J03	CB869218, BJ549838	hypothetical protein OsJ_15627 (Oryza sativa, Japonica)	565
102	1811		679j03	BQ658680	Far1 [Triticum aestivum]	183
60	1550		436p04	GH220600	GATA zinc finger family protein (Zea mays)	253
29	1499			BY852017	No significant similarity	
30	1499	BI958325	436P04, 252o06	BI958325	hypothetical protein SORBIDRAFT_06g023950 (Sorghum bicolor)	302
32	1499	BI958325	436P04, 252o06	GH228028	OSJNBa0011L07.8 (Oryza sativa, Japonica)	283
104	1658		252o06	CB881126	ORF (Triticum aestivum)	123
25	1729		252o06, 131N15, 727j05	CX626672	No significant similarity	
24	1729		252o06, 131N15, 727j05	no hits	No significant similarity	
70	1437	BF625659	727j05, 131n15	BY847183, BF625659	H0115B09.3 (Oryza sativa, Indica)	264
69	1437			no hits	No significant similarity	
88	1858		727J05, 131N15	no hits	No significant similarity	
99	122		131n15	no hits	No significant similarity	
9	1485	BE194244	52L22	no hits	gt-2 (Oryza sativa, Indica)	262
15	1557	MWG865	52L22, 663N09	CB866125	UDP-glycosyltransferase UGT88C4 (Avena strigosa)	155
14	1557			BU992850	No significant similarity	
101	1766		52L22, 663N09	no hits	No significant similarity	
93	1537		90n08	no hits	No significant similarity	
7	111	BE601445	90n08, 657c16	CB883689, BE601445	Os04g0543200 (Oryza sativa, Japonica)	939
96	1819		462n21	BU986685	hypothetical protein OsI_17244 (Oryza sativa, Indica)	885
3	49	BI948584	462N21	GH224396, BI948584	Os04g0541900 (Oryza sativa, Japonica)	268
5	94		462N21	BF628929	putative far-red impaired response protein (Oryza sativa, Japonica	504
6	94		462N21-end	FD527594	hypothetical protein (Oryza sativa, Japonica)	103
46	41			no hits	No significant similarity	
47	41			no hits	No significant similarity	
48	41			no hits	No significant similarity	
75	60			no hits	No significant similarity	
55	126			BY840630	hypothetical protein OsJ_15628 (Oryza sativa, Japonica)	87
58	126			no hits	No significant similarity	
39	1450			BQ470966	hypothetical protein OsI_16839 (Oryza sativa, Indica)	885
40	1450			BG366371	No significant similarity	
11	1501			DN177342	RecName: Full=Formin-like protein 3; AltName: Full=OsFH3	587
13	1501			no hits	No significant similarity	
81	1543			no hits	No significant similarity	
64	1554			BU993407, BF618043	hypothetical protein (Beta vulgaris)	1001
43	1603			no hits	Os07g0285700 (Oryza sativa, Japonica)	147
66	1606			EX578159	forminy 2 domain-containing expressed protein (Oryza sativa, Jap	226
63	1608			CB865507	No significant similarity	
44	1630			CA014815	CK2 regulatory subunit B1 (Zea mays)	379
45	1630			no hits	hypothetical protein SORBIDRAFT_06g024070 (Sorghum bicolor)	874
52	1722			EX573578	No significant similarity	
50	1722			no hits	No significant similarity	

FIGHTING AGAINST FHB – AN EXCELLENT NOVEL RESISTANCE SOURCE FOR FUTURE WHEAT BREEDING

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ABSTRACT

Fusarium head blight (FHB) is a devastating wheat disease that causes tremendous economic losses by reducing grain yield and quality in wheat-growing areas worldwide. Sources of resistance are critical for genetically improving wheat for resistance to FHB. From a large-scale evaluation of tetraploid wheat (*Triticum turgidum*) germplasm for resistance reactions to FHB, we identified an accession (PI 277012) that consistently showed a high level of resistance across all environments in both greenhouse and field experiments. PI 277012 is currently classified as tetraploid emmer wheat (*T. turgidum* subsp. *dicoccum*) in the National Small Grains Collection, but somatic chromosome counts revealed that this accession was actually a hexaploid wheat. To characterize the FHB resistance in this accession, we developed a doubled haploid (DH) mapping population consisting of 130 lines from the cross between PI 277012 and the hard red spring wheat cultivar 'Grandin'. The DH population was then evaluated for reaction to FHB under three greenhouse seasons and five field environments. Based on whole genome linkage maps that consisted of 350 SSR markers spanning 2,703 cM of genetic distance, two major FHB resistance QTLs were identified on chromosome arms 5AS and 5AL. The 5AS QTL peaked at the marker interval between *Xbarc180* and *Xwmc795*, and explained up to 25% of the phenotypic variation. The 5AL QTL explained up to 35% of the trait variation and peaked at the interval between markers *Xwmc470* and *Xgwm595*. FHB resistance has not previously been reported to be associated with this particular genomic region of chromosome arm 5AL, thus indicating the novelty of FHB resistance in PI 277012. Furthermore, the FHB resistance effects of neither QTL were associated with plant height and maturity. Elite agronomic traits were observed among several FHB-resistant DH lines. Therefore, these results suggest that PI 277012 is an excellent source for improving FHB resistance in wheat. The markers identified in this research are being used for marker-assisted introgression of the QTLs into adapted durum and hard red spring wheat cultivars.

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USING DOUBLED HAPLOIDS TO SPEED UP GENETIC ANALYSIS FOR RESISTANCE TO FHB AND OTHER COMPLEX TRAITS IN WHEAT

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ABSTRACT

Doubled haploids (DHs) or recombinant inbred lines (RILs) are useful in wheat for analyzing resistance to Fusarium head blight (FHB) or other complex traits that are governed by multiple genes and/or subject to environmental influences. This is because DHs or RILs allow highly-confident assessment of phenotypic differences in replicated trials under different conditions. DHs, obtained by doubling the chromosome number of haploids, are genetically pure and obtained in a single generation. This makes DHs much better for genetic analysis than RILs, which take far longer to produce and contain some residual heterozygosity. The complete homozygosity of DHs enables accurate evaluation of genetic effects, thus facilitating identification of genes controlling a complex trait such as resistance to FHB. Highly-efficient use of DHs in identifying novel gene sources for FHB resistance or other complex traits in wheat has recently been demonstrated. However, DHs have not been widely used in public wheat breeding programs in the United States mainly due to the complexity of producing DHs. In order to produce DHs on a scale that meets the requirements of wheat breeders, Heartland Plant Innovations (HPI) Inc. has launched a doubled haploid laboratory devoted to providing DHs on a fee-for-service basis for both public and private customers. Located on the campus of Kansas State University in Manhattan, Kansas, HPI is a unique collaboration of public and private partners consisting of a team of agricultural producers, public research institutions and plant science technology companies. Besides providing DH service for wheat breeders and geneticists, the DH lab in HPI will also focus on improving methods for highly-efficient production of wheat DHs, with the goal of greatly reducing the cost of wheat DH lines.

TESTING TRANSGENIC SPRING BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2010 FIELD NURSERY REPORT

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ABSTRACT

The 2010 field screening nursery, with 88 barley plots was located at UMore Park, Rosemount MN. Trial entries (n=18 transgenic) and an untransformed 2-row control Conlon (susceptible) were submitted by USDA-ARS, RRVARC Fargo. Barley lines with known reactions to Fusarium head blight (FHB) were also included as checks. The checks used were the moderately resistant cultivar Quest (included in previous nurseries as breeding line M122) and the susceptible cultivars Robust and Stander. The experimental design was a randomized block with four replicates. Plots were 2.4 m long single rows. The trial was planted on May 4, 2010. All plots were inoculated twice, with the first inoculation applied at head emergence. The second inoculation was applied three days after the initial inoculation (dai) for each plot. The inoculum was a composite of 51 *F. graminearum* isolates at a concentration of 200,000 macroconidia ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml L^{-1} as a wetting agent. The inoculum was applied at a rate of ca. 30 ml per meter of plot row. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10ml sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on June 28 till July 15 to facilitate FHB development. FHB incidence (FHBI) and severity (FHBS) were assessed visually 14 dai on 20 arbitrarily selected spikes per plot. FHBI was determined by the percentage of spikes with visually symptomatic spikelets of the 20 spikes observed. FHBS was determined as the percentage symptomatic spikelets of the total of all spikelets observed on the 20 spikes. Plots were harvested at maturity on August 5. The harvested seed from each plot was split to obtain a 25 g sub-sample, which was then cleaned by hand. The samples were ground and submitted for deoxynivalenol (DON) analysis. FHBI for all treatments ranged from 86 to 99%. FHBS ranged from 13 to 36% for the 18 entries examined. The FHBS for the untransformed control Conlon was 23%. The FHBS for the moderately resistant check Quest was 15% while FHBS for the susceptible checks Robust and Stander were 15% and 22%, respectively. The level of disease was similar to the 2009 nursery. We anticipate the DON data (not yet available) will provide additional information on the response of these entries to FHB.

ACKNOWLEDGEMENT

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CHROMOSOME ENGINEERING AND TRANSFER OF ALIEN SOURCES FOR FUSARIUM HEAD BLIGHT RESISTANCE IN HARD RED WINTER WHEAT

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ABSTRACT

We report on progress made in incorporating two new sources of resistance to Fusarium head blight (FHB) from *Leymus racemosus* and *Elymus tsukushiense* to hard red winter wheat. FHB resistance gene *Fhb3* from *L. racemosus* was transferred to wheat in the form of a compensating Robertsonian translocation T7AL·7Lr#1S. The *Fhb3* gene is located on the short arm of *L. racemosus* chromosome 7Lr#1S translocated to the long arm of wheat chromosome 7AL. The 7AL and 7Lr#1S arms are joined at the centromere. *Fhb3* confers resistance to single-point inoculation in the greenhouse. Ten lines homozygous for *Fhb3* in Jagger and Overley background were evaluated for their resistance to FHB and DON accumulation under field conditions in the 2008-9 growing season. Although the lines were homozygous for *Fhb3*, variable reaction to FHB and DON accumulation was observed, which could be caused by genetic background effects. Lines 08-193 (in Jagger) and 08-184 (in Overley) with higher levels of resistance were evaluated in the field FHB nursery in Manhattan in the 2009-10 growing season. The line 08-193 gave a disease index rating of 27.6% as compared to 36.8% for Jagger. The line 08-184 gave an index rating of 33.1% as compared to 50.2% for Overley. Both of these differences were significantly different ($P<0.05$, LSD=5.66). For further evaluating the genetic background effects we are backcrossing 08-193 to the cultivar Fuller. After a second backcross to Fuller, the selfed progenies will be screened for isolating homozygous *Fhb3* lines and will be evaluated for FHB resistance and DON accumulation under greenhouse and field conditions.

Chromosome engineering was used to isolate three recombinant chromosomes, one proximal rec124 (T7AL·7Lr#1S-7AS), and two distal rec989 and rec679 (T7AL·7AS-7Lr#1S) and homozygous lines were developed in Overley background. In the 2009-10 growing season, all the recombinant lines along with Overley were evaluated for FHB and DON. Rec124 had an FHB index rating of 27.6% as compared to rec679 (38.1%), rec989 (48.8%) and Overley (50.2%; LSD=5.66). DON ranged from 7.4 to 14.6 ppm as compared to 19.7 ppm for Overley (LSD=4.22). It appears that based on this and previous data, *Fhb3* is located in the interstitial region (FL 0.45-0.80) of the 7Lr#1S arm.

Another source of FHB resistance is derived from *Elymus tsukushiense* and was transferred to wheat in the form of a disomic chromosome addition stock (DA1Est#1), a ditelosomic addition stock (DtA1Est#1S), and a disomic addition/translocation stock DATW1Est#1S. Testing of the DATW1Est#1S stock from 2005 to 2007 indicated that this line conferred resistance to FHB under greenhouse conditions. In a spring 2010 greenhouse test, the lines T7AL·7Lr#1S (*Fhb3*), DA1Est#1, DtA1Est#1S, and DATW1Est#1S gave average spike index ratings of 19.2%, 26.2%, 23.5%, and 10.0%, respectively, whereas the resistant check Sumai#3 and the moderate susceptible check Chinese Spring had ratings of 5.3% and 32.0%, respectively (LSD=9.05). We are presently using directed chromosome engineering to produce recombinants with shortened *E. tsukushiense* chromatin, which will then be evaluated for their resistance to FHB and DON accumulation.

PUTATIVE ROLE FOR ETHYLENE SIGNALING IN TYPE II RESISTANCE TO *FUSARIUM GRAMINEARUM* IN WHEAT USING VIRUS INDUCED GENE SILENCING (VIGS) AND A GASEOUS INHIBITOR OF ETHYLENE PERCEPTION

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ABSTRACT

Ethylene (ET) has been shown to be important for resistance to necrotrophic pathogens in Arabidopsis. While it remains unclear as to whether *Fusarium graminearum* is a hemibiotroph or a necrotroph, its necrotrophic mode of growth is most damaging. Thus, ET is a potential candidate for disease resistance signaling. We have used a Virus-Induced Gene Silencing (VIGS) system to silence genes in both the ethylene biosynthesis pathway and the ethylene signaling pathway. Preliminary results indicate that a number of these genes may indeed be important for defense signaling against *Fusarium graminearum*. The genes were silenced in the resistant variety 'Ning' 7840. Upon application of the virus, containing a portion of a wheat gene, the plants were screened for conversion from resistance to susceptibility. The genes involved in ET signaling screened thus far include SAMs, ACS, ETO, CTR, EIN2, and an ERF. SAMs and the ERF in particular demonstrated remarkable conversion to susceptibility upon silencing. The importance of ethylene signaling in the resistant genotype was also observed using the gaseous inhibitor of ethylene signaling 1-Methylcyclopropene (1-MCP). Ning plants exposed to this inhibitor became significantly more susceptible to Fusarium than control plants.

CHARACTERIZATION OF FUSARIUM HEAD BLIGHT-RESPONSIVE GENES IN DIVERSE WILD AND CULTIVATED BARLEY

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ABSTRACT

Wild (*Hordeum vulgare* subsp. *spontaneum*) and cultivated barley (*Hordeum vulgare*) accessions possess various degrees of resistance to Fusarium head blight (FHB). Integration of resistance from diverse sources has the potential to enhance resistance, ultimately helping barley producers manage FHB. To identify genetically diverse barley lines carrying FHB resistance, DArT markers were used to genotype 102 wild or cultivated barley lines (80 FHB-resistant and 22 FHB-susceptible). Population structure was analyzed using two methods. First, phylogenetic analysis was conducted with MrBayes. Second, software STRUCTURE was used to infer distinct genetic populations in the germplasm collections. Both methods suggest the presence of four populations which comprised : (1) spring six-row, (2) spring two-row, (3) winter six-row and (4) wild two-row. Multiple wild and cultivated lines, including parents of mapping populations, were selected from across these major groups for haplotype and association mapping analysis. Previous GeneChip experiments have identified over 100 barley genes with significantly up-regulated transcript levels in response to treatment with *Fusarium graminearum* or DON. Thirty-nine of these genes, including those implicated in defense responses such as P450s, glutathione-S-transferases, and UDP-glucosyltransferases, have been sequenced from 24 diverse barley lines (13 resistant and 11 susceptible) and analyzed using an association-based approach. Seventeen of these genes were genetically mapped using the Oregon Wolfe Barley population. Map locations of twelve genes are within previously identified FHB QTLs. Whole genome and gene-based association mapping identified associations that are coincident with previous reported FHB QTL regions as well as potentially novel locations. The DArT marker haplotype diversity at FHB resistance QTL regions was analyzed and will be reported.

COMPREHENSIVE METABOLOMICS AND PROTEOMICS FOR FUSARIUM HEAD BLIGHT RESISTANCE GENE DISCOVERY AND FUNCTION IN TRITICEAE

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OBJECTIVES

To identify mechanisms of resistance in Triticeae against *Fusarium graminearum*, through mass spectrometry based comprehensive metabolomics and proteomics.

INTRODUCTION

The resistance in Triticeae (wheat, barley and triticale), to fusarium head blight (FHB, scab) caused by *Gibberella zeae* (anamorph: *Fusarium graminearum*), is quantitatively inherited and is controlled by several genes (3). The resistance mechanisms in Triticeae to FHB can be apparent or true. The cleistogamous florets in two row-barley lead to apparent resistance while the chasmogamous florets in six-row barley and wheat lead to susceptibility. In the chasmogamous floret genotypes, unlike in cleistogamous floret genotypes, the biochemical resistance in lemma and palea do not serve as a barrier that prevents the pathogen from attacking the ovary during anthesis. Excellent work has been done on apparent resistance including discovery of genes for cleistogamy. Other apparent resistance mechanisms include occurrence of less favourable environment for infection by pathogen due to plant height, spikelet density, etc. which mainly reduce duration of wetness. The true resistance is mainly due to structural or biochemical, which can be either constitutive or induced following pathogen inoculation. The biochemicals may be either proteins/enzymes/peptides or metabolites. Breeding for resistance requires high throughput selection parameters. Accordingly, the resistance is classified into type I = resistance to initial infection and type II = resistance to spread of infection within spike. Barley has very high type II resistance, while wheat varies among genotypes. More than 100 FHB resistance quantitative trait loci (QTLs) have been identified, in all the seven chromosomes, but only one third of these are stable and/or not associated with apparent resistance (3). The mechanism of resistance has been partially elucidated for one QTL on chromosome 3BS, which is due to an enzyme, DON-3-*O*-glucosyltransferase that converts the virulence factor DON to DON-3-O-glucoside (D3G) (7); the trichothecene lacking mutants fail to progress to the rachis in wheat. Though the cultivar ranking for type II resistance based on individual floret inoculation is quite consistent, the ranking of type I resistance is very inconsistent over years and seasons. Though marker assisted selection facilitates plant breeding it is often associated with apparent resistance mechanisms and the true mechanisms are not revealed. It is also possible that a given resistance mechanism can be controlled by more than one QTL. Thus, molecular breeders are looking for novel tools which also would lead to the identification of genes. In this study, our focus was to apply mass spectrometry based comprehensive metabolomics and proteomics to better understand the mechanisms of resistance in Triticeae against FHB.

MATERIALS AND METHODS

Comprehensive metabolomics and proteomics of biotic stress involved ten steps:

Step 1. Plant-pathogen systems for comparison: Metabolic and proteomic profiles of resistant and susceptible plant genotypes, inoculated with mock and pathogen, were compared. However, the effect of genetic background was not controlled. To address this problem, several genotypes and pairs of near isogenic lines (NILs) with FHB resistance QTLs were compared, as a precursor to evaluation of a population of recombinant inbred lines (RILs). Additionally, further protocol standardization is required. Use of cultivars, with several mechanisms of resistance, can help detecting several known mechanisms of resistance. Transgenic mutants lacking one target gene would also be ideal. However, not all the mechanisms are controlled by biochemicals. Other mechanisms also must be studied.

Step 2. Plant and pathogen production and inoculation conditions: Plant-pathogen interaction to produce disease depends on three components of disease triangle: host, pathogen and environment. Plants varying in resistance to FHB were produced under greenhouse conditions to reduce temperature variation. A single spore isolate of *F. graminearum* was used to avoid mixture of chemotypes. Trichothecene non-producing mutants were used to investigate mechanisms not involving trichothecenes. Spikelets were spray inoculated to assess spikelet resistance, and single spikelets were inoculated to assess disease spread within a spike (4).

Step 3. Assessment of resistance in plants against biotic stress: The number of spikelets diseased at 3d intervals until 21 dpi was assessed. The proportion of spikelets diseased (PSD) on 21 dpi or area under the disease progress curve (AUDPC) was calculated (4). The experimental design was a randomized complete block design with blocks over time of 3-5 d. Up to 30 spikelets were used to assess disease severity. Pair (Res. vs Sus.) wise comparison based on students *t*-tests, or grouping based on canonical discriminant analysis (CDA), using SAS (4) were performed.

Step 4. Sample collection for biochemical analysis: Spray or single spikelet inoculated, spikelets or rachis, at GS=65 or 75, from mid third of spike, were harvested at 48-72 hpi. Lemma+palea, lemma+palea+caryopsis or rachis were separately placed in vials, liquid nitrogen was added and stored at -80C. The experiment was conducted as a RCBD with five blocks over time. Each experimental unit consisted of 50-60 spikelets or 10-15 rachises.

Step 5. Metabolite and protein extraction: Samples were crushed in liquid nitrogen, 100 mg samples were taken, internal standards were added, extracted with methanol+water, and centrifuged (1). For GC/MS analysis the polar and apolar metabolites were separated using chloroform. The pellet was used for protein extraction and digested with tripsin to breakdown the proteins into peptides (5).

Step 6. Mass spectrometer analysis: Liquid chromatography and hybrid mass spectrometry (LC-nESI-LTQ-Orbitrap) was used for metabolite and protein/peptide analysis. Polar and semipolar columns were used. Metabolites were analyzed in negative ion mode at 60 000 resolution (at m/z = 400). Peaks were fragmented using 35 eV CID energy (1).

Step 7. Mass spectral output processing: The output from LC/MS was converted to netCDF using Bioworks (Thermos), imported to XCMS for deconvolution and alignment of peaks across samples. Peaks with signal to noise ratio s/n < 5 were used to avoid higher probability of extraction of peaks with high background spectra. Peaks with adducts and isotopes were identified using CAMERA, sieved using MS-EXCEL (1). The output from GC/MS was converted to netCDF using MassLynx, imported to AMDIS and peaks deconvoluted. Peaks were aligned and the abundance was calculated using MetIdea (4).

Step 8. Information extraction: Abundances of peaks (metabolites and peptides) were subjected to *t*-tests to sieve treatment significant peaks: RP

vs RM, SP vs SM, RP vs SP and RM vs SM, where R=resistant, S=susceptible, M=mock inoculated and P=pathogen inoculated. These were further subjected to canonical discriminant analysis (CDA) to cluster observations. CDA uses first a non-supervised principal component analysis to cluster observations and then a supervised analysis to reduce the distance within clusters. The CAN-vector scores and their associated loading to metabolites/proteins/peptides were used to identify the biomarker metabolites (1, 4).

Identification of pathogenesis (PR) and resistance related (RR) metabolites/proteins: *t*-test was used to identify PR and RR metabolites/proteins. Biochemicals with higher abundance in pathogen than in mock inoculated were designated as PR, and those in resistant genotype as PRr (RP>RM) and in susceptible as PRs (SP > SM). Biochemicals with higher abundance in resistant than in susceptible genotypes were designated as RR, and those based on mock inoculation were designated as constitutive (RRC = RM > SM) and on pathogen inoculated as induced (RRI = RP > RM and RP > SP) (1, 4). The median accurate masses obtained from XCMS linked to METLIN which was further linked to various databases (8) were used to putatively identify metabolites. Metabolites were identified based on three criteria: i) accurate mass (AME<5ppm), ii) isotope ratio based number of carbons in the formula; iii) fragmentation pattern using InteliXtract (ACDlab, Torornto). Some of these metabolites were matched to purified metabolites. The GC/MS peaks were identified based on fragmentation patterns (4). The peptides were imported to Mascot and the proteins were identified (Raghavendra – unpublished).

Identification of resistance indicator induced (RII) metabolites and proteins: The trichothecenes produced by the pathogen in the plant, especially the virulence factors, and their degradation products as a result of enzymes produced by resistant hosts were designated here as RII metabolites. Similarly, the enzymes that catalyze reactions of RR metabolites were designated as RII proteins/enzymes as they are not directly involved in resistance.

Step 9. New knowledge generation and its validation: The PR and RR biochemicals identified here were linked in their metabolic pathway to identify the precursors and the end products. These were searched in the literature for their role in plant defence, as plant metabolomics may be new but not the metabolites. For some RR metabolites the ability to reduce biomass was determined. This was combined with the abundance of the compound in host to derive resistance equivalence (RE).

Step 10. Application of knowledge and technology transfer: Use of RR metabolites and proteins in crop improvement require cost effective tools. The potential use of HPLC, qRT-PCR, molecular markers, etc., will be discussed.

RESULTS AND DISCUSSION

Disease severity: In spray inoculated barley the PSD and AUDPC varied significantly among genotypes tested. Barley has high type II resistance, however, in wheat the PSD varied among genotypes. For the evaluation of type I resistance, inoculation of spikelets during anthesis is not a suitable growth stage for chasmogamous florets as the pathogen avoids biochemical resistance in glume, lemma and palea. Inoculation at later stages can reveal resistance in caryopsis.

Metabolic and proteomic profiles: Metabolomic and proteomic/peptide profiles yielded more than 3000 peaks. About 1000 metabolites had significant treatment effects. These were further subjected to univariate and multivariate analyses. Peptides were used to identify proteins (analysis in progress).

PR/RR metabolites and their functions: The CDA of the abundances of treatment significant metabolites discriminated resistant and susceptible cultivars. In general the CAN vectors discriminated constitutive resistance and pathogenicity functions. Induced resistance was not well separated (1)

More than 300 metabolites were identified as PR or RR metabolites. Out of these about 200 metabol-

ites were putatively identified (Table 1). In general, more metabolites were RRC and very few were RRI, meaning barley genotypes have constitutive resistance to defend against this necrotrophic pathogen. However, the use of Tri5- mutant increased RRI metabolites, meaning otherwise the enzymatic reactions were prevented by DON. Biomass inhibition and the resistance equivalence of RR metabolites varied. The RR metabolites identified here belonged to mainly three metabolic pathways: phenylpropanoid-flavonoid, terpenoid and fatty acid pathways. These metabolites are known to have several plant defence functions, including pathogen biomass reduction, trichothecene toxin synthesis inhibition, signalling, etc. In addition, trichothecenes and their degradation products were detected and these are discussed below, under RII metabolites.

Phenylpropanoid and flavonoid pathway - Phenylpropanoid and flavonoid or phenyl ammonia lyase (PAL) pathway is activated through over expression of PAL enzymes. Several phenylpropanoids were RR metabolites, including phenylalanine, *p*-coumaric acid, sinapate, ferulic acid, etc. Among the monolignols (*p*-coumaryl, coniferyl, and synapyl alcohols) only coniferyl alcohol was detected, though several lignans, such as diphyllin, phyllanthin, eucommin A, and tuberculatin, were detected (6). The flavonoids that were RR metabolites were: kaempferol along with several of its conjugates, quercetin, naringenin, catechin, etc. The phenolics are known for their antioxidant properties and also lead to the formation of lignin which acts as a cell wall barrier to pathogen penetration. Though only ferulic acid has been shown to inhibit synthesis of trichothecene toxins (2), it is possible that several other phenolic and other compounds with antioxidant properties would also be able to inhibit toxin synthesis by the pathogen. These phenolic and flavonoid compounds are also known for their antimicrobial properties (1, 2). Accordingly, the metabolite abundance and its antimicrobial property were combined to derive resistance equivalence (1).

Terpenoid pathway - These metabolites contain isoprene units and are produced through farnesyl diphosphate downstream to the mevalonate pathway. Several mono, di, and tri-terpenes are known for their antimicrobial activities. Those identified as RR metabolites in barley against FHB include rishitin, polygodial, polygonic acid, boschnialactone, thymoquinol dimethyl ether, and cuauhtemone (1, 6).

Fatty acid pathway - Also known as octadecanoic acid pathway, this pathway leads to the production of jasmonic acid (JA), an important signal molecule. In barley, several precursors of JA, including linoleic and linolenic acid were produced, and all had high antimicrobial activity. In addition, capric acid and lauric acid were detected, and the capric acid was the most effective among the 20 RR metabolites evaluated.

PR and RR proteins and their functions: A preliminary proteomics analysis of wheat rachis detected more than 3000 peaks of peptides and identified about 100 proteins, including several PR proteins (Raghavendra - comparative analysis is in progress).*RII metabolites and their functions:* DON and 3ADON, along with their acetate adducts, were the only trichothecenes detected in this barley study. The plant enzymatic DON conversion product, D3G was also detected along with its acetate adduct. In addition, a novel plant enzymatic conversion product, S-methyl-DON (SMD) was detected, both in barley and in wheat. This can be traced back to the responsible enzyme and gene, and lead to the discovery of a novel gene. Total DON produced (TDP) and the proportion of total DON converted (PTDC) to D3G and also to SMD can be used as biomarkers in the evaluation of breeding lines. The TDP was the lowest in barley CIho 4196, and this could be due to the inhibition of trichothecenes through antioxidant properties of phenylpropanoids (2). The PTDC was the highest in Zhedar-2 and moderate in CIho 4196, though even a susceptible genotype had PTDC=0.4 (6). However, our proteomic analysis has yet to detect these enzymes. This indicates that PTDC is not the sole mechanism for type II resistance. *Future applications:* The RR metabolites identified here, and also the RR proteins to be identified, can be used as biomarkers to screen breeding lines, following validation of the occurrence of these biochemicals in other resistant genotypes. Most of the RR metabolites identified here have small and additive effects; thus, demonstrating their role indi-

vidually is difficult. Mass spectrometry technology is not suitable for plant breeding as it is expensive However, it could be used to establish the functions of the many QTLs already identified and lead to simpler methods suitable for the selection for disease resistance characteristics used for crop improvement.

Mass spectrometry for biochemical analysis is a powerful tool. With the advent of genome sequencing, including wheat and barley, MS can be used for functional genomic studies. Following gene identification, more specific molecular markers can be identified and used in trait transfer to elite cultivars. Additionally, the knowledgebase generated here can be used in metabolic pathway engineering.

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Table 1. Resistance related (RR) and resistance indicator induced (RII) metabolites in barley-Fusarium interaction detected based on LC hybrid MS.

Chemical group	RR and RII metabolites
Phenolics	RR: phenylalanine, *p*-coumaric acid, sinapate, ferulic acid, diphyllin, phyllanthin, eucommin A, tuberculatin, coniferyl alcohol, syringetin 3-rutinoside, quinic acid, scoparin
Flavonoids	RR: quercetin, naringenin, catechin, kaempferol 3-O-rhamnoside, naringenin 7-glucoside
Terpenes	RR: rishitin, polygodial, polygonic acid RII: deoxynivalenol, DON-3-*O*-glucoside, S-methyl-DON
Fatty Acids	RR: jasmonic acid, capric acid, lauric acid, linolenic acid, undecanoic acid

ACTIVATION TAG SCREENING TO IDENTIFY NOVEL GENES FOR TRICHOTHECENE RESISTANCE

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ABSTRACT

The goal of our research is to identify plant genes which enhance trichothecene resistance and, ultimately, Fusarium Head Blight resistance in wheat and barley. We are taking a two pronged approach using *Arabidopsis* to identify plant genes which confer resistance to trichothecenes. The first approach identified *Arabidopsis* orthologs of previously identified yeast knockouts from a viability screen using a trichothecene mycotoxin, trichothecin. An alternative method to identify trichothecene targets in plants is to perform mutagenesis followed by selection. T-DNA mutagenesis coupled with phenotypic selection has proven to be an extremely successful strategy to identify and isolate genes. One drawback from screening traditional T-DNA mutants is the problem of gene redundancy whereby knockouts do not present identifiable phenotypes. An alternative version of T-DNA mutatgenesis, termed activation tagging, provides an effective approach to overcome this limitation. Activation tagging uses a modified T-DNA vector which contains multiple copies of the cauliflower mosaic virus (CaMV) 35S gene enhancer arranged in tandem. In addition to knocking out genes, the modified T-DNA vector can also function as an enhancer when inserted either upstream or downstream of a gene to produce gain-of-function phenotypes. The genomic location of the tag is readily identifiable by thermal asymmetric interlaced (TAIL) PCR. Using this approach, we have screened >45,000 activation tagged *Arabidopsis* seeds for resistance to trichothecin and identified 15 lines that showed a very high level of resistance. These plants were able to form roots on 4 μM Tcin, a concentration which severely inhibits germination and prevents root formation of the Col-0 wild type. We will present the preliminary characterization of two of these mutants. Sequence analysis of the resistant lines by TAIL-PCR demonstrated T-DNA insertions in two novel genes, termed *Arabidopsis thaliana resistant root formation1* and *5* (*AtTRRF1* and *AtTRRF5*). *Arabidopsis* plants with independently generated knockouts (T-DNA) in these two genes are currently being tested for resistance. In addition, we are testing expression of neighboring genes by qPCR for upregulation due to the enhancer sequences. We propose that screening a large activation tagged *Arabidopsis* collection on media containing trichothecene mycotoxins provides an extremely flexible and efficient method to identify novel genes for trichothecene resistance in plants.

ENGINEERING DEFENSE REGULATORY GENES AND HOST SUSCEPTIBILITY FACTORS FOR ENHANCING FHB RESISTANCE

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ABSTRACT

Fusarium head blight (FHB)/scab caused by the fungus *Fusarium graminearum* is a destructive disease of wheat and barley. Due to the lack of monogenic gene-for-gene resistance to FHB, the mechanism(s) involved in signaling and activation of plant defense against *F. graminearum* are poorly understood. The utilization of a host-fungus system consisting of *Arabidopsis* and *F. graminearum* has provided an excellent model system to identify and characterize defense regulatory genes and genes that contribute to host susceptibility to *F. graminearum* that could be targeted for enhancing FHB resistance in wheat. Salicylic acid (SA) signaling was previously shown to promote resistance against *F. graminearum* in Arabidopsis (Makandar et al. 2010) and overexpression of the *NPR1* gene, which is a key regulator of SA signaling, was shown to enhance resistance against *F. graminearum* in Arabidopsis and wheat (Makandar et al., 2006, 2010). This interaction between Arabidopsis and *F. graminearum* has been utilized to identify additional genes (*PAD4*, *WRKY18*, and *LOX*s), that offer promising targets for enhancing FHB resistance in wheat. *PAD4* regulates multiple defense mechanisms, including SA synthesis and signaling in Arabidopsis, and *WRKY18* encodes transcription factor that regulates defense gene expression. In contrast to *NPR1*, *PAD4*, and *WRKY18*, which promote defense against *F. graminearum*, a lipoxygenases activity contributes to host susceptibility to this fungus. To determine if altered expression of these genes can promote FHB resistance in wheat, we have generated transgenic wheat plants that constitutively express *PAD4* and *WRKY18* from the ubiquitously expressed *Ubi* promoter. In addition, transgenic wheat plants that are silenced for expression of various lipoxygenases encoding genes have also been generated. Results on the analysis of these plants will be presented. In addition, progress on targeting non-host resistance mechanism for engineering FHB resistance in wheat will also be presented.

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DEVELOPMENT AND TESTING OF IMPROVED ENZYMES FOR TRANSGENIC CONTROL OF FHB

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ABSTRACT

The primary goal of the present study is to develop improved enzymes for the inactivation of trichothecene mycotoxins associated with Fusarium head blight and test their efficacy in barley. Trichothecene mycotoxins such as DON play a prominent role in the establishment of FHB and have been implicated in pathogen virulence. A primary agent for the inactivation of trichothecene mycotoxins is the trichothecene 3-*O*-acetylase (TRI101) enzyme. TRI101 catalyzes the acetylation of the 3-OH on the trichothecene toxin resulting in a 100-fold decrease in toxicity. Therefore efforts to use TRI101 from *Fusarium sporotrichioides* as a transgenic resistance factor have been implemented in wheat (*Triticum aestivum)*, barley (*Hordeum vulgare*), and rice (*Oryza sativa*). These transgenic cereals have shown moderate resistance to FHB in greenhouse tests, but have shown little success in field trials. *In vitro* kinetic analysis of the TRI101 enzymes from *Fusarium sporotrichioides* (FsTRI101) and *Fusarium graminearum* (FgTRI101) reveal that FgTRI101 has 100-fold greater efficiency (k_{cat}/K_M) for the acetylation of DON. It is proposed that this significant kinetic difference accounts for the poor performance of transgenic cereals in field trials. Consequently the present work is focused on optimization of the kinetically superior FgTRI101 for expression in barley. The 3-dimensional structure of FgTRI101 was used to engineer several point mutations to improve the stability and solubility of the enzyme in its transgenic host. Strategies such as entropic stabilization, consensus mutagenesis, and surface charge introduction were employed to create an optimized FgTRI101. An increase of 4.7 °C in enzyme melting temperature and a catalytic efficiency comparable to the wild type FgTRI101 were observed for the optimized enzyme. Both the wild type and optimized FgTRI101 have been inserted into plasmid pBract214 and have been utilized in *Agrobacterium*-mediated transformation of barley to create transgenic strains. Transformed plants have been obtained and will be analyzed for resistance to FHB once homozygous lines are identified. Additionally, an antibody-based purification protocol has been established for TRI101 expressed in transgenic barley. This protocol has been used to isolate FsTRI101 from barley and has shown that the transgenic enzyme has retained enzymatic activity although western blots indicate that the enzyme has been post-translationally modified. Future studies will examine the nature of this modification and also characterize the optimized and wild type FgTRI101 enzymes from transgenic barley. These studies will establish a connection between the *in vitro* and *in vivo* studies of TRI101.

GREENHOUSE EVALUATION OF TRANSGENIC BARLEY EXPRESSING *GASTRODIANIN* FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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OBJECTIVE

To develop transgenic barley expressing *gastrodianin* for resistance against *Fusarium* head blight (FHB).

INTRODUCTION

The filamentous ascomycete *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is the major pathogen causing Fusarium head blight (FHB) in barley, wheat, oats and other cereals (McMullen *et al*., 1997). FHB-infected plants have reduced yield due to sterile florets and shriveled kernels. Furthermore, infected kernels are contaminated with trichothecene mycotoxins such as deoxynivalenol (DON) and 15- acetyldeoxynivalenol (15-ADON; Desjardins, *2006*) that are harmful to humans and animals (Rocha *et al*., 2005).

Barley has very limited resistance to FHB (Bai and Shaner, 2004). FHB resistance in barley primarily involves restriction of initial infection known as type I resistance (Steffenson, 2003). Quantitative trait loci (QTLs) for FHB resistance often map to the location of other QTLs including those for heading date, plant height, spike angle (Bai and Shaner, 2004), and two-row spike type (Mesfin *et al*., *2003)*. This relationship between QTLs for FHB resistance and other traits is due to tight linkage rather than a single QTL controlling several phenotypes (Nduulu *et al*., *2007*). Identification of resistance QTLs is pivotal for the development of germplasm with improved resistance to FHB through breeding. Advances in genome-wide association mapping may facilitate development of effective markers for breeding resistant barley (Massman *et al*., 2010). However, progress in breeding resistant lines has been slow. Introduction of anti-*Fusarium* genes through genetic engineering may complement breeding efforts to enhance resistance to FHB.

We have transformed barley with *gastrodianin*, an anti-fungal gene isolated from *Gastrodia elata,* for resistance against FHB. Gastrodianin is a 12 kDa, non-agglutinating, monomeric, mannose and chitin-binding lectin that belongs to the superfamily of monocot mannose-specific lectins (Wang *et al*., 2001). *In vivo* studies have established that gastrodianin inhibits the growth of saprophytic fungi including *F. graminearum* (Wang *et al*., 2001). *In vitro* studies using transgenic tobacco and plum showed that gastrodianin inhibits root rot caused by the fungal pathogens *Rhizoctonia solani, Phytophthora nicotianae* and *P. cinnamomi* (Cox *et al*., 2006; Nagel *et al*., 2008). Field tests in cotton have also demonstrated that transgenic plants expressing *gastrodianin* are more resistant to *Verticillium* wilt (Wang *et al*., 2004). The role of gastrodianin in fighting fungal pathogens is probably attributable to its ability to bind to fungal cell walls and slow hyphal growth. Gastrodianin is stable at fluctuating temperatures (Wang *et al*., 2001). Its stability and inhibitory effects on fungal pathogens makes *gastrodianin* an attractive gene for engineering resistance to FHB. In this study we report preliminary results in the response of transgenic barley expressing the *gastrodianin* gene against *F. graminearum* infection under greenhouse conditions.

MATERIALS AND METHODS

Transformation and Cytological Analysis of Transgenic Plants - Immature barley (*Hordeum vulgare* cv. Golden Promise) embryos were transformed with an expression vector pLem2VGM2 containing the *gastrodianin* gene (Ng *et al.*, 2007). Expression of *gastrodianin* was driven by a spike-specific Lem2 promoter (Abebe *et al.*, 2005). To assess ploidy, chromosome number was counted from root tips of transgenic seedlings. T_2 seeds were germinated in Petri dishes in the dark for 1 to 2 days. Root tips were pre-treated with saturated 1-bromonapthalene solution overnight at 4°C. Root tips were then fixed in 1:3 glacial acetic acid:95% ethanol solution at 4°C, hydrolyzed in 1M HCl at 60°C for 5 minutes, and stained in Fuelgen solution. The root tips were squashed on glass slides in a drop of 1% aceto-carmine and chromosomes were visualized under a microscope.

Greenhouse Screening of Transgenic Barley for FHB Resistance - Screening of transgenic Golden Promise barley plants for resistance to FHB was performed in a greenhouse at the University of Minnesota, St. Paul, MN. T_2 plants from seven events (event numbers 48, 50, 51, 52, 53, 56, and 58) were each grown in eight pots with five plants per pot. Non-transformed (wild-type) Golden Promise and transgenic Golden Promise expressing only *gfp* (Lem2Bgfp-GP) were included as negative controls. Conlon (FHB susceptible two-row), M122 (FHB moderately resistant six-row), Stander (moderately susceptible six-row), and Robust (FHB moderately susceptible six-row) were included as checks. Macroconidia of *F. graminearum* isolate Butte86ADA-11, cultured on mung bean agar (Evans *et al.*, 2000) were used as inoculum. Plants were spray inoculated at anthesis using 2 ml /head of a 1 x 10^5/ml macroconidial suspension, which was applied to both sides of the head with an airbrush sprayer. To facilitate infection, inoculated plants were kept at 100% humidity for 72 hours in a dew chamber. Following the incubation period plants were maintained in a greenhouse until assessed. FHB incidence and severity were assessed visually 14 days post-inoculation. FHB severity was calculated as the percentage of symptomatic spikelets/spike. The mean FHB severity for each transgenic line, Lem2Bgfp-GP (negative transformed control), and checks was compared with the wild type Golden Promise using Student's *t*-test.

RESULTS AND DISCUSSION

Phenotype of transgenic plants - We recovered fertile plants from 10 transformation events. Most had abnormal phenotypes including slow maturation, stunted growth, reduced seed set, twisted leaves, and bushy growth habit. Cytological analysis of T_2 plants revealed that transgenic plants with abnormal phenotypes had a tetraploid chromosome set (Table 1). Plants from event 58 had normal phenotypes with normal chromosome numbers. Observation of abnormal phenotypes is in agreement with many studies that used particle bombardment (Choi *et al.*, 2000; Filipecki and Melepszy, 2006). Particle bombardment often leads to complex patterns (multiple copies) of transgene integration (Filipecki and Malepszy, 2006). Moreover, the transgenes can disrupt endogenous genes, which can contribute to the development of strange phenotypes. Regeneration of plants from *in vitro* cultures exposes transformants to extra stresses due to selection agents (herbicide), osmotic effects from culture media, and insufficient nutrient supply or uptake (Latham *et al.*, 2006). These stress factors could lead to polyploidy, aneuploidy, chromosome rearrangements, somatic recombination, gene amplification, excision and insertion of retro-transposons, DNA methylation, and histone modifications (Filipecki and Malepszy, 2006). Mutations may also accumulate as time in tissue culture increases (Fukui, 1983). In our study, transgenic calli were maintained in tissue culture for 4 to 5 months, which may have increased somaclonal variation and contributed to the observed polyploidy.

Resistance of transgenic plants to FHB - T_2 plants from seven transformation events (two lines per event) expressing *gastrodianin* were evaluated for resistance to FHB (Table 2). Comparison of disease severity in transgenic plants expressing *gastrodianin*, the negative control (Lem2Bgfp-GP) and checks with the wild type Golden Promise indicated that the transgenic lines 50A4, 50D3, and 51E2 had significantly higher levels of FHB infection. Transgenic lines from event

Table 1. Chromosome number and phenotypes of transgenic barley plants expressing *gastrodianin*.

Transformation event	Transgenic lines	Plant[§]										No. of chromosomes
		1	2	3	4	5	6	7	8	9	10	
48	48C1	nh sh	nh sh tl	*	*	ns *	*	*	*	missing	missing	31 chromosomes
	48D1	hb *	ns *	ns tl	ns sh	*	ns *	*	missing	missing	missing	
50	50A4	*	*	*	*	*	*	*	*	missing	missing	
51	51E5	*	*	*	*	*	*			missing	missing	
52	52D6	*	*	*	*	*	nh sh	sh *	*	missing	missing	
	52G2	*	*	*	*	*	*	*		missing	missing	
53	53A1	ns sh tl	*	*	*	*	*	*	*	ns sh *	*	29 chromosomes
	53B1	sh *	sh *	sh *	ns sh *	missing	missing	missing	missing	missing	missing	
56	56A1	*	*	*	*	*	*	*	*	*	tl *	30 chromosomes
	56C1	*	*	*	*	*	*	*	*	*	missing	32 chromosomes
58	58B5	n	n	n	n	n	n	n	n	n	missing	Normal
	58D5	n	n	n	n	n	n	n	n	n	n	Normal

[§] hb, head branching; n, normal; nh, no head; ns, no seed; sh, short; tl, thin leaves; *, broad leaves, large seed, partial sterility, resembles tetraploid.

Table 2. FHB severity (% symptomatic spikelets/spike) for transgenic barley lines expressing *gastrodianin* and FHB checks. Values are means of 6–8 replications, with each replicate consisting of 3–5 plants.

Plants	FHB severity (%)
A. Check lines	
1. Non-transgenic	
Golden Promise	55.9
Conlon (FHB susceptible, 2-row)	89.0*
Robust (FHB moderately susceptible, 6-row)	69.7
Stander (FHB moderately susceptible, 6-row)	60.9
M122 (FHB moderately resistant, 6-row)	74.4
2. Transgenic (expressing gfp only)	
Lem2Bgfp-GP	43.5
B. Transgenic lines expressing gastrodianin	
48A1	77.7
48B3	72.6
50A4	81.5*
50D3	84.3*
51E2	80.3*
52D6	77.1
52G2	73.2
53A1	55.5
56A1	61.2
56D3	73.6
58B5	45.5
58D5	24.0*

**Indicates significantly different FHB severity ($p<0.05$) compared with non-transgenic Golden Promise.*

58 either had similar (58B5) or lower (58D5) FHB severity. Among the checks, only Conlon had significantly higher FHB infection than the wild-type Golden Promise (Table 2).

In our study, the average percentage of FHB infection varied from 24% (plant 58D5) to 84% (plant 50D3). One major difference between transgenic plants from event 58 and the susceptible transgenic plants was that the former had normal sets of chromosomes whereas the latter were tetraploids (Table 1). It is possible that abnormal chromosome numbers may have made plants more susceptible to FHB. Another reason why the response to FHB infection varied widely among the transgenic lines may be differences in the location of transgene insertions (position effect). Sequences surrounding the transgene will likely influence transgene expression and stability. Independent transgenic lines that contain the same copy number sometimes show differences in expression by as much as a 100-fold due to positional effects (Filipecki and Malepszy, 2006).

FUTURE PLANS

Our greenhouse evaluation of transgenic plants expressing *gastrodianin* has produced at least one line (58D5) that has improved resistance to FHB. However, the test needs to be repeated to make sure the response observed is real. We are currently repeating the greenhouse experiment. Field testing of transformants is the best way to accurately determine FHB resistance under natural conditions. Most of our transformants are tetraploids and, even with the diploid plants, crossing to an elite variety is necessary to remove any unwanted traits such as low seed

setting, stunted growth and slow maturity (Bregitzer *et al*., 2008). Dr. Lynn Dahleen has crossed selected transformants into Conlon (female parent). Field evaluation of the crosses is underway.

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ASSOCIATION ANALYSIS OF FHB RESISTANCE DERIVED FROM TUNISIAN 108 IN DURUM WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of wheat (*Triticum aestivum* L.) world-wide, causing tremendous losses in grain yield and quality. The main species that causes FHB in the United States is *Fusarium graminearum* (Scab). This destructive fungal disease caused two billion dollars (direct revenue loss) in the period of 1993 to 2001 in the United States alone, while indirect loss estimated almost three times of this amount. Durum wheat has been heavily impacted, with a 44% loss of value in the U.S. crop, which is grown primarily in North Dakota. Thus, it is critical to identify means of defeating this disease or reducing its pathogenic effect to enhance wheat production.

In the previous report we used 171 BC_1F_6 and 169 BC_1F_7 lines derived from crossing of four Tunisian tetraploid sources of resistance (Tun7, Tun18, Tun34, Tun36) with durum cultivars 'Ben', 'Maier', 'Lebsock' and 'Mountrail' for association studies. The Tun18 and Tun7 expressed similar resistance level to FHB as compared with the best hexaploid wheat sources (i.e. Sumai3 and Wangshuibai). A new significant QTL for FHB resistance was identified on the long arm of chromosome 5B (*Qfhs.ndsu-5BL*) with association mapping analysis. Linkage disequilibrium (LD) blocks extending from 40 to70 cM were evident in these populations. The results of association mapping analysis also demonstrated a region on the short arm of chromosome 3B as potentially linked to FHB resistance. This region is in proximity of major FHB resistance gene "*fhb1*" reported in hexaploid wheat. This finding was surprising considering the distance and lack of relationship between Tunisian tetraploid sources studied here and Chinese sources used to identify *fhb1*.

In the current study, two additional Tunisian- derived advanced backcross populations, Tun 108 × Lebsock/Lebsock and Tun 108 × Ben/Ben, were screened for FHB resistance in both greenhouse and field. Although there are obvious discrepancies between the two set of data because of environmental effect, on the average, 53 out of 173 (30.64%) and 57 out of 170 (33.53%) lines showed less than 20% infection in Tun 108 × Lebsock/Lebsock and Tun 108 × Ben/Ben populations, respectively. Both populations were genotyped using DArT (Diversity Array Technology®) clones and resulted in 553 polymorphic loci that mapped on the A and B genomes. Preliminary pedigree based association analysis of QTL results on these populations will be presented and compared with our previous results on Tunisian-derived lines.

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INDUCTION OF PLANT DEFENCE GENE EXPRESSION BY ANTAGONISTIC LIPOPEPTIDES FROM *PAENIBACILLUS* SP. STRAIN B2

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ABSTRACT

With the aim of obtaining new strategies to control plant diseases, we investigated the ability of antagonistic lipopolypeptides (paenimyxin) from *Paenibacillus* sp. strain B2 to elicit hydrogen peroxide (H_2O_2) production and several defence-related genes in the model legume *Medicago truncatula*. For this purpose, *M. truncatula* cell suspensions were used and a pathosystem between *M. truncatula* and *Fusarium acuminatum* was established. In *M. truncatula* cell cultures, the induction of H_2O_2 reached a maximum 20 min after elicitation with paenimyxin, whereas concentrations higher than 20 μM inhibited H_2O_2 induction and this was correlated with a lethal effect. In plant roots incubated with different concentrations of paenimyxin for 24 h before inoculation with *F. acuminatum*, paenimyxin at low concentration (c.a. 1 μM) had a protective effect and suppressed 95% of the necrotic symptoms, whereas a concentration higher than 10 μM had an inhibitory effect on plant growth. Gene responses were quantified in *M. truncatula* by semi-quantitative reverse transcription–polymerase chain reaction (RT–PCR). Genes involved in the biosynthesis of phytoalexins (phenylalanine ammonia-lyase, chalcone synthase, chalcone reductase), antifungal activity (pathogenesis-related proteins, chitinase) or cell wall (invertase) were highly up-regulated in root or cells after paenimyxin treatment. The mechanisms potentially involved in plant protection are discussed.

IDENTIFYING AND CHARACTERIZING BARLEY GENES THAT PROTECT AGAINST TRICHOTHECENES

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ABSTRACT

Our overall goal is to identify genes that play a role in resistance to Fusarium Head Blight (FHB) and to develop and test transgenic wheat carrying these genes. In particular, we are interested in identifying genes that protect barley and wheat from the effects of trichothecenes. Previously, we conducted a large set of RNA profiling experiments during *Fusarium graminearum* infection of barley and inoculation with the trichothecene deoxynivalenol (DON). We identified a set of potential resistance genes that respond to trichothecene accumulation. The potential resistance genes encode a cysteine synthase, ABC transporters, UDP-glucosyltransferases, cytochrome P450s, and glutathione-S-transferases (GST). From our RNA profiling experiments, we identified ten barley UDP-glucosyltransferases and cloned eight full-length cDNAs for testing in yeast. We identified a barley UDP-glucosyltransferase gene that exhibits DON resistance based on the yeast assay. As proof of concept, we generated transgenic *Arabidopsis* overexpressing the barley UDP-glucosyltransferase and tested these plants for their ability to grow on media containing trichothecene mycotoxins such as deoxynivalenol (DON) and 4,15-diacetoxyscirpenol (DAS). After 4 weeks of growth on DON-containing media, the wild-type seedlings were albino and had ceased growing. Shoot and root growth were not inhibited in the UDP-glucosyltransferase overexpression lines grown on media containing 10, 15 and 20 ppm of DON. During DAS treatment, the seedlings of these overexpression lines showed an obvious difference for root length (longer) and general plant health compared with the control. These results showed that overexpression of UDP-glucosyltransferase in transgenic *Arabidopsis* protect plants from the deleterious effects of DON and DAS. We are developing transgenic wheat plants upregulating this UDP-glucosyltransferase gene. Currently, we have isolated three barley genes encoding GSTs and are developing transgenic *Arabidopsis* carrying these genes.

TRANSPOSONS BASED SATURATION MUTAGENESIS TO EXPLORE FHB RESISTANCE IN BARLEY

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ABSTRACT

Fusarium head blight is a devastating epidemic disease of wheat and barley that causes heavy economic losses to farmers due to yield decreases and production of mycotoxin that renders the grain useless for flour and malt products. Barley varieties resistant to FHB is a matter of high priority in many areas where they are grown, but the complex nature of resistance make this a highly challenging task. Two major QTL's have been identified viz. QTL1 and QTL2 on chromosome 6H and 2H respectively which have a large effect on kernel discoloration. The resistant allele of QTL2 decreases the occurrence of head blight by nearly 50% in varieties in which it is present thus proving its importance. Efforts have been made to clone important QTLs for better understanding of the mechanisms involved for FHB tolerance. Maize *Ac*/*Ds* system is one of the important tools that can be utilized for dissecting and saturating QTLs through saturation mutagenesis. Previous and ongoing mapping studies in our lab indicate an added advantage of *Ds* transpositions, in gene rich linked positions; making this technique appropriate to dissect FHB QTLs. Currently, our main focus is to saturate QTL2 region using maize *Ds* elements eventually facilitating identification and characterization of genes associated with FHB resistance. Plants with single *Ds* insertions (TNPs), mapping near QTLs of interest are important vehicles for gene identification through re-activation and transposition of *Ds*. *Ds* elements from TNP 41 (mapped near QTL2) were re-activated by crossing them with *Ac*TPase-expressing plants. In this population, we have identified some phenotypes, morphology of which may be associated with FHB tolerance. This effort of saturation mutagenesis with *Ds* transposons will lead to a better understanding of FHB resistance and candidate genes that display quantitative variation.

GENE EXPRESSION ANALYSIS OF RELATED WHEAT LINES WITH CONTRASTING LEVELS OF HEAD BLIGHT RESISTANCE AFTER *FUSARIUM GRAMINEARUM* INOCULATION

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ABSTRACT

Eight spring wheat genotypes with contrasting phenotypes for FHB resistance were used in this study: the highly resistant line CM82036, the highly susceptible cultivar Remus, four BC_5F_2 near isogenic lines (NILs) for *Fhb1* and *Qfhs.ifa-5A* and two doubled haploid (DH) lines from a CM82036/Remus mapping population differing in *Fhb1* and *Qfhs.ifa-5A*.

At anthesis the flowering ears of the plants were single floret inoculated by *F. graminearum* or water. The inoculated spikelets were harvested at several time points after inoculation and dissected into the generative and vegetative parts for RNA preparation. Differential gene expression was monitored with two complementary methods: 1) cDNA-AFLPs or 2) using the Affymetrix wheat GeneChip. At early time points (8-24 hpi) after inoculation only few genes were differentially expressed, at later time points (48-72 hpi) an increasing number of differentially expressed transcripts was evident. A comparative analysis of the data on identified candidate genes gained by the two complementary approaches will be presented.

ACKNOWLEDGEMENTS

We acknowledge funding of this work by FWF (Austrian Science Fund), project numbers: P16724-B05 and F3711-B11; and the Federal State of Lower Austria.

CHARACTERIZATION OF AN MRP INVOLVED IN THE WHEAT RESPONSE TO THE MYCOTOXIN DEOXYNIVALENOL

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ABSTRACT

Previously we identified several wheat genes that were responsive to the Fusarium mycotoxin deoxynivalenol (DON) and that were associated with the ability of the wheat cultivar CM82036 to resist the deleterious effects of this toxin. We have cloned and sequenced one such gene, namely a multidrug resistant protein (MRP) ABC transporter. Phylogenetic analysis indicated that it clusters with clade II and the MRP3 subfamily of MRP transporters. Gene expression studies indicated that it is more DON-up-regulated in cultivar CM82036 as compared to the DON-susceptible cultivar Remus. Additionally, it is up-regulated in response to jasmonic acid. The effect of DON on TaMRP3 transcript accumulation in wheat was more pronounced than that of the more potent protein synthesis inhibitor CHX, suggesting that its activation is not merely a secondary effect of toxin-mediated inhibition of protein synthesis. Ongoing work is determining the functional significance of the encoded protein in plant responses to xenobiotics.

UNRAVELLING THE WHEAT RESPONSE TO THE PROTEIN SYNTHESIS INHIBITOR DEOXYNIVALENOL

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ABSTRACT

Several wheat genes, including a MRP ABC transporter gene and two cytochrome P450 genes are responsive to the *Fusarium* mycotoxin deoxynivalenol (DON). We found that the accumulation of these transcripts in response to the *Fusarium* mycotoxin DON was significantly higher, and occurred earlier, in the DON-resistant cultivar CM82036 as compared to susceptible cultivar Remus, as revealed using gene expression studies. Based on the nature of these transcripts, insights are gained into how plants respond to, transform, and resist the harmful effects of, the toxin. Analysis of the effect of DON on the transcriptome of cultivar Remus yielded further insights into how a susceptible host responds to DON. The results support the theory that ubiquitin-proteasome system components play an important role in the plant response to DON. Furthermore, they provide evidence that jasmonates and phenylpropanoids contribute to the host response to this toxin.

ASSOCIATION STUDIES VALIDATE AND DISCOVER GENETIC LOCI FOR WHEAT FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Wheat Fusarium head blight (FHB) is a destructive wheat disease worldwide. To validate and identify quantitative trait loci (QTL) for wheat FHB resistance (type II), association study was conducted using a collection of 149 Asian wheat accessions and 205 U.S. hard and soft winter wheat breeding lines. FHB was evaluated for three greenhouse seasons from 2008-2010 by injecting ~1000 conidiospores into the central spikelet of a spike and measuring the proportion of symptomatic spikelets (PSS) at 16 day after inoculation (DAI) at Kansas State University. In general, Asian accessions had a relatively higher type II resistance than that of U.S. accessions. A total of 282 SSR markers covering all wheat chromosomes including those linked to known QTL for FHB resistance were used to genotype the population. Statistical model tests selected the unified linear mixed model (ULMM) for association computation. Eighteen marker alleles showed significant association with FHB resistance in Asian population including three previously reported QTLs on 3BS, 3BSc, and 5AS. Four marker alleles for 5AS QTL linked to FHB susceptibility in the Asian group suggested most of Asian accessions in this study may lack the resistance allele on chromosome 5AS. Marker *Xgwm276* on 7A was significantly associated with FHB resistance in the Asian group, which has not been reported previously. Twelve accessions with the *Xgwm276*-110 allele had a mean PSS of 0.14 that is lower than these accessions with marker allele *Xgwm533*-159 (PSS= 0.21) on 3BS. In the U.S. population, 18 alleles from 17 markers were significant associated with FHB resistance. Two previously reported QTLs on 3BS (*Xgwm493* and *Xbac102*) and 4D (*Xbarc98*, *Xwmc473*, and *Xgwm608*) were validated. Among all 17 significant markers, two novel marker alleles, *Xcfa2263*-140 (2A) and *Xgwm320* -274 (2D), showed the largest effect on FHB resistance in the U.S. population with a mean of PSS of 0.38. Therefore, the QTL on 2A and 2D are likely new QTL for FHB resistance in U.S. accessions. The results not only validated previously reported important QTL, but also discovered some new QTL in germplasm from both Asian and the U.S. wheat.

ACKNOWLEDGEMENT AND DISCLAIMER

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SESSION 2:

PATHOGEN BIOLOGY AND GENETICS

Chairperson: David Schmale

GENETIC BASIS FOR THE 3-ADON AND 15-ADON TRICHOTHECENE CHEMOTYPES IN *FUSARIUM*

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ABSTRACT

Fungi in the *Fusarium graminearum* species complex (FGSC) and the related species *F. cerealis* (synonym *F. crookwellense*) and *F. culmorum* can cause *Fusarium* head blight (FHB) of wheat, barley, and other small cereal grain crops worldwide and contaminate grain with trichothecene mycotoxins. In general, *Fusarium* species that cause FHB exhibit three trichothecene production phenotypes (chemotypes): nivalenol (NIV) production, 3-acetyldeoxynivalenol (3-ADON) production, or 15-acetyldeoxynivalenol (15-ADON) production. The genetic basis for the NIV versus 3-ADON/15ADON chemotypes has been demonstrated previously. However, until now, the genetic basis for the 3-ADON and 15ADON chemotypes has not been identified. Two genes, *TRI3* and *TRI8*, have been proposed to affect 3-ADON and 15-ADON production based on functional analysis of the genes in 15-ADON strains of the FHB pathogen *F. graminearum sensu stricto* and in *F. sporotrichioides*, which produces another type of trichothecene, T-2 toxin. The analyses indicate that *TRI3* encodes an enzyme that catalyzes acetylation of trichothecenes at carbon atom 15 (C-15) and that *TRI8* encodes an enzyme that deacetylases trichothecenes at C-3. Here, we identified consistent DNA sequence differences in the coding region of the trichothecene biosynthetic gene *TRI8* in 3-ADON and 15-ADON strains. Functional analyses of the *TRI8* enzyme (Tri8), including gene disruption, cell-free feeding, yeast expression, and fungal transgenic expression, revealed that Tri8 from 3-ADON strains catalyzes deacetylation of the trichothecene biosynthetic intermediate 3,15-diacetyldeoxynivalenol at C-15 to yield 3-ADON, whereas Tri8 from 15-ADON strains catalyzes deacetylation of 3,15-diacetyldeoxynivalenol at carbon 3 to yield 15-ADON. In contrast, the function of *TRI3* was the same in NIV, 3-ADON and 15-ADON strains, and the function of *TRI8* was the same in NIV strains as it was in 15-ADON strains. Together, our data indicate that differential activity of Tri8 determines the 3-ADON and 15-ADON chemotypes in *Fusarium*.

A CROSS BETWEEN TWO GENETICALLY SIMILAR *FUSARIUM GRAMINEARUM* STRAINS PRODUCES STABLE TRANSGRESSIVE SEGREGANTS FOR FHB PATHOGENICITY RELATED TRAITS

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ABSTRACT

Epidemics of Fusarium Head Blight reduce the production and quality of wheat and small grains grown in North America and worldwide. *Fusarium graminearum (*teleomorph*: Gibberella zeae)* is the principal causal agent of FHB in the United States. Population studies have demonstrated that there are high levels of genetic diversity among North American *F. graminearum* field isolates. However, almost all isolates belong to a single genetic lineage known as lineage seven. *F. graminearum* is a homothallic ascomycete with the ability to outcross in culture and in nature. Sexual recombination is an important source of genetic diversity. The objective of this study was to study the genetic regulation of traits impacting fertility, pathogenicity, and aggressiveness among progeny generated by crossing two closely related lineage 7 *F. graminearum* strains. The *F. graminearum* strains chosen as parents, PH-1 (NRRL 31084) and Gz3639, have been used as models by different laboratories studying FHB, and both have available genome sequences. A GFP-tagged strain of Gz3639 was used to aid in screening for heterothallic perithecia. Segregation of SNPs and polymorphic repetitive DNA sequences was used to identify perithecia that resulted from heterothallic matings. Ninety-four progeny strains were isolated from four of these perithecia. The progeny isolates varied significantly in spore production and fertility, and in aggressiveness on the susceptible wheat variety Pioneer 2555. Transgressive segregants were observed for each phenotype. Four isolates with significantly higher, and four with significantly lower levels of aggressiveness, compared with the parental strains, were chosen for further analysis. Preliminary mycotoxin measurements demonstrated a correlation between DON production in planta and the severity of FHB symptoms. Fecundity, aggressiveness and DON production traits in the selected progeny strains were heritable among single-spored progeny of the transgressive segregant strains. These results demonstrate that crosses among phenotypically and genotypically similar strains have the potential to produce a highly diverse progeny population, including strains that are significantly more aggressive and toxigenic than either parent, although the significance of this in the field is unknown.

INFECTION STRUCTURES AND MYCOTOXIN INDUCTION OF *FUSARIUM GRAMINEARUM* ON WHEAT FLORETS

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ABSTRACT

F. graminearum is one of the best investigated phytopathogens however detailed information about fungal development on host surfaces and the penetration strategy of the pathogen is limited. Therefore, a bioassay was established to investigate inoculated floret tissues of wheat. Detection of mycelium was facilitated by constitutive expression of the *dsRed* reporter gene. Bright field, fluorescence microscopy, and confocal laser microscopy was used to study fungal development and host symptoms during infection. Trichothecene production of the fungus was monitored by a *GFP* coupled *TRI5*-promoter. Combining bioimaging with scanning electron microscopy we identified different infection structures and subcuticular growth. In addition to short infection hyphae foot-like structures, lobate appressoria, and infection cushions were observed. Monitoring of *GFP* fluorescence visualized a specific induction of *TRI5* gene expression in infection structures. Interestingly, a *TRI5* deletion mutant exhibits the same infection strategy and efficacy. We conclude that trichothecene biosynthesis is specifically induced in infection structures, but is neither necessary for their development nor for disease symptoms of wheat florets.

A SUBSET OF THE NEWLY DISCOVERED NORTHLAND POPULATION OF *FUSARIUM GRAMINEARUM* FROM THE U.S. DOES NOT PRODUCE THE B-TYPE TRICHOTHECENES DON, 15ADON, 3ADON OR NIV

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ABSTRACT

Between 2003 and 2006, large-scale population surveys of *Fusarium graminearum* from North Dakota (ND), South Dakota (SD) and Minnesota (MN) were conducted to determine the spatial and temporal dynamics of the emergent population of *F. graminearum* that has been determined to be more toxigenic than the pre-existing and widespread Midwestern (MW) 15ADON population. To efficiently determine population membership, we developed three VNTR markers with alleles that are specific to the known populations. After genotyping and determination of trichothecene type of more than 6,000 isolates, and after identifying and excluding species other than *F. graminearum* (e.g. *F. culmorum*, *F. poae*) and clones from repeat isolations from the same wheat head, we identified roughly 400 *F. graminearum* isolates that did not display population specific VNTR patterns. Further genotyping these with PCR-RFLPs, together with members from known populations for comparison, and subsequent analysis with STRUCTURE, a Bayesian model-based clustering software that assigns multilocus genotypes probabilistically to *K* populations, revealed the presence of three populations, the MW15ADON population, the emergent population (including 3ADON and 15ADON types) and a newly identified population that we named the Northland population. We currently have identified 176 isolates in our collection that belong to this population, whereby the majority originated from MN (77%), followed by ND (19%), and SD and WI (2% each). Isolates were not only recovered from wheat, but also from grasses in non-agricultural regions such as the Arrowhead region of MN (extreme Northeast MN). About 2/3 of these isolates were typed as 15ADON and 1/3 as 3ADON. Twelve isolates of each trichothecene type were then examined for aggressiveness and mycotoxin potential on the susceptible wheat cultivar Norm in the greenhouse. While all spikelets inoculated with the 15ADON types of the Northland population contained as excepted DON>15ADON>3ADON, spikelets inoculated with eleven of the twelve 3ADON isolates of the Northland population did not contain detectable levels of any of the common trichothecenes. These 3ADON type isolates all had different PCR-RFLP genotypes, and were therefore not clonally related; they were also geographically widespread. These observations lead us to hypothesize that this phenotype is heritable and that there may not be a selective disadvantage against this phenotype. After point-inoculation into a central spikelet, these isolates also spread in the spike and symptoms caused by these isolates could visually not be distinguished from isolates that produce the common trichothecene toxins. Inoculations with only one of the 3ADON Northland isolates yielded plant material with the expected trichothecenes DON>3ADON>15ADON. We sequenced most of the TRI cluster (*TRI*8-*TRI*13) from one of the 3ADON Northland isolate that did not produce the common trichothecene toxins and compared that sequence to the previously published sequence (*TRI*3-*TRI*12) for NRRL 28336, a 3ADON isolate from Ohio. There were less than 70 SNPs over the 18.5kb of sequence and the very few indels were all in intergenic regions or introns. As expected, *TRI*7 was missing and *TRI*13 was a pseudogene. All other genes in the gene cluster had complete ORFs and appear to code for normal

proteins. Therefore, the cause of repression of common trichothecene production may reside outside of the trichothecene gene cluster. Current efforts focus on determining whether any other and potentially unknown toxins are produced by this group of isolates and what molecular mechanisms are responsible to generate this unusual phenotype.

DON BIOSYNTHESIS IN WHEAT

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ABSTRACT

Deoxynivalenol (DON) is a potent mycotoxin and a virulence factor and thus causes great concern in wheat and barley cultivation. Treatment of wheat with fungicides offers some relief from scab disease, although use of strobilurins has been reported to increase DON accumulation in wheat. The DON biosynthetic pathway is well characterized, with the core cluster gene, *Tri5,* catalyzing the formation of trichodiene, the first pathway-specific intermediate. Few studies have focused on the expression of *Tri5* during infection and colonization of wheat heads, despite its importance in DON accumulation. Using quantitative RT-PCR, we examined the expression of *Tri5* during wheat head infection and colonization of susceptible and resistant cultivars and susceptible cultivars treated with strobilurin fungicides. DON was then also quantified to correlate expression with toxin accumulation. The highest *Tri5* expression relative to housekeeping genes was observed in asymptomatic kernels at the infection front and immediately behind the infection front. As infection progressed, kernels closest to the inoculation point showed diminished *Tri5* expression relative to housekeeping gene expression, but *Tri5* expression never ceased during the 21 days observed. Relative *Tri5* expression in Quadris-treated Wheaten did not differ significantly from that in untreated Wheaten. In resistant cultivar Alsen, there was a reduced presence of the fungus and of *Tri5* expression. Interestingly, for the final day of the time-course, 21 dpi, no fungus was detected in Alsen. Importantly, *Tri5* continues to be transcribed even in fully senesced kernels 21 dpi. In addition, strobilurin treatment did not increase *tri5* expression or alter DON accumulation in treated plants in comparison to untreated plants.

TRACKING RELEASED CLONES OF *GIBBERELLA ZEAE* WITHIN WHEAT AND BARLEY FIELDS

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ABSTRACT

Previous research has made significant progress in defining the spatial dissemination of inoculum sources of *G. zeae* within agricultural fields, but it has been unable to unambiguously distinguish between within-field and background sources. We used a technique known as amplified fragment length polymorphisms (AFLPs) to track released clones of *G. zeae* within commercial wheat fields. This strategy allowed us to determine the contribution of released clones to FHB, compared to that of background inocula. Corn stalk pieces infested with clones of *G. zeae* were released in winter wheat fields in New York and Virginia in 2007 and 2008. Recovery of released clones decreased an average of 90% between 3 and 6 m from area sources of inoculum. In 2008-2010, varying amounts of corn stalk pieces (45, 200, or 400 g) infested with a single clone of *G. zeae* were released in winter wheat and barley fields in Virginia. The influence of the infested corn residues was dependent upon the environmental conditions within the growing season. Our work contributes to an increased understanding of the influence of overwintered corn residues to FHB and DON. Understanding the contribution of *Fusarium*-infested corn residues will enable future research to reduce the inoculum potential from within-field sources.

ACKNOWLEDGEMENTS

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UNDERSTANDING THE MOLECULAR MECHANISMS OF *FUSARIUM OXYSPORUM*-MEDIATED DEGRADATION OF WHEAT STRAW

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ABSTRACT

Fusarium oxysporum is a facultative pathogen that degrades lignin and complex carbohydrates in plants and plant debris, thus facilitating its pathogenicity and persistence in the environment. We found that although two genetically distinct strains of this fungus were equally efficient in colonizing wheat straw (glucosamine content), they differed with respect to their ability to degrade this substrate. The ability to release phenolics during colonization was 2 times higher in strain 11C, as compared to strain 7E. The specific activity of the cellulases [endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), β-glucosidase (EC 3.2.1.21)] secreted by strain 11C was significantly higher than those of 7E. However, there was no difference in the specific activity of hemicellulases [endoxylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37)] secreted by the two strains. **Suppression subtractive hybridization (SSH**) analysis of gene expression during wheat straw colonization identified several genes up-regulated in strain 11C, as compared to 7E, including those involved in gene transposition, saccharification of cellulose and hemicellulose, cellular transport of sugars and pentose catabolism. These results give insights into how plant pathogens have evolved and can be exploited and manipulated for the purposes of lignocellulose degradation.

THE *HDF1* HISTONE DEACETYLASE GENE IS IMPORTANT FOR CONIDIATION, SEXUAL REPRODUCTION, AND PATHOGENESIS IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Head blight caused by *Fusarium graminearum* is an important disease of wheat and barley. Its genome contains chromosomal regions with higher genetic variation and enriched for genes expressed *in planta*, suggesting a role of chromatin modification in the regulation of infection-related genes. In a previous study, the *FTL1* gene was characterized as a novel virulence factor in the head blight fungus. *FTL1* is homologous to yeast *SIF2*, which is a component of the Set3 complex. Many members of the yeast Set3 complex, including Hos2 histone deacetylase (HDAC), are conserved in *F. graminearum*. In this study, we characterized the *HDF1* gene that is orthologous to *HOS2*. *HDF1* physically interacted with *FTL1* in yeast two-hybrid assays. Deletion of *HDF1* resulted in a significant reduction in virulence and DON production. The Δ*hdf1* mutant failed to spread from the inoculation site to other parts of wheat heads or corn stalks. It was defective in sexual reproduction and significantly reduced in conidiation. Expression of *HDF1* was highest in conidia in comparison with germlings and hyphae. Deletion of *HDF1* also resulted in a 60% reduction in HDAC activity. Microarray analysis revealed that 149 and 253 genes were down- and up-regulated, respectively, over 5-fold in the Δ*hdf1* mutant. Consistent with up-regulation of putative catalase and peroxidase genes, the Δ*hdf1* mutant was more tolerant to H_2O_2 than the wild type. Deletion of the other two class II HDAC genes had no obvious effect on vegetative growth and resulted in only a minor reduction in conidiation and virulence in the Δ*hdf2* mutant. Overall, our results indicate that *HDF1* is the major class II HDAC gene in *F. graminearum*. It may interact with *FTL1* and function as a component in a well conserved HDAC complex in the regulation of conidiation, DON production, and pathogenesis.

FUSARIUM TRI8 DETERMINES 3-ACETYLDEOXYNIVALENOL (3ADON) OR 15ADON PRODUCTION

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ABSTRACT

Trichothecene mycotoxins produced by *Fusarium* species can promote disease in small grain crops such as wheat and barley. Two main trichothecene production phenotypes (chemotypes) have been identified among strains of *Fusarium graminearum* and closely related species: strains produce either deoxynivalenol (DON) or nivalenol (NIV) trichothecenes. The DON phenotype can be further subdivided into the 3-acetyldeoxynivalenol (3ADON) chemotype and the 15-acetyldeoxynivalenol (15ADON) chemotype. However, grain infected by strains with either the 3ADON or 15ADON chemotype is typically contaminated with DON rather than the acetylated derivatives.

DON and NIV are identical in structure except for the presence (NIV) and absence (DON) of a hydroxyl function at carbon atom 4 (C-4) of the trichothecene molecule. The basis for DON and NIV chemotypes resides in the trichothecene C-4 hydroxylase gene *TRI13*. In DON-producing strains of *F. graminearum*, *TRI13* is nonfunctional because of multiple insertions and deletions within its protein coding region. As a result DON-producing strains are unable to hydroxylate trichothecenes at C-4. In contrast, NIV-producing strains have a functional *TRI13* and, therefore, can hydroxylate trichothecenes at C-4. For greater efficiency in chemotype classification, differences in *TRI13*, as well as the C-4 acetyl transferase gene (TRI7), sequences have been used to develop PCR markers to predict DON and NIV chemotypes.

During the last several years, PCR markers for *TRI3* and *TRI12* have been used to predict 3ADON and 15ADON chemotypes in *Fusarium graminearum*. In order to determine the genetic basis for these chemotypes, we examined differences in the sequences and functions of *TRI3* and *TRI8*, two trichothecene biosynthetic genes that have been proposed to play a role in production of 3ADON versus 15ADON in *Fusarium*. *TRI3* was functional in both 3ADON and 15ADON strains and had the same function, namely trichothecene C-15 acetyltransferase, in strains with either chemotype. *TRI8* was also functional in strains with both chemotypes; however, its function differed in the two types of strains. In 15ADON-producing strains, the TRI8 enzyme is a trichothecene C-3 esterase; it catalyzes removal of an acetyl group from the C-3 position. In contrast, in 3ADON strains, the *TRI8* enzyme homolog is a C-15 esterase; it catalyzes removal of an acetyl group from the C-15 position. These results indicate that *TRI8*, but not *TRI3*, determines whether Fusarium produces 3ADON or 15ADON. Furthermore, expression studies with *TRI8* chimeras containing a portion of *TRI8* from a 3ADON strain and a portion from a 15ADON strain indicated that sequence differences in the middle of the coding region are responsible for determining the 3ADON versus 15ADON chemotype. These results should contribute to understanding the role of the 3ADON and 15ADON chemotypes in the ecology of *Fusarium* species that cause wheat head blight.

FUSARIUM SPP. ASSOCIATED WITH HEAD BLIGHT IN SOUTH AFRICAN WHEAT PRODUCTION AREAS

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ABSTRACT

Fusarium head blight (FHB) of wheat is a complex disease caused by a number of *Fusarium* species. Several of these *Fusarium* species produce mycotoxins, mainly trichothecenes, which are associated with human and animal mycotoxicoses. In South Africa, FHB is primarily associated with wheat planted under irrigation and in fields where wheat is rotated with crops like barley, maize, soybean and sunflower. The aim of this study was to determine the distribution of *Fusarium* spp. associated with FHB in all wheat production areas of South Africa during the 2008 and 2009 growing seasons. Wheat heads showing FHB symptoms were collected from four cultivars in fields under irrigation in the Northern Cape, KwaZulu-Natal (KZN), the Bushveld and the Free State, and under dry land conditions in the Western Cape. Twenty diseased heads from each cultivar were collected per location, and kernels from each head were plated on potato dextrose agar and selective *Fusarium* media. Single-spore isolates representing each *Fusarium* colony were identified using morphological and molecular techniques. *Fusarium graminearum* species complex was most commonly associated with FHB of wheat in South Africa, and was found in 75.6% of the kernels analysed in 2008, and in 86.2% in 2009. Other known *Fusarium* spp., such as *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. lunulosporum*, *F. oxysporum*, *F. poae*, *F. pseudograminearum*, *F. sambucinum*, *F. scirpi*, *F. semitectum*, *F. solani* and *F. subglutinans*, were also isolated. At one location in the Free State, and also at the location in the Western Cape, the dominating species was *F. pseudograminearum*, occurring at higher frequencies than *F. graminearum* species complex. Chemotyping of the toxin-producing *Fusarium* isolates revealed that the 15-acetyl deoxynivalenol (15-ADON) chemotype was the most common in both years at most locations. However, in the Western Cape, and at one location in the Free State, the most common chemotype was 3-acetyl deoxynivalenol (3-ADON). The nivalenol (NIV) chemotype was most prevalent at one site in KZN (62.9%) in 2009. Information on the distribution of *Fusarium* species in South Africa, as well as the toxins they produce may be helpful for the future development of resistant wheat cultivars and sustainable disease management practices.

SYSTEMATIC CHARACTERIZATION OF THE KINOME OF THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*

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ABSTRACT

Wheat scab caused by *Fusarium graminearum* is one of the most important diseases of wheat. Beside yield losses, infested wheat kernels are often contaminated with mycotoxins. Like in many other eukaryotes, protein kinases play major regulatory roles in filamentous fungi. In *F. graminearum*, there are 126 predicted protein kinases that belong to different protein kinase groups and families. Like other fungi, *F. graminearum* lacks tyrosine kinase and kinases belonging to the **RGC and TKL group**. To determine their functions, we have undertaken a systematic approach to generate gene replacement mutants. To date, we have isolated mutants for over 100 kinase genes. For six of them, their orthologs are essential genes in the budding yeast. Sixteen of these predicted protein kinase genes appeared to be essential in *F. graminearum*. All the mutants have been assayed for their defects in wheat head infection, DON production, conidiogenesis, sexual reproduction, responses to various stresses, conidium germination, and hyphal growth. Twenty one of them were significantly reduced in virulence or non-pathogenic. Four of them did not produce DON in colonized wheat kernels. One of them produced predominantly single-celled conidia in CMC cultures. We also identified several mutants that germinated faster than the wild type or were more resistant to hyperosmotic or other stresses. Further characterization of these mutants is in progress. A database has been developed to include gene annotation and all the phenotypes of the *F. graminearum* kinase genes. In addition, the interaction among these protein kinases and their association with other *F. graminearum* proteins were predicted based on their yeast orthologs using the interlog approach. For a few predicted pathways or networks that are important for plant infection, affinity purification and co-immunoprecipitation assays will be used to determine their interactions *in vivo*. Overall, this is the first systematic characterization of protein kinase genes in filamentous fungi. We have identified protein kinase genes that regulate conidiogenesis, conidium germination, responses to hyperosmotic and ROS stresses, pathogenesis, sexual reproduction, hyphal growth, and mycotoxins production.

PREINOCULATION OF WHEAT HEADS WITH A NONTOXIGENIC *FUSARIUM* ISOLATE INHIBITS DEOXYNIVALENOL PRODUCTION BY A TOXIGENIC PATHOGEN

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OBJECTIVE

To evaluate the potential of using a naturally-occurring, nontoxigenic isolates of *Fusarium graminearuum* to reduce Fusarium head blight and deoxynivalenol accumulation.

INTRODUCTION

Despite the effectiveness of new scab resistant wheat cultivars and new fungicides in reducing the severity of Fusarium head blight (FHB) and reducing deoxynivalenol (DON) levels in grain, economic loss from DON accumulation can still occur under disease favorable conditions. Biocontrol agents are being investigated as a strategy to augment host resistance and fungicides, but they have not been consistently effective in the field. This may be related to the agents being very dissimilar ecologically to the pathogen *Fusarium graminearum* (*Fg*). A solution to finding more effective biological control agents against FHB may be to use agents that share the same physical niches and environment conditions as *Fg*. Many examples can be found in the biological control literature of related incompatible pathogens (i.e. pathogens of another host) or hypovirulent pathogen isolates being used to control a virulent pathogen (Cook and Baker, 1983). This strategy is being applied commercially in the management of aflatoxin in peanut and corn using aflatoxin-nonproducing isolates of *Aspergillus flavus* (EPA, 2010). Disease control using hypovirulent pathogen isolates is attributed to niche competition and induction of host resistance (Whipps, 2001). Infection by *Fg* activates plant resistance responses (Pritsch et al., 2000). Therefore, the ideal biological agent for FHB control and DON reduction would be a strain of *Fg* that is non-toxigenic (Tox-) and hypovirulent.

A naturally-occurring, presumably Tox- isolate of *Fg* (WG-9) was isolated from a wild grass from a remote non-agricultural area in northern Minnesota by L. R. Gale and associates. Although DNA isolated from WG-9 amplified well for ten highly polymorphic PCR-RFLP primer pairs developed specifically for *F. graminearum sensu stricto* (Gale et al., 2010), the resulting genotype was not typical of any described U.S. population of *Fg*. Additional isolates that group with WG-9 have since been detected and found to represent a new population of *F. graminearum*, the Northland population (see abstract in this volume by Gale et al. "A Subset of the Newly Discovered Northland Population of *Fusarium graminearum* from the U.S. Does Not Produce the B-Type Trichothecenes DON, 15ADON, 3ADON or NIV"). In greenhouse experiments, WG-9 did not produce any detectable amounts of DON or other derivatives (3ADON, 15ADON, NIV) in inoculated spikelets. Furthermore spread of WG-9 on inoculated wheat heads from point inoculations at individual florets ranged from low to moderate compared to the standard virulent isolate PH-1. In this study, WG-9 was used to test the concept that preapplication of a Tox- hypovirulent strain to wheat heads can inhibit floret infection by a toxigenic (Tox+) virulent pathogen resulting in reduced DON accumulation in the grain.

MATERIALS AND METHODS

Two sets of experiments were conducted, the first involving preinoculation of Tox- isolate WG-9 onto the flowering heads followed by a challenge inocula-

tion with Tox+ isolate PH-1 one day later. Inoculations were made by spraying conidial suspensions containing 10^4 spores/ml. Water was applied as the preinoculation and challenge inoculation controls. In the second set of experiments, the Tox- and Tox+ isolates were co-inoculated by injecting 10 µl volumes of spore suspension into the center florets in each wheat head. The treatments tested included equal volume mixtures of Tox- and Tox+ spore suspensions (10^4 spores/ml), Tox- spore suspension diluted with water, Tox+ spore suspension diluted with water, and a water only control. Both sets of experiments were conducted on susceptible hard red spring wheat cultivars Wheaton and Bobwhite grown in 20 cm pots (6 plants per pot) in a greenhouse, with 6 replicate pots per treatment. Inoculated plants were kept in a humid growth chamber for 2 days and then moved to a greenhouse for symptom development. After 1 week, inoculated heads were rated for numbers of spikelets exhibiting scab lesions. Seed was harvested at maturity for determination of Fusarium diseased kernels (FDK). Diseased and asymptomatic seed were assayed separately for DON concentration by the University of Minnesota Diagnostic Lab. All data was analyzed with SAS ProcMixed, with the LSD test used to separate means. In addition, the proportion of kernels infected by WG-9, PH-1, or both isolates were determined in the first set of experiments. Infected seeds were cultured separately on half-strength PDA in 24-well plates for 2 days, and then the seed and mycelium extracted for DNA. The extracts were used as templates in multiplex PCR amplification using 3ADON and 15ADON chemotype-specific primers based on *TRI3* and *TRI12* gene sequences (Starkey et al. 2007). WG-9 and PH-1 were distinguished on the basis of amplicons produced in the *TRI3* and *TRI12* multiplexes, with WG-9 producing 243 and 410 bp amplicons and PH-1 yielding 610 and 670 bp amplicons, respectively (Figure 1).

RESULTS AND DISCUSSION

We found that sequential spray inoculation of wheat heads with Tox- isolate WG-9 followed by Tox+ isolate PH-1 or point inoculation with the two isolates together significantly reduced DON concentrations in the mature seed compared to inoculation with the Tox+ isolate alone (Tables 1 and 2). The reductions in DON concentrations were measured in asymptomatic and scabby wheat kernels. In the first set of experiments, preinoculation with WG-9 prior to challenge inoculation with PH-1 suppressed kernel infection by PH-1, as revealed by PCR analysis of fungi in individual seeds. Among seeds from heads inoculated with both isolates, more than of 50% were infected with WG-9 while only 5% were infected with PH-1 (Table1). Similar proportions of WG-9 and PH-1 infections were found in the WG-9/PH-1 treatment in other experiments in which nearly 100% of seeds from heads inoculated with one isolate alone were infected by the respective isolate (data not shown). However, measurements of scab severity and proportions of FDK in experiment set 1 revealed that preinoculation with WG-9 prior to inoculation with PH-1 was ineffective in suppressing total symptom development compared to inoculation with PH-1 or WG-9 alone (Table 1). It appeared that, although WG-9 is assumed to be Tox-, it is virulent in respects to infection of individual florets, supporting previous research on other DON nonproducing *Fg* (Bai et al., 2002). In contrast, the ability of WG-9 to spread through wheat heads from point inoculation was significantly reduced compared to PH-1 (Table 2), which is consistent with DON production being important in the spread of *Fg* through the rachis. But point inoculation with the two isolates together had no effect on subsequent symptom severity compared to inoculation with PH-1 alone. We speculate that DON produced by PH-1 in association with WG-9 might have allowed both fungi to spread through the rachis, but eventually, fewer florets were infected by PH-1 via the rachis. This would be consistent with lower DON concentrations being detected in the seed following coinoculation.

The results from this study support the hypothesis that a Tox- isolate of *Fg* could be used as a biological control agent to compete with or exclude Tox+ pathogens strains and, thus, reduce DON levels in the harvested grain. The high disease severity and expected yield loss caused by WG-9 alone is an obvious drawback to using WG-9 as a biocontrol agent. But we surmise this problem could possibly

be overcome by using Tox- strains of *Fg* with lower virulence than WG-9, by applying Tox- isolates at lower spore concentrations or at different time intervals relative to flowering, or by integrating applications of Tox- strains with scab resistant cultivars. Field experimentation also is essential to confirm the benefits of pretreatment with Tox- strains. Other alternatives might be identified when the mechanisms by which WG-9 and other Tox- isolates exert their suppressive effects become known.

ACKNOWLEDGEMENT AND DISCLAIMER

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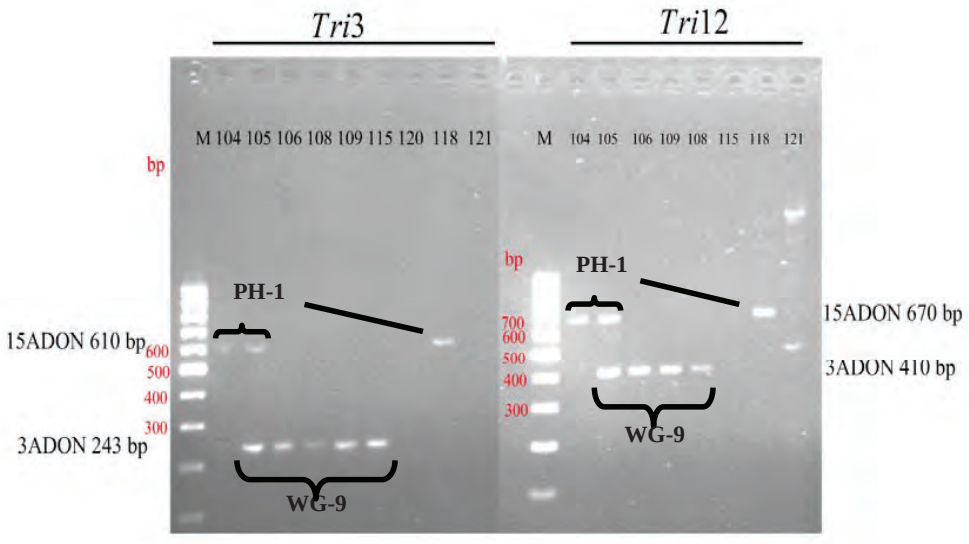

Figure 1. Representative electrophoresis results from *TRI3* (left) and *TRI12* (right) multiplex PCR of DNA extracted from plated seed and mycelia. *Fg* PH-1 (TOX+) and *Fq* WG-9 (TOX-) are indicated by amplicons of different size in each multiplex.

Table 1. Effects of preinoculation of wheat heads with Tox- WG-9 and challenge inoculation with Tox+ PH-1 on scab severity and DON.

Cultivar	Pretreatment/ challenge	% infected spikelets 1 week after challenge	Proportion of seed with: WG-9	PH-1	% FDK	DON in diseased kernels (ppm)	DON in asymptomatic kernels (ppm)
'Bobwhite'	WG-9/PH-1	70.7 A[†]	12/19	1/19	79.3 AB	24.9 B	0.8 B
	Water/PH-1	34.4 B	-*	-	65.6 B	176.0 A	3.1 A
	WG-9/water	78.0 A	-	-	93.2 A	<0.5[#]	<0.5
	Water/water	3.4 C	-	-	4.6 C	-	-
'Wheaton'	WG-9/PH-1	64.8 A	11/20	1/20	91.7 A	12.1 B	2.8 B
	Water/PH-1	12.0 B	-	-	86.4 A	97.9 A	16.5 A
	WG-9/water	78.5 A	-	-	96.5 A	1.7 C	<0.5
	Water/water	8.0 B	-	-	7.5 B	-	-

[†] Letters denote significant differences at P=0.05 based on LSD test.
* Not determined.
[#] Values below detection level of 0.5 ppm were not used in statistical analysis.

Table 2. Results of point inoculations with Tox- WG-9 and Tox+ PH-1.

Cultivar	Inoculum	Percent infected spikelets 1 week after inoculation	% FDK	DON in diseased kernels (ppm)	DON in asymptomatic kernels (ppm)
'Bobwhite'	PH-1 only	38.4 A[†]	32.9 A	202.4 A	2.5 A
	WG9 + PH-1	53.9 A	46.7 A	119.8 A	0.5 B
	WG9 only	4.6 B	4.5 B	<0.5#	<0.5#
	Water	0.4 B	0.3 B	-*	-
'Wheaton'	PH-1 only	68.0 A	75.1 A	48.4 A	1.0 A
	WG9 + PH-1	56.7 A	81.1 A	18.7 B	0.3 B
	WG9 only	23.3 B	46.5 B	<0.5#	<0.5#
	Water	2.8 C	9.4 C	-	-

[†] Letters denote significant differences at P=0.05 based on LSD test.
[#] Values below detection level of 0.5 ppm were not used in statistical analysis.
* Not determined.

SESSION 3:

FHB MANAGEMENT

Co-Chairpersons: Marcia McMullen
and Pierce Paul

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

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INTRODUCTION

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

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

MATERIALS AND METHODS

Plant materials, Inoculations, and FHB Disease Rating: Two wheat cultivars, Briggs (FHB susceptible) and Alsen (FHB moderately resistant with fhb1 gene from Sumai 3), were planted in a randomly complete block design with a split plot arrangement with three replications, at the NDSU Agricultural Research Station at Fargo on April 20, 2010. Wheat cultivars served as the main plot, and inoculum type and fungicide application were treated as the subplots. The test plots were planted on 2009 soybean ground, to minimize the chances of a previous year's inoculum effect. The plot size

was 10 x10 feet. Each field plot was separated with a 20 feet strip planted with Alsen to minimize the chance of inoculum interference from one treatment to the other. Three plots of each cultivar with and without fungicide treatment were spray-inoculated with a mixture of ten 3ADON isolates (A), or a mixture of ten 15ADON isolates (B), or an equal mixture of A and B at 100,000 spores/ml, when the plants were at the flowering stage (Feekes GS 10.52). Non-inoculated and non-sprayed plots of each cultivar were used as checks. All twenty isolates used in this study were recovered from FHB infected heads collected from various locations of North Dakota in 2008 and characterized for chemotype and aggressiveness in the greenhouse (Puri and Zhong, 2010; unpublished).

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

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

RESULTS AND DISCUSSSION

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

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

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

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

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

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

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

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

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

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

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

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ABSTRACT

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

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

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

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

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VALIDATION OF BARLEY DON RISK PREDICTION MODEL

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INTRODUCTION

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

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

OBJECTIVE

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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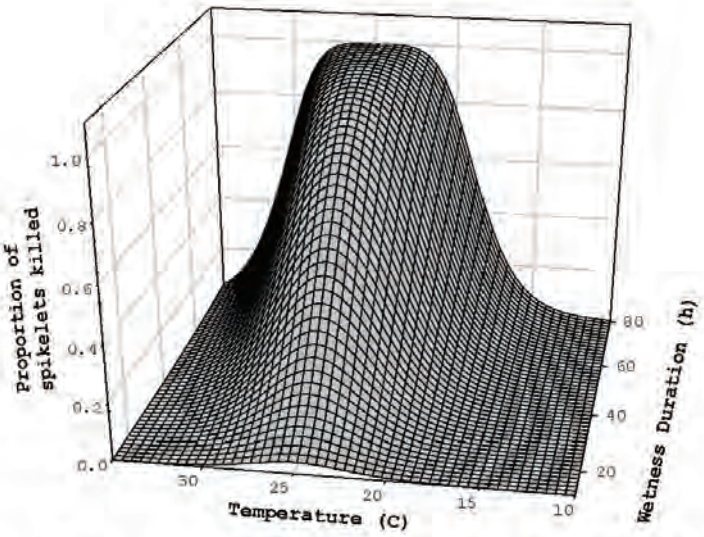

Figure 1: Response surface generated the Weibull function given in Eq. 1. This models FHB incidence in wheat following inoculation and incubation at different combinations of temperature and wetness duration.

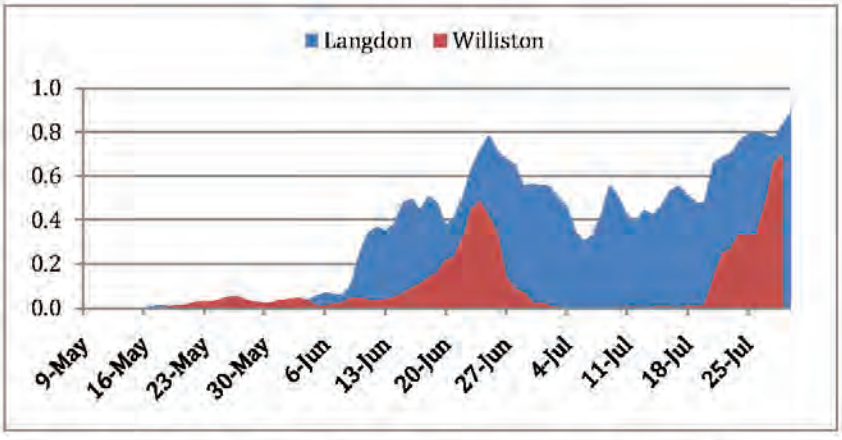

Figure 2: The distribution of the WEIB_WRH90 at two locations in North Dakota between May 10, 2010 and July 31, 2010. The cut-off threshold to classify the day as risk is 0.63.

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

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ABSTRACT

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

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

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ABSTRACT

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

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

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ABSTRACT

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

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

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

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

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

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

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ABSTRACT

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

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

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

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ABSTRACT

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

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INTEGRATED MANAGEMENT FOR FUSARIUM HEAD BLIGHT IN WISCONSIN

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ABSTRACT

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

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

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OBJECTIVES

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

INTRODUCTION

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

MATERIALS AND METHODS

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

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

Nine fungicide treatments were applied (Table 1). Two registered products, Prosaro (prothioconazole + tebuconazole) from Bayer, and Caramba (metconazole) from BASF, were applied at three growth stages, head half emerged (Feekes 10.3), head fully emerged (Feekes 10.5) and kernel watery ripe (Feekes 10.54). Two unregistered products also were tested and applied at Feekes 10.5. LEM 17 is a penthiopyrad chemistry from DuPont, and A9234D is a mixture of difenoconazole + tebuconazole, from Syngenta. Headline (pyraclostrobin) from BASF, a Quinone outside inhibitor (QoI) fungicide, also was applied at Feekes 9 to see if mid-season application of this product would result in DON levels greater than the

untreated check. All fungicide applications applied to grain heads were applied with a CO2 backpack-type sprayer equipped with XR8001 flat fan nozzles angled 30 degrees from the horizontal in 18 gpa, at 40 psi. The Headline application was applied with XR8002 flat fan nozzles oriented straight down.

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

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

RESULTS AND DISCUSSSION

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

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

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

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

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

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

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

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

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ABSTRACT

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

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

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ABSTRACT

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

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

DETERMINANTS OF ADOPTION OF SCAB MANAGEMENT TECHNIQUES

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OBJECTIVES

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

INTRODUCTION

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

Estimated Coefficients of Logistic Model:

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

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

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

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

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

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

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ABSTRACT

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

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

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

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ABSTRACT

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

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

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

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ABSTRACT

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

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

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ABSTRACT

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

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

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ABSTRACT

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

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

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

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ABSTRACT

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

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

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

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ABSTRACT

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

ACKNOWLEDGEMENT AND DISCLAIMER

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

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

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OBJECTIVES

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

INTRODUCTION

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

MATERIALS AND METHODS

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

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

RESULTS AND DISCUSSION

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

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

incidence compared to the control (22%, 49% and 61%, respectively). As was the case in the 2009 studies, PTCT variant 3C of OH 182.9 often exhibited efficacy in reducing FHB and mixed results in enhancing the performance of Prosaro® used at full or reduced rates.

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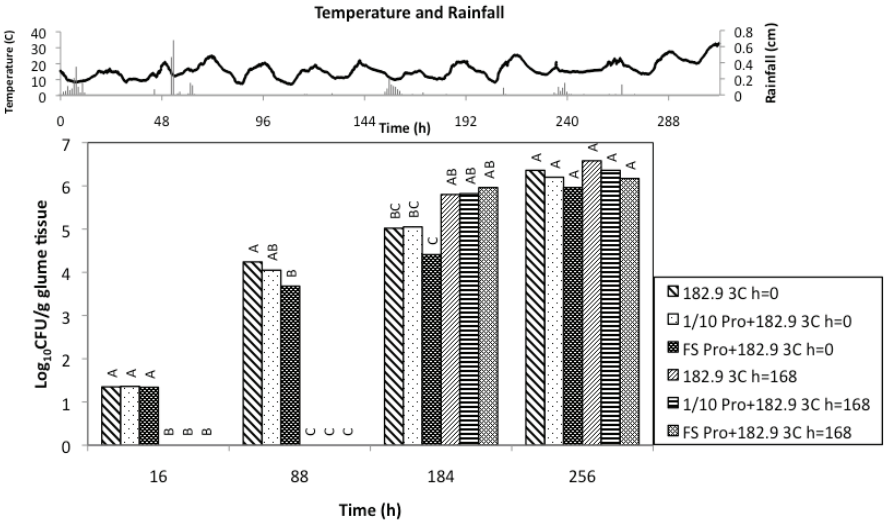
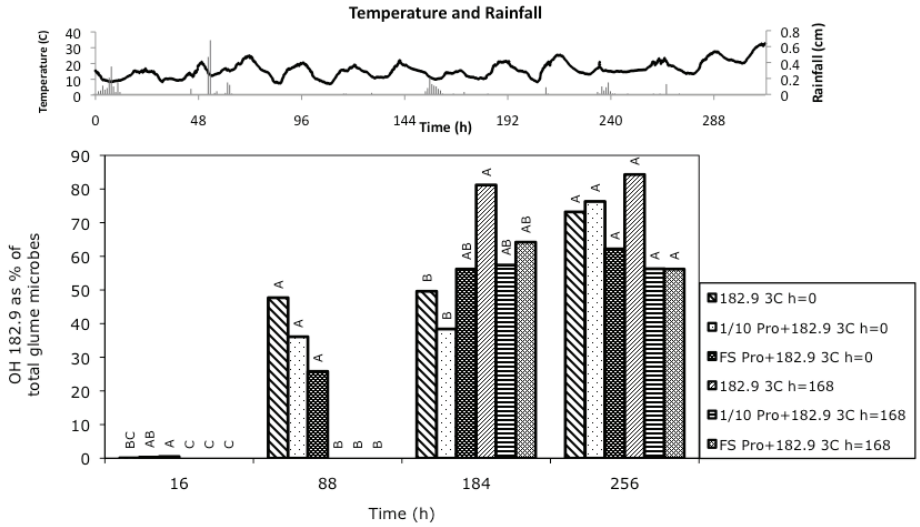


[a] Between 220 hours and 230 hours a total of 0.5 cm of irrigation was applied aerially during six separate irrigation events, each of 3 minutes duration. Within plating times, bars with no letters in common are significantly different ($p<0.05$, FPLSD).

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



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

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

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

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

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

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

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

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

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ABSTRACT

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

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

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

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

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

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OBJECTIVE

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

INTRODUCTION

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

MATERIALS AND METHODS

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

RESULTS AND DISCUSSION

Although planting dates varied by a week, any developmental head start in the earlier planted environment was inconsequential by spring. Flowering occurred simultaneously in both environments during a dry and hot period unfavorable to FHB development. The two weeks following flowering were considered medium risk of FHB due to increased rainfall and more moderate temperatures. The overall average of FHB incidence observed for both environments was 20%. Inoculation with the conidial suspension proved fruitful as only 2% FHB incidence was observed in adjacent non-inoculated experiments. There is little evidence to suggest inocula from within-plot crop residues impacted FHB development. Only two cultivars, Jensen and Richland, had significantly higher FHB indexes in

the soybean residue plot (environment 1). There were no significant differences observed in the DON levels between the two environments for all cultivars. Due to the similar levels of FHB development in both environments, FHB is unlikely an explanation to the greater yields observed in environment 1. One potential explanation of yield differences is that more foliar disease, especially Stagonospora leaf blotch, was observed in the previously fallow plot (environment 2). In both environments, foliar diseases were reduced significantly by application of any of the fungicides.

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

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

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

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

ACKNOWLEDGEMENT AND DISCLAIMER

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

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

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

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

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

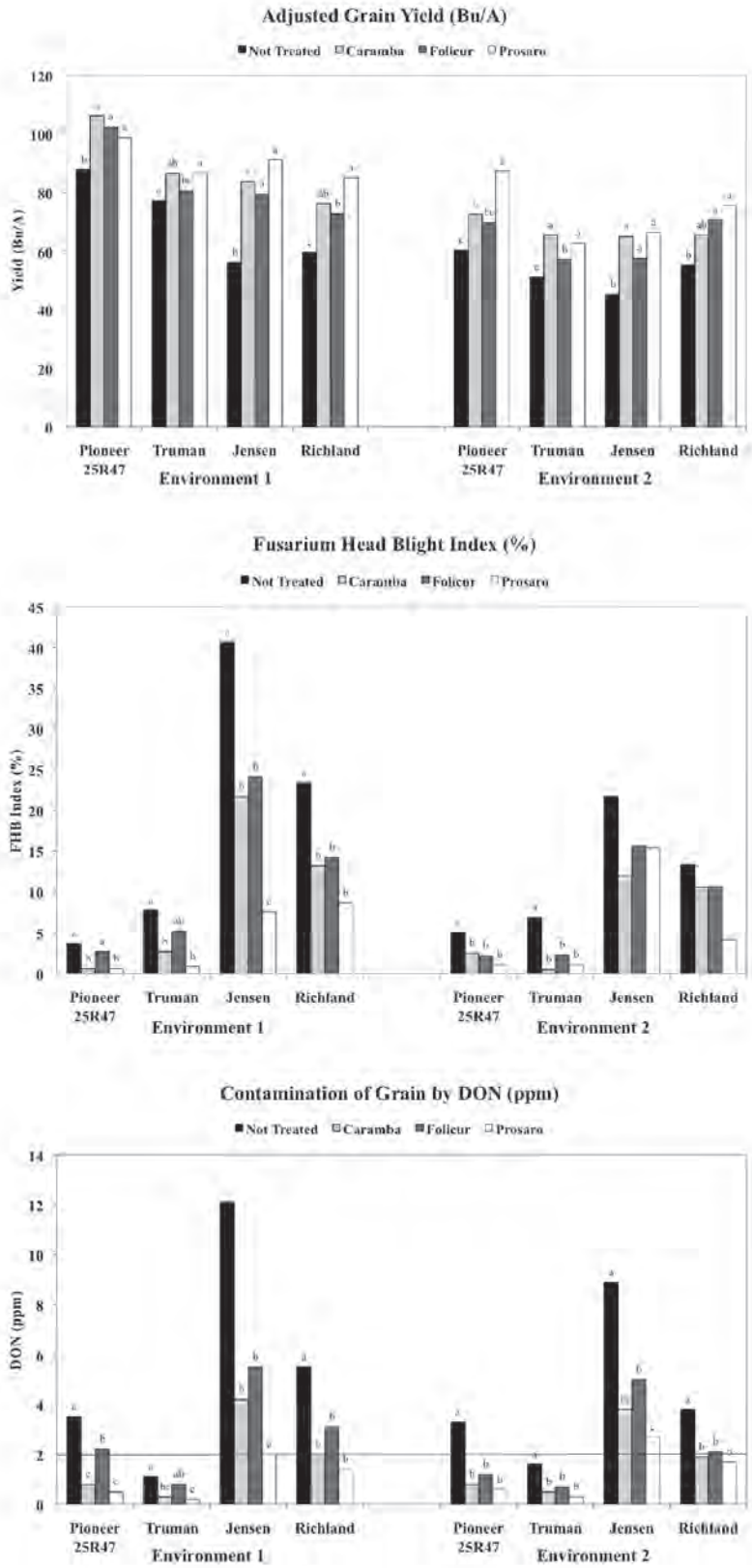

Figure 1. Effect of flowering stage application of Prosaro fungicide on yield, FHB index and DON contamination of four winter wheat cultivars in Aurora, NY. Letters denote treatment means that differ significantly at P=0.05.

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

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ABSTRACT

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

ACKNOWLEDGMENT AND DISCLAMER

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

INOCULATED FIELD TRIALS FOR EVALUATING FHB/DON INTEGRATED MANAGEMENT STRATEGIES

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ABSTRACT

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

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

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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 author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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

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ABSTRACT

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

Main findings are:

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

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

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

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

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

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

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

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

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

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

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

2010 UNIFORM BIOLOGICAL CONTROL TRIALS - PRELIMINARY RESULTS

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OBJECTIVE

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

INTRODUCTION

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

MATERIALS AND METHODS

Six trials involving a range of wheat market classes were conducted across four states (Table 1). The biological materials tested were the double yeast, supplied by D. Schisler in frozen cell concentrate form, and Taegro, supplied as a dry formulation by Novozymes Biologicals, Salem, VA). The same set of treatments (Table 2) was tested in every trial. All treatment liquids were amended with 0.125% Induce. One application was made per treatment at early flowering (Feekes 10.51) or 5 days later (late-bloom) in 20 gal/acre using a CO_2-pressurized sprayer. The size and number of replicate plots varied among trials, as did the use of mist irrigation systems to stimulate infection and the application

of *Fusarium graminearum*-infested corn kernels as a source of pathogen inoculum. In all trials, FHB incidence, severity, and index were determined from at least 40 heads per plot around 3 weeks after anthesis. Plot yields, test weight, and the incidence of Fusarium-damaged kernels (FDK) were determined after harvest. Kernel samples from each plot were analyzed for DON content by the North Dakota State University Veterinary Diagnostic Laboratory in Fargo. Data from each trial were analyzed separately by analysis of variance or, in cases in which data was incomplete, by ProcMixed (SAS), with arithmetic means or LSmeans being separated by the LSD test at the 95% confidence level. Data from all experiments also were pooled together and analysis by ProcMixed.

RESULTS AND DISCUSSION

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

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

ACKNOWLEDGEMENT AND DISCLAIMER

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

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

Table 2. Treatments tested in 2009 uniform trials.

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

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

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

Table 3 (continued)

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

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

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

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ABSTRACT

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

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SESSION 4:

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Chairperson: Dave Kendra

PREDICTING WHEAT MYCOTOXIN CONTENT USING NEAR INFRARED REFLECTANCE SPECTROSCOPY

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ABSTRACT

Among the limiting factors for wheat production in Southern Brazil, are wet and warm springs in some years, that favor the occurrence of Fusarium head blight (FHB) outbreaks. *Fusarium graminearum*, the main FHB causal agent in the region, is able to produce different mycotoxins such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZON), which are considered the most important ones because of their widespread occurrence and toxicity. Among the methods available for the rapid screening of contaminated samples, Near Infrared Reflectance Spectroscopy (NIRs), which is based on the absorption of near-infrared light by organic compounds, presents promising results. The aim of this study was to verify the presence of mycotoxins in different wheat samples using reference chromatographic methods and to adjust and validate a NIR-based method for quick screening of these contaminants, as well as genotype evaluation in breeding programs. A total of 196 and 120 wheat samples were analysed for DON and for ZON content, respectively. Wheat samples were obtained from commercial fields naturally infected by *Fusarium graminearum* and from breeding trials conducted by Embrapa in 2008 and 2009 growing seasons. The procedure required, firstly, the whole kernel wheat samples (125 g each), to be scanned by NIR. Secondly, the same samples were milled and homogenized, resulting in particles with less than 1.0 mm in diameter, and they were scanned again by NIR. Finally, the milled samples were sent to a reference laboratory for chemical analyses using liquid chromatography-tandem mass spectrometry (LCMS/MS) equipment. The NIR instrument used was a FOSS XDS – RCA (FOSS NIRSystems, Hoganas, Sweden), coupled with the module XDS Monochromator double detection system (Silicon 400–1100 nm) and (Lead Sulphide 1100–2500 nm). The accuracy of each calibration model was tested by using cross-validation in groups and selected based on the highest coefficient of determination of calibration (r^2) and the lowest standard error of cross validation (SECV). The calibrations were performed by partial least square (PLS) and modified partial least square (MPLS). Spectral outliers (standardized H>3) were removed and samples with large residuals (T≥2.5) were omitted from the population. In our study, the r^2 obtained for DON content in wheat kernel was 0.89 and SECV 612.05 μg kg^{-1} and in milled wheat r^2 was 0.91 and SECV 578.33 μg kg^{-1}, indicating a very good prediction using the NIR calibration model MPLS and PLS, respectively. Furthermore, a r^2: 0.86 with SECV 254.29 μg kg^{-1} and r^2: 0.87 with SECV 231.85 μg kg^{-1} obtained in wheat kernel and milled wheat, respectively, represented an acceptable prediction of ZON content by NIR using MPLS calibration models. These results indicated reasonable prediction for both DON and ZON content, in unprocessed wheat. Further tests will demonstrate the potential to incorporate this methodology for quick screening of contaminated commercial samples as well as early generation breeding samples.

ACKNOWLEDGEMENT

The authors would like to thank Wheat Embrapa Team for providing wheat samples for mycotoxin analysis.

STABILITY OF THE TRICHOTHECENE, DEOXYNIVALENOL IN PROCESSED FOODS AND WHEAT FLAKE CEREAL

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ABSTRACT

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by Fusarium species, principally F. graminearum and F. culmorum. These fungi are natural contaminants of wheat, barley and corn and, consequently, DON is found in cereal-based foods. The effect of thermal processing on DON is variable: some methods have been shown to reduce DON concentrations whereas others have had little effect. To determine if DON is stable during the production of selected foods, its concentrations in flour, wheat and processed food items prepared using commercially relevant conditions were compared using a gas-chromatographic method. The mean DON concentrations (n=9/item) in cookies, crackers, and pretzels were 61% (cookies) to 111% (pretzels) that of the unprocessed flour (100% = 0.46 ppm). Lower concentrations were found in donuts and bread. Their respective DON concentrations were 44% and 30%, respectively, that of flour. Mass balance estimations indicated that the total amount of DON (ppm flour equivalents) remaining in the flour-based products was as low as 50% (bread, 0.23 ppm flour equivalents) and as high as 120 % (donuts). This suggests that dilution of the flour by other ingredients significantly contributed to reducing DON concentrations in the bread and accounted for the entire reduction found in donuts. The mass balance results for the other flour products were in the range of 76% to 107%. The concentration of DON was higher in cereal flakes (0.55 ppm) than in the wheat (0.40 ppm) Likewise, the total amount of DON remaining in the finished flakes (mass balance result = 0.58 ppm) was also higher. In summary, DON concentrations were reduced ≥50% in only in bread and donuts and evidence for "loss" of DON through decomposition, interaction with food matrix components or other mechanisms was obtained only for bread. The findings for this series of products are consistent with earlier reports and provide additional evidence that DON is generally stable during the preparation of heat processed foods made from flour or whole wheat.

SESSION 5:

VARIETY DEVELOPMENT AND HOST RESISTANCE

Co-Chairpersons: Bill Berzonsky
and Jose Costa

VALIDATION OF *FHB1* AND *QFHS.NAU-2DL* IN SEVERAL SRW WHEAT BREEDING POPULATIONS

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ABSTRACT

Fhb1 is the most widely used exotic Fusarium Head Blight (FHB) resistance quantitative trait locus (QTL). Located on the short arm of chromosome 3B, this QTL is derived from Sumai-3. *QFhs.nau-2DL* is a non-Sumai-3 QTL that has been suggested as a complement to *Fhb1* in conferring FHB resistance, specifically by reducing deoxynivalenol (DON) concentrations. To validate the effects of both QTLs, five populations were evaluated in the Lexington scab nursery in 2010: Cross 12 (26R58/VA01W-476//KY97C-0574-01), Cross 17 (25R54/VA01W-476//KY97C-0574-01), Cross 19 (25R54/VA01W-476//KY97C-0554-02), Cross 40 (25R78/Cumberland//VA01W-476) and Cross 42 (25R23/KY93C-1238-17-1//VA01W-476). Traits measured included incidence, severity, FHB index, Fusarium damaged kernels (FDK) and deoxynivalenol (DON) concentration. It is expensive and time consuming to quantify FDK and DON. Thus, rapid and non-destructive methods for predicting these traits are of great interest. Near infrared (NIR) calibrations to predict FDK and DON have been recently developed at the University of Kentucky. The objectives of this study are (i) to investigate the impact of *Fhb1* and *QFhs.nau-2DL* on FHB resistance and (ii) to improve current NIR calibrations to predict FDK and DON. FDK was significantly reduced between 37 and 41 % due to the effect of *Fhb1* in all five populations. *Fhb1* also reduced FHB index between 18 and 49 % in four of five populations and it reduced DON 23 % in Cross 19 and 33 % in Cross 42. *QFhs.nau-2DL* reduced severity 31 % in Cross 19 and FDK 35 % in Cross 40. The correlation between FDK measured using air separation and using NIR spectroscopy was 0.66 and 0.73 for Crosses 19 and 42, respectively.

ACKNOWLEDGEMENT AND DISCLAIMER

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ASSOCIATION ANALYSIS OF MARKERS FOR BREEDING SCAB RESISTANCE IN WINTER WHEAT

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ABSTRACT

Marker assisted breeding is suited for low heritability traits and QTL of moderate to small effect. In order for markers to be applied, they must exhibit a trait association and be able to differentiate the target allele in a variety of germplasm. Bi-parental mapping is the conventional approach to identify marker-trait associations, although LD is maximized and polymorphism is limited to the parental alleles contributed. Association analysis is an approach that can determine the diagnostic ability of markers in a wide collection of material, in addition to determining or validating marker-trait association. An association analysis in wheat is needed because over 250 QTL for FHB resistance have been reported in bi-parental maps; few of which have been validated in alternate market class germplasm.

We report the results of an association study with advanced breeding lines from 19 Eastern United States breeding programs in relation to Fusarium Head blight resistance. Our analysis combined the phenotypic results of 258 lines from three uniform disease nurseries over three years. These advanced breeding lines were genotyped with 112 SSR and 2072 DArT markers. The population stratification and marker-trait associations were analyzed with TASSEL (v3.0.46). The results of the genome wide association study will be presented.

PHENOTYPIC CHARACTERIZATION OF FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN HULLED AND HULLESS WINTER BARLEY GROWN IN THE MID-ATLANTIC REGION

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ABSTRACT

Interest in use of winter barley (*Hordeum vulgare*) for ethanol production has heightened research focusing on its improvement, production, grain composition, utilization, and high value byproducts. Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a serious fungal pathogen that produces the mycotoxin deoxynivalenol (DON), which is known to accumulate in barley grain and can become concentrated in dried distiller's grain with solubles (DDGS). These high value DDGS produced as a byproduct of ethanol production are used in animal feeds and have potential for use in human foods. High DON concentration in DDGS can render them unmarketable. Information is needed regarding the accumulation, fate and changes in DON concentration in barley grain and during ethanol production. Potential ways to reduce DON concentration in grain and/or to degrade it during ethanol production include use of FHB resistant cultivars, milling or pearling to remove hulls where DON is concentrated, and development of yeast strains having the capability to degrade DON. Currently, little is known about FHB resistance in hulled and hulless winter barley grown in Virginia. Nine winter (hulled and hulless) barley genotypes including three putatively resistant, moderately resistant, and susceptible lines were selected from the Virginia Tech barley breeding program to further characterize FHB resistance. Genotypes were planted in a randomized complete block with two replications in mist-irrigated nurseries at Blacksburg and Mt. Holly, VA. Plots were 1.5 m x 13.4 m to produce sufficient grain for analysis of DON concentration in barley grain, during ethanol fermentation, and in DDGS. *Fusarium graminearum* colonized corn (*Zea mays*) kernels were applied to plots at the boot stage at both locations, and the test at Blacksburg was spray inoculated using conidia (1×10^4) applied at 50% flowering stage. Plots were rated for FHB incidence, proportion of 30 heads infected with FHB per plot, and FHB severity, number of infected spikelets divided by the total number of spikelets for thirty heads per plot. Additional data collected included grain yield, test weight, 1000 kernel weight, Fusarium damaged kernels (FDK), and DON concentration. Analysis of variance showed significant differences ($P \leq 0.05$) among genotypes for all traits. A significant ($P \leq 0.05$) genotype x environment interaction occurred for yield, test weight, and incidence. All FHB measurement parameters were significantly ($P \leq 0.0001$) correlated with each other. Pearson correlation coefficients ranged from r = 0.43 to 0.91. All FHB parameters had a significant ($P \leq 0.05$) negative effect on yield, test weight, and 1000 kernel weight, except for incidence, which did not have a significant effect on yield. Correlation coefficients ranged from r = -0.24 to -0.73. Results indicate that a range in resistance exists among both hulled and hulless winter barley lines and cultivars grown in the Mid-Atlantic region. FHB has a significant negative effect on important agronomic traits. Additional research is underway to track DON concentration in barley grain, during ethanol fermentation, and in DDGS.

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COMPARISON OF TWO METHODS FOR ESTIMATING FUSARIUM DAMAGED KERNELS IN SOFT RED WINTER WHEAT

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ABSTRACT

Evaluating seed samples for Fusarium damaged kernels (FDK) by visual estimates is a tedious, highly subjective task. High-tech devices, such as spectral imaging and optical sorters, and low-tech machines, such as gravity tables, are available for detecting and/or separating FDK from healthy kernels, but are cost prohibitive or do not work well with our small sample sizes. A low-cost specific gravity separation device, developed at the University of Kentucky, uses a vacuum and air column to separate the FDK from the healthy kernels. Both portions of the seeds are weighed, and a percentage (W/W) is calculated. Our objective was to see how well visual estimates and air separation percentages correlated to known FDK scores.

The test was a completely randomized design of 80 lines grown in three replications in the 2009 Fusarium head blight (FHB) disease nursery in Urbana, IL. Two samples of each line were scored for FDK by three methods: 100 seed count, visual estimate, and air separation. Seed from each line were then sent to the University of Minnesota DON testing lab for deoxynivalenol quantification. Data were analyzed using PROC CORR in SAS 9.2. We used Spearman's rank correlation to compare the methods. All methods had highly significant correlations ($P < 0.001$). Both visual estimates and air separation percentages had strong correlations to the actual FDK count ($r = 0.81$ and 0.73, respectively). DON levels were not as closely correlated to the 100 seed count ($r = 0.59$), visual estimate ($r = 0.58$), or air separation ($r = 0.51$) method, but correlations were similar among methods.

These data support the use of an air column separation device or visual estimates for evaluating FDK. While a single air column separation device may not hasten the evaluation of FDK samples, it provides objective and reproducible data, and can be operated by hourly employees. Operating several separation devices would speed up the process and allow for evaluation of more samples. Obtaining the DON data was ancillary to the main objective of the study, but the moderate correlations between DON level and visual kernel damage serve are a reminder that visual kernel damage may not always be a good indicator of DON content in the grain.

CURRENT KNOWLEDGE ON THE GENETICS OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT - IMPLICATIONS FOR RESISTANCE BREEDING

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INTRODUCTION

In the wheat gene pool significant variation for resistance to Fusarium head blight has been discovered and documented for several decades. Resistance to Fusarium head blight is a quantitative trait controlled by polygenes and modulated by the environment. Numerous studies were performed to decipher the inheritance of Fusarium head blight resistance in wheat. Quantitative trait loci (QTL) mapping became the standard method for genetic analyses of FHB resistance. Most of the published studies were based on 'classical' QTL mapping using segregating populations from bi-parental crosses of a resistant with a susceptible parental line (Tanksley 1993). More recently, methods of association mapping have to been proposed for genetic analysis of quantitative traits. In this case sets of genotypes, which may be cultivars, breeding lines, or introduced germplasm collections, with or without pedigree and kinship information, are used for genetic analysis. This approach aims to associate the occurrence of certain marker haplotypes with trait expression (Gupta et al. 2005, Rostoks et al. 2006). Initial studies applying association mapping for FHB resistance in winter wheat were recently published by Zwart et al. (2008) and Miedaner et al. (2010).

MAPPED QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE

A detailed review on FHB resistance QTL was recently compiled by Buerstmayr et al. (2009). In this article we summarized the relevant findings from 51 quantitative trait loci (QTL) mapping studies and included 9 research articles on marker assisted selection and 7 on marker assisted germplasm evaluation. Up to now QTL for FHB resistance were reported on almost all wheat chromosomes. Some QTL were found in several independent mapping studies indicating that such QTL are stable and appear therefore useful in breeding programs. We summarize and update current knowledge on the genetics of Fusarium head blight resistance in wheat and review breeding strategies based on the available information and DNA markers. Two independent meta-QTL studies were published recently (Löffler et al. 2009, Liu et al. 2009), which to a large extent agree with each other and with the review by Buerstmayr et al. (2009)

Obviously, the most repeatable QTL are those on chromosomes 3BS (*Fhb1*), 5AS (*Qfhs.ifa-5A*), and 6BS (*Fhb2*) all based on resistance sources from Asia (Buerstmayr et al. 2009). In addition, *Fhb4* (*Qfhi.nau-4B*) has been fine mapped by Xue et al. (2010). Recently several QTL from European winter wheat have been mapped and validated, *e.g.* on chromosomes 6AL and 7BS (Häberle et al. 2007, Wilde et al. 2008) and on 1BL (Häberle et al. 2009). For further details on FHB resistance QTL see Buerstmayr et al. (2009), Löffler et al. (2009) and Liu et al. (2009).

MOLECULAR BREEDING FOR FHB RESISTANCE

For the purpose of marker assisted selection, diagnostic markers are currently available for only *Fhb1*. Other FHB QTL have also been used in MAS programs, especially in cases where breeders are familiar with marker allele types of the QTL donors and the recipient germplasm. More diagnostic markers should be developed for QTL to be easily adopted by breeders. Therefore, the emphasis of future research

activities should be to discover '*perfect*' markers for the most repeatable QTL.

While in hexaploid wheat both conventional and marker assisted breeding for improving FHB resistance has made significant progress, in tetraploid durum wheat good sources of resistance are still sparse and more work is needed to identify resistant germplasm and to decipher its FHB resistance.

Marker assisted selection for major QTL has proven very efficient. For example marker assisted incorporation of *Fhb1* and *Qfhs.ifa-5A* in winter wheat cultivars resulted in an average reduction of the disease severity in the range 20-40%, relative to lines without the resistance QTL alleles (Salameh et al. 2010, von der Ohe et al. 2010). Currently, for practical cultivar improvement a skilful combination of marker assisted selection for relatively large effect QTL with phenotypic selection is a very useful strategy. Because of the quantitative nature of FHB resistance in the different wheat gene pools, the adoption of novel 'genomic selection' methods appears a very valuable research field and could increase the gain by selection.

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ADVANCED BACK-CROSS QTL MAPPING OF RESISTANCE TO FUSARIUM HEAD BLIGHT DERIVED FROM *TRITICUM MACHA* (GEORGIAN SPELT WHEAT)

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ABSTRACT

While many reports on genetic analysis of Fusarium resistance in bread wheat have been published during the past decade, only limited information is available on FHB resistance derived from wheat relatives. In this contribution we report about genetic analysis of FHB resistance derived from *Triticum macha* (Georgian spelt wheat). As the origin of *T. macha* is in the Caucasian region it is supposed, that its FHB resistance differs from other well investigated resistance sources. In order to introduce valuable alleles from the landrace *T. macha* into a modern genetic background we adopted an advanced back-cross QTL mapping scheme (Tanksley and Nelson 1996).

A large back-cross-two derived recombinant inbred line population of over 300 BC_2F_3 lines was developed from a cross of *T. macha* with the Austrian winter wheat cultivar 'Furore'. The population was evaluated for Fusarium resistance in six field experiments during three seasons using spray inoculations. The population was genetically fingerprinted using SSR and AFLP markers.

Map construction was done with an updated version of *CarthaGène* (De Givry et al. 2005). For QTL mapping *QGene* (Nelson 1997) was used. The obtained linkage map covered 37 linkage groups with 563 markers. Five novel FHB resistance QTL, all descending from *T. macha*, were found on four chromosomes (2A, 2B, 5A, 5B). The largest effect QTL overlapped with the *Q-locus* (spelt type) on chromosome 5A and appears therefore an interesting QTL especially for spelt wheat improvement.

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EVALUATION OF BACK-CROSS-TWO DERIVED SISTER LINES CARRYING *RHT-B1A/B* OR *RHT-D1A/B* IN A HIGHLY FUSARIUM RESISTANT DONOR BACKGROUND

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ABSTRACT

Recent publications have shown that the widely used dwarfing genes *Rht-B1* (syn. *Rht1*) and *Rht-D1* (syn. *Rht2*) are associated with Fusarium head blight (FHB) resistance. The semi-dwarf allele *Rht-D1b* and to a lesser extent *Rht-B1b* appear to increase FHB susceptibility in wheat (Miedaner and Voss 2008, Holzapfel et al. 2008, Srinivasachary et al. 2009). In order to further evaluate the effects of these alleles we developed and tested back-cross derived sister lines differing in their *Rht* alleles.

As donor line we used the highly FHB resistant breeding line '20812.2.2' derived from the cross 'Capo' x 'Sumai-3', which possesses the tall alleles *Rht-B1a* and *Rht-D1a*. As donors for the semi-dwarf alleles we used 'Hermann' (*Rht-B1b*, *Rht-D1a*), 'Toras' (*Rht-B1a*, *Rht-D1b*) and 'Courtot' (*Rht-B1b*, *Rht-D1b*). Using diagnostic PCR markers for the *Rht* alleles, we generated back-cross-two derived sister lines ($BC_2F_{2:3}$ lines) which were homozygous for contrasting *Rht* alleles and tested these in one field trial at IFA-Tulln using spray inoculations.

On average across seven NIL-pairs for *Rht-B1* we found that lines with the semi-dwarf allele *Rht-B1b* showed about 90% increased FHB severity compared to their sister lines which had the tall allele *Rht-B1a*. The difference was even more pronounced for *Rht-D1*, where on average across six NIL-pairs lines with the semi-dwarf allele *Rht-D1b* had about 160% higher FHB severity compared to lines with the *Rht-D1a* allele. Our data are in agreement with previous findings that semi-dwarfing alleles reduce FHB resistance and that *Rht-B1b* is less damaging than *Rht-D1b*. However, the negative effect of the semi-dwarf alleles can be balanced by selecting lines with other known or unknown FHB resistance QTL in their genome. Therefore, selection of semi-dwarf cultivars with good FHB resistance is quite difficult but feasible.

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DEVELOPMENT OF ADVANCED SPRING WHEAT LINES WITH FHB RESISTANCE THROUGH ALIEN INTROGRESSION

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ABSTRACT

One hundred and fourteen advanced spring wheat lines with various levels of FHB resistance were developed from crosses of adapted hard red spring wheat varieties with different wheat-alien species derivatives. These advanced breeding lines were evaluated for FHB resistance in multiple greenhouse seasons and at three field locations (Jianyang, Fujian Province, China; Prosper, ND; and Langdon, ND). Thirty-six of them exhibited good agronomic characteristics, such as maturity, test weight, and yield, in addition to FHB resistance in the 2009 field trial at Prosper, ND. One of the advanced lines showed significantly higher yield than the corresponding wheat parents when grown at Prosper, ND. The 36 advanced lines were further evaluated for FHB resistance and agronomic performance in a replicated yield trial at Langdon, ND in 2010. Most of the lines consistently showed FHB resistance and yield advantage when grown at Langdon, ND. Genotyping of these lines at *umn10* locus, a molecular marker tagging Sumai 3-derived FHB resistance gene *Fhb1*, indicated some of the advanced lines did not contain the "Sumai 3" allele at this marker locus. Deoxynivalenol (DON) content was measured in seed samples that were collected from146 alien introgression lines in a field experiment with three replicates. Some of the lines had significantly lower DON content than corresponding wheat parents. In addition, some of the advanced spring wheat lines exhibited advantages over wheat parents on some of the end-use quality characteristics. FHB resistance of these lines will be further verified in a replicated field experiment at Jianyang, China in 2011. Some of the lines have been provided to wheat breeding programs to be used for variety development. We anticipate releasing several spring wheat germplasm with FHB resistance and good agronomic characteristics in 2011.

EVALUATION OF SCAB RESISTANCE QTLS ON AGRONOMIC AND QUALITY TRAITS OF SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a devastating fungal disease which affects wheat crops worldwide. While many quantitative trait loci (QTL) responsible for FHB resistance have been reported, some of the most widely used sources are from exotic varieties. Ning 7840, a Chinese hard red spring wheat, contains a major QTL on the 3BS chromosome, as well as two minor QTL on the 5A and 2DL chromosomes. Ning 7840 was crossed with Pioneer 2643, a soft red winter wheat, and a recombinant inbred line population was derived. The effect of the Ning7840 alleles on agronomic traits and milling and baking quality was examined over three growing seasons in Maryland. In 2009 and 2010, height was reduced by the 3BS QTL, incidence was decreased by the 5A QTL, and seed weight was decreased by both the 2DL QTL and the 5A QTL. These results suggest that the introduction of FHB resistance QTLs into soft red winter wheat may have consequences on agronomic and quality traits.

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MAPPING SCAB RESISTANCE IN THE WINTER WHEAT LINE MD01W233-06-1

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OBJECTIVE

To map resistance to scab in a doubled haploid population derived from the resistant soft winter wheat genotype MD01W233-06-1 and the susceptible genotype SS8641 under field conditions.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a devastating disease of wheat (*Triticum aestivum* L.) in the United States and around the world that affects grain yield and quality. High levels of the toxin deoxynivalenol (DON) in the grain are often associated with this disease (Bai and Shaner, 1994). Infection of wheat spikes may cause significant grain yield and quality losses due to poor grain fill, high percentage of Fusarium damaged kernels (FDK), and low test weights. Development of resistant cultivars is an important means of controlling FHB. Sources of resistance to FHB are limited to a few wheat cultivars, such as 'Sumai 3' (Anderson et al., 2001) and 'Frontana' (Steiner et al., 2004), which hinders the development of improved cultivars. New resistance sources are needed for breeding wheat with FHB resistance. 'MD01W233-06-1', a soft red winter wheat (SRWW) (*Triticum aestivum* L.) germplasm, is a US native source of FHB resistance that does not carry Sumai 3 alleles (Costa et al., 2010). Here, we make an initial evaluation of a doubled haploid population derived from the cross of MD01W233-06-1 with SS8641, a scab susceptible cultivar.

MATERIALS AND METHODS

Plant material and experimental design - A doubled haploid (DH) population that consisted of 90 DH lines obtained via the maize method (Laurie and Bennett, 1988) from an F1 resulting from a cross between: 'SS8641', a FHB scab susceptible cultivar, and the SRWW scab resistant MD01W233-06-1 cultivar. Field plots were established in Salisbury (MD) in a conventionally tilled field, previously planted with corn. A total of 184 plots (5-ft long) were planted on October 13, 2009. The experimental design was a randomized complete block, with two replications. The experiment was inoculated with Fusarium colonized corn (*Zea mays*) kernels prior to anthesis and was mist-irrigated from late April through May and early June 2010.

Trait analysis - Heading date (HD) and plant height (HT) were measured by hand in the field. Incidence (I) and severity (S) were visually estimated in two groups of 20 spikes randomly chosen within each plot from which FHB index (IND) was calculated. *Fusarium* damaged kernels (FDK) were based on counting 200 seeds (%). ISK was calculated by estimating incidence and severity and FDK. DON extraction and analysis were as described in Mirocha et al. (1998). Statistical analysis of phenotypic data was carried out using SAS version 9.2 (SAS Institute, Cary, NC) where block were considered as random effects and genotype and environment as fixed effects. Coleoptiles (red or white color) were visually scored in the greenhouse.

Genetic map construction and QTL analysis - Extraction of genomic DNA, PCR amplification, PCR screening and genotyping data was performed as previously described by Somers et al. (2004). One phenotypic and 29 SSR markers were scored. A genetic map was constructed with MAPMANAGER QTXb20. The mapping function was used to convert recombination fractions into centiMorgans (cM) as map distance. (Kosambi, 1944)

RESULTS AND DISCUSSION

Phenotypic variation of parental lines and DH population - In the 2009-2010 growing season, SS8641 had similar heading date, was taller, had higher incidence, severity, index, *Fusarium* damaged kernels, ISK, and DON than MD01W233-06-1 (Table 1). In the DH population, measured traits varied over a wide range and were normally distributed (Table 1). Significant differences were found among DHs. For most of the traits analyzed, positive and negative transgressive segregants were observed in the DH population, which suggest that positive and negative alleles may be found in both parental lines. Correlation coefficients among the average values of measured traits are presented in Table 2. Heading date had a positive correlation with INC, FDK and DON. Height was not correlated with other traits. INC exhibited a strong correlation with SEV, Index and ISK. SEV showed a positive correlation with Index and ISK. Index had a strong positive correlation with ISK. Another strong positive correlation was observed between FDK and DON. Additionally, there was a positive correlation between ISK and DON.

QTL mapping - Twenty nine SSR markers, and one morphological marker (red coleoptile) were used for linkage analysis and mapping of the quality traits for scab resistance. Seven linkage groups with 2 to 3 markers and one group of unlinked 15 markers were found. A total of 26 QTLs were detected by Map manager analysis (Table 3). The QTL with the highest effect was for ISK, FDK, and DON on 1A near SSR marker wmc278Fd (for ISK) and wmc496Nd (for FDK and DON) near the 1AL.1RS translocation. Oliver et al. (2005) similarly suggested that the 1AL.1RS translocation may be responsible for FHB resistance in the wheat line Amigo.

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Table 1. Mean, range, CV, LSD of heading date (HD), height (HT), incidence (I), severity (S), *Fusarium* damaged kernels (FDK) and DON from an inoculated and misted study in Salisbury (MD) in 2010.

	PARENTAL LINES		DHs(90)		WHOLE POPULATION		
	MD01W 233-06-1	SS8641	AVERAGE	RANGE	MEAN	CV	LSD
HD(d)	123	123	123	120-129	123	0.9	2.1
HT(inches)	33.0	34.5	30.4	23.0-36.5	30.4	6.0	3.6
I (%)	8.0	10.0	15.2	2.5-67.5	15.4	66.8	20.4
S (%)	10.0	30.0	19.9	5.0-60.0	20.2	55.5	22.3
IND	1.2	2.8	4.3	0.1-38.0	4.4	126.1	11
FDK (%)	3.8	15.0	29.4	2.8-53.0	14.7	43.9	12.9
ISK	0.3	1.5	3.8	0.0-71.8	3.7	192.2	14.2
DON (ppm)	2.5	8.8	8.3	0.5-40.3	8.2	65.0	10.6

Table 2. Correlation coefficients among the traits measured.

	Height	INC	SEV	Index	FDK	ISK	DON
Julian	0.01^{NS}	0.39**	0.17*	0.26**	0.59**	0.30**	0.63**
Height		0.06^{NS}	0.14^{NS}	0.02^{NS}	-0.07^{NS}	-0.01^{NS}	-0.07^{NS}
INC			0.65**	0.89**	0.55**	0.78**	0.35**
SEV				0.78**	0.36**	0.58**	0.23**
Index					0.48**	0.90**	0.26**
FDK						0.59**	0.81**
ISK							0.36**

**Correlation significantly different from zero at the 0.01 level, *Correlation significantly different from zero at the 0.05, NS: non significantly different from zero.

INC: incidence, SEV: severity, FDK: *Fusarium* Damaged Kernels.

Table 3. Quantitative trait loci detected in the MD01W233-06-1×SS8641 wheat population by composite cumulative mapping.

Trait	Chromosome	Position	Flanking markers	R^2	LRS
Julian	1A	61.7	wmc278Fd	5	4.9
	1A		wmc496Nd	9	8.6
	1A	58.2	barc28Fd	9	7.9
Height	1A	61.7	wmc278Fd	3	2.8
			wmc471Vd	5	4.6
	7D		Red coleoptile	2	2.0
Incidence	1A	61.7	wmc278Fd	5	4.9
	1A		wmc496Nd	5	4.9
	1A	58.2	barc28Fd	5	4.7
	4B	30.9	gwm149Fd	4	3.7
Severity	4B	30.9	gwm149Fd	5	4.4
			wmc471Vd	5	4.5
Index	1A	61.7	wmc278Fd	4	4.0
	4B	30.9	gwm149Fd	6	5.1
ISK	1A	61.7	wmc278Fd	12	11.4
	1A		wmc496Nd	4	3.9
	1A	58.2	barc28Fd	4	3.5
	4B	30.9	gwm149Fd	5	5.1
FDK	1A	61.7	wmc278Fd	9	7.9
	1A		wmc496Nd	11	11.0
	1A	58.2	barc28Fd	10	9.4
	4B	30.9	gwm149Fd	4	3.3
DON	1A	61.7	wmc278Fd	6	5.6
	1A		wmc496Nd	8	7.0
	1A	58.2	barc28Fd	7	5.8
	4B	30.9	gwm149Fd	3	2.5

A NOVEL GENOME MUTATION IN WHEAT INCREASES FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

We sought to validate an FHB resistance QTL reported to be on chromosome 2A in the soft red winter wheat cultivar Freedom, by introducing it into the highly susceptible rapid maturing dwarf wheat Apogee. Marker-assisted backcrossing with an SSR marker reported to be associated with this QTL was undertaken. One backcross four-derived Apogee near-isogenic line (NIL), designated A30, exhibited improved type II FHB resistance. Other independently derived NILs harboring the introgressed Freedom SSR marker did not exhibit improved FHB resistance, suggesting that the FHB resistance of A30 could be due to the presence of an unlinked major FHB resistance QTL from Freedom. A project was undertaken to identify marker loci for this putative new FHB resistance QTL. Ninety F2:3 families derived from the cross Apogee x A30 were evaluated for type II FHB resistance in two greenhouse evaluations. SSR marker polymorphisms between A30 and an FHB susceptible sib line, A31, were identified to provide targets for mapping the putative new QTL in the Apogee x A30 population. Sampling of markers in major intervals of A30 deriving from Freedom did not reveal an association with FHB resistance in the mapping population. However, when a set of segregating SSR markers present in Apogee (and A31) but null in A30 was examined, a significant relationship with FHB resistance was detected, with a positive effect associated with the null allele state. On average, F2:3 families homozygous for the null alleles were approximately 50% more resistant than Apogee in the greenhouse evaluations. Aneuploid analysis with Chinese Spring cytogenetic stocks mapped the null markers to chromosome arm 3DL. Evaluation of other markers previously mapped to this chromosome arm identified a new set that were null in A30 and present in Apogee. The null marker loci in A30 were localized to bin 3 of the Chinese Spring chromosome arm 3DL segmental deletion line series. Interestingly, Freedom is not null for these same SSR markers. We conclude that during the course of our backcrossing efforts between Apogee and Freedom, a segment of the terminal end of chromosome arm 3DL of Apogee was deleted. Deletion of this chromosome segment has a significant positive effect on type II FHB resistance. The gain of FHB resistance associated with the loss of a chromosome segment suggests that the region contains either a suppressor of FHB resistance or a gene that promotes virulence of *F. graminearum*. We are now examining whether this effect occurs in other genetic backgrounds.

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FUSARIUM HEAD BLIGHT IN BARLEY: IDENTIFICATION OF THE CAUSAL FUSARIUM SPECIES IN EUROPE AND TESTING OF FHB RESISTANCE USING ARTIFICIAL INOCULATION

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ABSTRACT

Fusarium head blight (FHB) in barley can be caused by different *Fusarium spp.* producing various mycotoxins. Breeding for resistance requires 1) resistance sources and 2) a reliable screening technique. Syngenta wanted to investigate FHB resistance of a barley nursery as a basis for future breeding programs. We also compared different inoculation methods and the resistance to DON/NIV and T2/HT2-producing *Fusarium spp.* We started with the isolation, purification and identification of *Fusarium* isolates from infected barley kernels originating from France, Germany and UK. In total 63 isolates were identified belonging to 8 different *Fusarium spp.* Most frequently detected isolates in Germany were *F. poae*, in France *F. cerealis* and *F. graminearum* and in the UK *F. avenaceum*. FHB resistance was tested with spray inoculation and with the kernel spawn method. Five different *Fusarium spp.* were used for inoculation. Scored was disease incidence and severity. ANOVA analyses showed highly significant differences between genotypes and treatments. Resistance data obtained with both inoculation techniques and with most *Fusarium spp.* were significantly related (r = 0.57-0.81). Correlation coefficients between disease incidence and severity data were highly significant (r=0.93-0.99). We could not find any evidence for specific plant resistance against a particular type of toxin producer.

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MOLECULAR MAPPING OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN TETRAPLOID WHEAT

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ABSTRACT

While many reports on genetic analysis of Fusarium resistance in bread wheat have been published, only limited information is available on FHB resistance derived from tetraploid wheats. In this contribution we report about genetic analysis of FHB resistance derived from two tetraploid *Triticum* sources: 1) *Triticum dicoccum* (cultivated emmer) and 2) *Triticum dicoccoides* (wild emmer). Back-cross-one derived recombinant inbred line populations were developed from crosses of the resistance donors with adapted Austrian durum wheat cultivars. The populations were evaluated for Fusarium resistance in well replicated experiments with artificial inoculation. The *T. dicoccum* derived populations were tested in field trials using spray inoculations and the *T. dicoccoides* derived mapping population was greenhouse tested using single-floret inoculations. The same lines were genetically analysed using SSR and AFLP markers. Map construction based on the back-cross derived RIL populations was done with *CarthaGène* (De Givry et al. 2005) and QTL mapping in *Qgene* (Nelson 1997). In *T. dicoccum* the most consistent QTL mapped to chromosome 4B, associated with the *Rht-B1a* allele. A second consistent QTL mapped to chromosome 6B. Significant QTL for type 2 FHB resistance were detected in wild emmer (*T. dicoccoides*) mapping to chromosomes 3A and 6B. Wild and cultivated emmer wheat are promising sources for improving FHB resistance in durum wheat.

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PRELIMINARY QTL ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN THE SOFT RED WINTER WHEAT, 'TRUMAN'

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ABSTRACT

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), the pathogen known to cause Fusarium head blight (FHB) or scab, is an increasingly important problem for wheat production in warm and humid regions of the world. To date, most advances in FHB-resistance breeding have been made through selection for type II resistance largely because this type of resistance is durable, reliably estimated, and less sensitive to non-genetic factors than other types of resistance. Combining type II resistance from various sources of resistance to FHB is expected to generate lines with higher levels of resistance, more effective resistance under high inoculum loads, and/or varieties in which resistance is more stable over broad geographic areas but is limited by a lack of highly effective, genetically different sources of type II resistance. 'Truman' soft red winter wheat was developed and released by the University of Missouri. Its resistance is broadly based combining good levels of types I and II resistance with low DON and good kernel quality retention under disease pressure. The type II resistance in Truman is comparable to that in Sumai 3 but appears to be conditioned by different genes and is also unique in that it appears to be highly penetrant with good breeding value. This research was conducted to identify QTL associated with type II resistances in a set of F_9 recombinant inbred lines developed from the cross Truman/MO 94-317. MO 94-317 is a highly susceptible inbred line developed in the University of Missouri's wheat breeding program. Two years (5 replications) of greenhouse type II phenotypic data were collected for this study. A Missouri isolate of *F. graminearum* previously tested for pathogenicity was used for all inoculations. Each plant was inoculated at first anthesis with 10 µL of a macroconidial suspension of this isolate concentrated to 50, 000 mL^{-1}. Inoculum was placed in the basal floret of a central spikelet. Plants were incubated in a mist chamber at 100% relative humidity for 72 h post-inoculation to initiate disease development and then returned to the greenhouse bench to enable the disease to progress in the head. Ratings for type II resistance were taken at 21 d post-inoculation. Fusarium head blight severity was determined as the ratio of disease spread to the total number of spikelets on the inoculated head expressed as percentage. Molecular marker analysis was conducted using SSR and DArT [Diversity Arrays Technology Pty Ltd, (Triticarte) Yarralumla, Australia] markers. Genetic linkage maps were constructed using MapMaker 3.0. QTL analysis for individual replications and across reps was conducted using composite interval mapping (CIM) with WinQTLCart 4.0. Preliminary data suggest QTL for type II resistance on chromosomes 1B, 2B, and 6B at the 0.05 significance level.

ENHANCEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT USING MARKER ASSISTED SELECTION

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ABSTRACT

Both exotic and native resistant sources have been employed in our breeding program to improve the local adaptive soft red winter wheat (*Triticum aestivum* L.) varieties for Fusarium head blight (FHB) resistance. Progenies of F_3 lines with desired agronomic traits were genotyped with molecular markers on chromosome 2A, 2DL, 3BS, 3BSc, and 5A. Elite lines with FHB QTL from Sumai3, Roane, and Truman3BS were identified and advanced into the next generation for further evaluations for FHB and pest resistance in the field and agronomic traits.

IDENTIFYING QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN A RIL POPULATION DERIVED FROM A THREE-WAY CROSS INVOLVING THREE RESISTANT PARENTS

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ABSTRACT

Fusarium head blight (FHB) of wheat has become an increasingly important disease over the past 25 years. Significant yield losses due to FHB can be observed when there is a favorable environment for disease development. Grain quality is also of great concern. Fusarium graminearum, the primary fungal pathogen that causes FHB produces deoxynivalenol, a mycotoxin that can cause serious health problems for both humans and livestock when consumed in FHB infected grain. While cultural practices and fungicide treatments can suppress FHB, the use of resistant varieties is the most effective method for control of FHB. Breeding for resistance to FHB has become a very large part of wheat and barley breeding programs in temperate climates. Various sources of resistance have been used to develop new varieties that have high levels of resistance. The primary objective of this study was to combine multiple sources of resistance using a recombinant inbred line (RIL) population derived from three FHB-resistant University of Illinois breeding lines (IL96-6472, IL97-6755 and IL97-1828) to obtain transgressive segregants that are significantly better than the three parents. The RIL population, consisting of 266 lines, was evaluated for FHB resistance in the greenhouse and in a mist irrigated, inoculated disease nursery. Forty-three simple sequence repeat (SSR) and 250 Diversity Arrays Technology (DArT) polymorphic markers were used to create a linkage map using Joinmap 3.0. PlabQTL was used for composite interval mapping and detection of significant QTL. QTL were found for all measured traits except formean severity in the 2008 and 2009 greenhouse evaluation. QTL on the short arm of chromosome 3B were identified for all measured traits and accounted for 6.8% to 10.1% of the phenotypic variation, depending on the trait. We believe that these markers are associated with Fhb1 or QTL tightly linked to Fhb1. Minor QTL were also found on chromosomes 7B and 1Aexplaining a smaller amount of phenotypic variation (between 5.3% and 8.2%). A total of 13 transgressive segregants were found that were significantly better than the mean of the three FHB-resistant parents for more than one trait. These thirteen lines were found to carry many of the resistance alleles associated with the QTL found in the study. Although the population was derived from three FHB-resistant parents, and there were likely QTL that were not detected due to a lack of polymorphism, we believe that multiple genes for resistance were combined in the transgressive segregants observed in the recombinant inbred line population.

A TRANSCRIPTOMIC APPROACH TO ELUCIDATE THE POSSIBLE ROLES OF BRASSINOSTEROIDS IN EARLY PLANT DEFENSE RESPONSES

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ABSTRACT

Brassinosteroids (BRs) are a class of plant hormones that have received great attention for their growth-promoting activities. They have pleotropic effects and can induce a broad spectrum of cellular processes. It has been demonstrated that BRs function in mediating an enhanced broad disease resistance in tobacco and rice. We investigated the effect of BR signalling on *Fusarium* seedling blight disease of barley. Results obtained using BR-insensitive mutant, isogenic wild type barley and brassinosteroid treatment in seedling blight disease trials indicated that this hormonal pathway influences cereal defense against *Fusarium culmorum*. The exact mechanism of BRs mediated plant defense responses is not yet elucidated. Using both microarray analyses and real time RT-PCR analyses, we investigated the effect of BR treatment and BR insensitivity on the transcriptome of healthy and *Fusarium*-inoculated barley stem base tissue (24 and 48 h post-treatment). The most striking result was the pronounced effect that both the BR-insensitive mutation and BR treatment had on defense gene expression.

EFFECT OF ISOLATED MICROSPORE CULTURE AND SILVER NITRATE PRE-TREATMENT ON IMPROVING *IN VITRO* SELECTION TO REDUCE DEOXYNIVALENOL ACCUMULATION IN BARLEY

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ABSTRACT

Fusarium head blight (FHB) incited by *Fusarium graminearum* Schwabe is presently the most damaging disease of barley in Canada, primarily due to grain contamination by mycotoxins. As resistance from exotic landraces has been difficult to incorporate in barley (*Hordeum vulgare* L.), *in vitro* selection (IVS) was applied in concurrence with doubled haploid production as an alternative to traditional breeding methods in attempt to reduce deoxynivalenol (DON) accumulation. A large FHB screening nursery at Brandon, MB was used to evaluate DON content in harvested grains of experimental lines. Previous attempts to include trichothecenes in anther culture culture media, had proven to be largely unsuccessful at selecting breeding lines with reduced DON content. A novel "bridge" method was developed to incorporate DON in liquid media during isolated microspore culture (IMC) which typically uses solid media. Barley cultivar Newdale and F_1 plants from the BM0332 (Svansota/Newdale) and BMO270 (TR04282/Newdale) crosses, were used as donors. Contrasts between selected lines and respective controls indicated that IVS was not effective in IMC using the new method. Barley cultivar Newdale and F_1 plants from the BM0362 (HDE84194-622-1/Newdale) and BM0525 (HB382/Newdale) crosses were used to investigate the effect of anther pre-treatment with silver nitrate on improving FHB resistance. Although the addition of DON to the silver nitrate pre-treatment mixture did not appear to lower DON content in the resulting DH plants, a trend was observed for silver nitrate pre-treated lines from Newdale and both F_1 populations with or without DON in the culture media to accumulate less DON than controls. Four of the Newdale DH lines subjected to silver nitrate pre-treatment will be evaluated further by the breeding program, and one of them may be advanced to registration trials in 2011.

EFFICACY OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY TO PREDICT *FUSARIUM* DAMAGED KERNELS AND DEOXYNIVELANOL IN RED AND WHITE WHEAT IN MICHIGAN

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ABSTRACT

Michigan State University annually evaluates the Michigan State Performance Trial (MSPT) entries for Fusarium head blight (FHB) resistance. The MSPT consists of both red and white genotypes that vary in visual field symptoms of FHB, *Fusarium* Damaged Kernels (FDK) and deoxynivalenol (DON) levels. It has been previously reported that white wheat accumulates higher amounts of DON than red wheat (Knott, Van Sanford et al. 2008). In addition, near-infrared spectroscopy (NIRS) predictions for DON levels have been investigated, in general. However, the effect of grain color (red vs. white) in NIRS predictions of FDK and DON have not, to our knowledge, been investigated. In this poster we will present data of visual field symptoms of FHB (incidence, severity, index), FDK, DON and NIRS predictions of both FDK and DON when grain color is considered for the 2009 MSPT.

REFERENCE

Knott, C. A., D. A. Van Sanford, et al. (2008). "Comparison of selection methods for the development of white-seeded lines from red x white soft winter wheat crosses." Crop Science **48**(5): 1807-1816.

EFFICIENT SELECTION FOR LOW DON LEVELS IN WHEAT

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ABSTRACT

Deoxynivalenol (DON) is the most common mycotoxin associated with wheat grain from fields affected by Fusarium head blight (FHB). Growing cultivars that are resistant to FHB is believed to be the key component for reducing DON levels in grain. Wheat breeding programs have been successful at reducing the levels of DON in some recently released cultivars relative to the DON levels in susceptible cultivars and are continuing efforts to reduce DON to even lower levels. Given that reducing DON levels in wheat grain is a high priority for breeders, millers, and consumers and that screening lines for DON level is expensive, becoming more efficient at selecting wheat lines with low DON levels should be a priority for breeding programs in the USWBSI. The objective of this study is to determine how to most efficiently select wheat lines that have low levels of DON in harvested grain. Twenty cultivars representative of those being grown in Arkansas in 2010 and 58 lines from the 2010 Southern Uniform Winter Wheat Scab Nursery (SUWWSN) were grown in early-planted and late-planted FHB nurseries at Fayetteville. Plots were two rows x 1 m long and replicated three times in each nursery. Plots were inoculated using infested corn kernels and misted to promote ascospore development and infection. Lines were evaluated for FHB severity at soft dough stage and harvested at maturity. A 50-g sample of grain was visually rated for percentage of scabby kernels by comparing samples to a set of standards, and this same sample was ground to determine DON level. A 4-g subsample of ground grain was sent to the mycotoxin lab at the University of Minnesota for DON analysis. To determine the relationships among FHB severity, percentage of scabby kernels and DON level, Pearson correlation coefficients were calculated for each pair of variables for the cultivars and the SUWWSN in the early- and late-planted FHB nurseries. The percentage of scabby kernels was positively correlated with FHB severity (range 0.68-0.85, mean 0.77), and DON level was positively correlated with FHB severity (range 0.61-0.82, mean 0.72) and Percentage of scabby kernels (range 0.84-0.91, mean 0.88). These results indicate that lines with high FHB severities do not need to be harvested and that harvested lines with high percentages of scabby kernels do not need to be assayed for DON level in order to identify lines with low DON level. Thresholds for deciding which lines should be harvested and sent for DON analysis should be based on values for resistant checks. Considerable savings can be realized by not harvesting or analyzing lines with a low probability for low DON level.

USEFULNESS OF GREENHOUSE EVALUATIONS AS A PREDICTOR OF WHEAT HEAD BLIGHT RESISTANCE IN THE FIELD

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ABSTRACT

Five types of resistance to Fusarium head blight (FHB) of wheat have been hypothesized. A new method for conducting greenhouse evaluations generates data on four of these types: resistance to initial infection, resistance of spread within a spike, tolerance (i.e. ability to yield when affected by FHB), and resistance to deoxynivalenol (DON) accumulation. The objective of this study is to determine how well the results from greenhouse evaluations predict results from evaluations in inoculated, misted field nurseries that are commonly used by breeding programs to select resistant lines. Two sets of wheat lines (20 cultivars commonly grown in Arkansas and 58 lines in the 2010 Southern Uniform Winter Wheat Scab Nursery (SUWWSN)) were evaluated in greenhouse/growth chambers and in early- and late-planted inoculated, misted nurseries at Fayetteville. Briefly, plants with spikes at flowering stage were spray-inoculated, bagged, and incubated at constant 23°C. Bags were removed after 48 h, and plants were moved to a greenhouse 5 days after inoculation (dai). The percentage of blighted primary florets was determined at 5, 14 and 21 dai, and the area under the disease progress curve (AUDPC) was calculated between 5 and 21 dai. The percentage of blighted florets at 5 dai estimates resistance to initial infection, and the AUDPC estimates resistance to spread. Grain was harvested at maturity, bulked across six reps, weighed to determine tolerance to FHB, ground, and sent for DON analysis. Three reps of two-row wide x 1-m long field plots were rated for percentage of blighted florets at soft dough stage, harvested at maturity, weighed, and a sample sent for DON analysis. Pearson and Spearman correlations were calculated to determine how well greenhouse/growth chamber results predicted field results for the 20 cultivars and 58 SUWWSN lines in each field nursery, except yield data from the early-planted nursery were not useable because of erratic stands. Pearson correlation coefficients were similar for the four correlations between field FHB severity and AUDPC, averaging 0.71, and for the two correlations between field yield and greenhouse yield, averaging 0.70. For comparing field DON to greenhouse DON, Pearson correlation coefficients were similar for the two correlations involving the SUWWSN lines, averaging 0.63, and for the two correlations involving the cultivars, only averaging 0.34. Spearman correlations gave similar results, and coefficients for both Pearson and Spearman correlations averaged 0.62 across all ten correlations. These results indicate that the new method for conducting greenhouse evaluations predicted field results moderately well and may be useful for quantifying four types of resistance. However, evaluations in inoculated, misted field nurseries can accommodate a large number of lines at less expense per line, and field evaluations across multiple locations and years may be the most efficient way to select resistant lines in breeding programs.

COMPARISON OF DIFFERENT INOCULATION METHODS FOR EVALUATION OF FHB RESISTANCE IN WHEAT VARIETIES

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ABSTRACT

Fusarium head blight (FHB) in wheat is caused by the fungus *Fusarium graminearum*, which infects wheat heads at anthesis. Weather conditions such as humidity and temperature play an important role in the extent of infection. Several mechanisms have been proposed for host resistance, including resistance to the incidence of disease (Type I) and resistance to spread of infection (Type II). Several screening methods have been researched for successful prediction of resistance to FHB infection; the most prominent being spray and point inoculation which measure Type I and Type II resistance respectively.

The objective of this study was to compare the efficiency of the following methods for evaluating resistance to FHB: spray inoculation in the greenhouse followed by bagging, spray inoculation in the field followed by bagging, and traditional field method using grain spawn inoculum. The study includes 16 varieties adapted to Michigan, which includes both soft red and soft white winter wheat lines with varying levels of resistance to FHB. Comparisons are made on % incidence, % severity and Fusarium damaged kernels (FDK).

THE 2009-10 SOUTHERN UNIFORM 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 2009-10 nursery comprised 54 advanced generation breeding lines and four check cultivars, 'Ernie', 'Bess', 'Jamestown' (partially resistant) and 'Coker 9835' (susceptible). Seven 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 one private company, Agripro-Coker, submitted entries. The nursery was distributed to 11 U.S., and one Romanian cooperator for field, and / or greenhouse evaluations. In addition, three USDA-ARS laboratories conducted evaluations for Hessian fly resistance, milling and baking quality and marker genoypes based on established diagnostic markers.

Copies of the full report will be available at the 2010 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

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Table 1. Mean performance of the 58 entries in the 2009-10 Southern Uniform Winter Wheat Scab Nursery evaluated at up to 11 locations for components of FHB resistance.

	Cultivar/ Designation	FHB Incidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	G'hse Severity	RANK	DON	RANK
1	ERNIE	39	1	20	4	9	1	18	12	25	3	16	14	11	6
2	COKER 9835	63	51	57	53	39	53	40	48	55	49	68	54	25	50
3	BESS	43	3	23	8	12	3	8	1	22	1	7	1	13	17
4	JAMESTOWN	47	6	22	5	13	4	14	5	28	9	18	19	11	6
5	LA01164D-94-2	54	31	34	37	23	34	28	33	37	27	23	29	14	21
6	03M1539#031	57	39	31	26	24	38	13	4	31	13	32	37	23	45
7	AR 99054-4-1	52	22	33	34	21	30	24	25	40	37	33	40	27	53
8	ARS03-4736	55	32	32	31	20	29	24	25	35	21	18	18	23	45
9	ARS05-1234	49	9	37	40	24	38	30	38	38	31	17	15	39	57
10	LA01141D-98-6-2	61	48	40	43	30	44	42	49	52	46	21	26	18	39
11	03M1539#019	53	29	32	31	19	23	24	25	37	27	23	30	16	29
12	AR99092-4-1	51	17	27	13	15	9	23	23	32	14	14	10	20	42
13	AR99102-4-1	53	29	40	43	27	42	34	44	47	43	47	47	16	29
14	AR99160-1-1-B	47	6	28	17	14	6	15	8	28	9	10	5	32	55
15	AR99264-8-1	56	35	31	26	21	30	15	8	34	17	18	20	18	39
16	AR99311-12-1	58	42	33	34	23	34	24	25	39	34	20	25	11	6
17	ARGE97-1042-4-5-20	39	1	17	1	10	2	22	18	25	3	15	11	11	6
18	ARGE97-1047-4-2-9	45	4	17	1	13	4	14	5	23	2	9	3	9	2
19	ARGE97-1048-3-6-7	52	22	26	11	16	16	22	18	34	17	23	31	8	1
20	ARS04-1267	49	9	30	22	15	9	17	11	27	6	14	7	16	29
21	ARS05-0005	58	42	48	48	34	49	38	46	52	46	48	48	15	26
22	ARS05-0043	50	13	27	13	19	23	33	43	39	34	18	21	15	26
23	ARS05-0277	64	53	51	49	38	52	28	33	48	44	62	53	21	44
24	ARS07 0095	62	50	45	47	29	43	30	38	46	42	35	41	26	51
25	ARS07-0203	59	45	53	50	35	51	38	46	55	49	40	44	16	29
26	GA031188-O15	69	58	64	55	45	57	58	57	68	57	88	58	24	49
27	GA031188-O16	65	55	64	55	43	54	47	52	63	55	68	55	23	45
28	GA031188-O17	65	55	66	58	44	56	51	53	63	55	77	56	23	45
29	GA041243-LE36	64	53	35	39	25	40	42	49	49	45	50	49	16	29
30	GA041260-Q19	59	45	56	52	31	47	53	55	58	52	59	52	26	51
31	GA041271-PL49	61	48	56	51	34	49	59	58	59	53	39	43	60	58
32	GA041271-Q23	63	51	65	57	47	58	56	56	69	58	78	57	35	56
33	GA041271-Q24	67	57	61	54	43	54	52	54	61	54	57	51	29	54
34	LA01141D-98-6-3	59	45	38	41	30	44	46	51	56	51	39	42	17	37
35	LA02058E63	49	9	34	37	18	18	30	38	38	31	23	28	14	21
36	LA02058E97	58	42	40	43	25	40	32	41	44	39	31	36	17	37
37	LA03130E68	51	17	22	5	15	9	24	25	36	23	14	9	15	26
38	LA03186E2	56	35	39	42	30	44	36	45	52	46	19	24	16	29
39	LA04142C-P5	57	39	31	26	23	34	29	36	44	39	44	46	14	21
40	M08*8005#	52	22	28	17	15	9	12	3	27	6	31	35	11	6
41	MD01W233-07-1	55	32	32	31	23	34	25	30	40	37	25	33	11	6
42	MD02W135-08-9	52	22	31	26	18	18	19	16	34	17	27	34	12	13
43	MD03W61-09-1	50	13	19	3	14	6	16	10	30	11	14	8	13	17
44	MD03W91-09-7	45	4	22	5	15	9	11	2	25	3	9	2	11	6
45	NC07-21036	51	17	30	22	19	23	22	18	37	27	17	17	13	17
46	NC07-23081	50	13	24	10	18	18	22	18	34	17	19	22	12	13
47	NC07-23126	50	13	28	17	15	9	23	23	35	21	41	45	16	29
48	NC07-23771	51	17	33	34	19	23	32	41	38	31	25	32	14	21
49	NC07-24445	55	32	41	46	31	47	26	32	44	39	52	50	13	17
50	VA06W-580	51	17	26	11	16	16	14	5	27	6	33	39	12	13
51	VA07W-569	52	22	30	22	18	18	25	30	37	27	19	23	14	21
52	VA08W-622	56	35	27	13	18	18	18	12	33	15	32	38	10	4
53	VA08W-630	57	39	31	26	22	33	28	33	39	34	10	4	16	29
54	VA08W-653	56	35	28	17	21	30	22	18	36	23	16	13	19	41
55	VA08W-709	48	8	27	13	15	9	18	12	30	11	15	12	12	13
56	VA09W-641	52	22	23	8	14	6	21	17	33	15	13	6	10	4
57	VA09W-654	52	22	28	17	19	23	18	12	36	23	21	27	9	2
58	W1104	49	9	30	22	19	23	29	36	36	23	17	16	20	42
	Mean	54		35		23		28		41		31		18	
	LSD (0.05)	23.9		24		22		25		19		29		15	
	CV%	22.4		35.4		48.6		45.5		23.7		43.2		41.9	

	Cultivar/ Designation	Heading Date	RANK	Plant Height	RANK	Spindle Streak 0-9	Hessian Fly Biotype L	MILLING QUALITY SCORE		BAKING QUALITY SCORE		SOFT. EQUIV. SCORE		Stripe Rust (0-9) F'VILLE (1) AR	Stripe Rust (0-9) F'VILLE (2) AR	Stem Rust % F'VILLE AR
1	ERNIE	127	6	33	20	4.0	0-14	56	D	51	D	60	C	63	15	2
2	COKER 9835	130	41	32	13	5.0	0-15	64	C	65	C	80	B	54	63	0
3	BESS	129	28	35	33	4.5	0-19	66	C	61	C	66	C	1	0	2
4	JAMESTOWN	125	2	31	9	4.5	0-12	62	C	52	D	64	C	0	0	2
5	LA01164D-94-2	129	28	37	50	5.5	0-16	74	B	53	D	49	E	23	43	0
6	03M1539#031	128	11	36	43	5.5	15-4	73	B	88	A	81	A	7	6	70
7	AR 99054-4-1	131	49	39	55	2.5	0-14	67	C	55	D	57	D	1	0	15
8	ARS03-4736	128	11	36	45	2.0	11-5	62	C	25	F	24	F	1	1	7
9	ARS05-1234	132	57	36	41	2.0	0-19	70	C	43	E	38	F	3	57	0
10	LA01141D-98-6-2	129	28	32	11	7.5	0-13	72	B	65	C	68	C	2	0	30
11	03M1539#019	129	28	36	46	6.5	14-0	62	C	75	B	78	B	10	68	2
12	AR99092-4-1	130	41	42	58	5.0	0-16	59	D	61	C	54	D	0	2	2
13	AR99102-4-1	130	41	36	44	3.5	0-18	67	C	50	E	49	E	1	5	0
14	AR99160-1-1-B	131	49	42	57	6.0	0-17	79	B	68	C	43	E	0	0	2
15	AR99264-8-1	130	41	42	56	4.5	0-12	69	C	70	B	68	C	0	0	30
16	AR99311-12-1	128	11	32	10	4.5	0-14	63	C	55	D	64	C	0	0	2
17	ARGE97-1042-4-5-20	128	11	35	34	7.5	0-16	59	D	35	F	23	F	0	0	2
18	ARGE97-1047-4-2-9	126	3	38	51	6.0	0-18	63	C	52	D	19	F	1	0	0
19	ARGE97-1048-3-6-7	127	6	38	53	6.5	0-16	52	D	38	F	44	E	3	1	7
20	ARS04-1267	128	11	33	23	2.0	0-15	62	C	25	F	15	F	0	0	2
21	ARS05-0005	129	28	34	28	4.5	0-14	57	D	44	E	30	F	1	0	2
22	ARS05-0043	128	11	34	27	4.0	0-17	57	D	43	E	31	F	0	0	7
23	ARS05-0277	129	28	32	12	4.5	0-15	64	C	62	C	54	D	1	0	0
24	ARS07 0095	131	49	34	29	3.0	0-14	66	C	64	C	62	C	1	0	2
25	ARS07-0203	131	49	33	16	4.5	0-17	76	B	65	C	60	C	0	0	2
26	GA031188-O15	128	11	36	49	2.5	0-15	76	B	72	B	57	D	1	0	0
27	GA031188-O16	128	11	34	31	3.0	0-14	72	B	64	C	55	D	1	0	0
28	GA031188-O17	129	28	34	24	3.0	0-15	73	B	69	C	56	D	1	0	0
29	GA041243-LE36	128	11	36	40	5.0	16-0	56	D	58	D	55	D	1	0	0
30	GA041260-Q19	129	28	33	17	6.5	0-19	70	B	63	C	63	C	10	0	0
31	GA041271-PL49	136	58	38	52	5.5	0-16	66	C	46	E	65	C	15	11	0
32	GA041271-Q23	131	49	36	48	4.5	0-19	65	C	44	E	57	D	49	29	7
33	GA041271-Q24	131	49	36	47	5.0	0-17	67	C	50	E	55	D	45	36	2
34	LA01141D-98-6-3	128	11	33	22	7.5	0-17	71	B	56	D	59	D	1	0	15
35	LA02058E63	127	6	33	19	5.5	0-17	67	C	34	F	44	E	2	1	0
36	LA02058E97	128	11	35	38	5.5	0-19	69	C	38	F	49	E	17	1	0
37	LA03130E68	124	1	35	36	4.0	0-18	69	C	58	D	51	D	11	0	0
38	LA03186E2	130	41	38	54	3.5	0-17	66	C	54	D	50	E	1	1	50
39	LA04142C-P5	128	11	36	42	4.0	0-15	62	C	51	D	54	D	1	0	2
40	M08*8005#	126	3	34	30	4.0	0-17	69	C	77	B	65	C	2	0	2
41	MD01W233-07-1	131	49	31	8	3.5	0-12	65	C	60	C	61	C	6	1	15
42	MD02W135-08-9	129	28	30	4	2.0	0-14	51	D	49	E	73	B	80	75	7
43	MD03W61-09-1	128	11	34	26	2.5	0-17	55	D	47	E	55	D	8	13	2
44	MD03W91-09-7	127	6	35	37	6.5	0-17	54	D	46	E	46	E	0	0	0
45	NC07-21036	130	41	30	5	5.0	16-0	63	C	56	D	62	C	1	0	7
46	NC07-23081	128	11	33	18	5.0	0-18	53	D	41	E	46	E	21	63	2
47	NC07-23126	129	28	32	15	5.5	0-17	58	D	49	E	56	D	6	5	0
48	NC07-23771	129	28	32	14	6.0	0-18	63	C	59	D	53	D	16	1	0
49	NC07-24445	127	6	31	7	5.0	0-19	62	C	59	D	56	D	1	0	0
50	VA06W-580	128	11	28	2	4.5	0-17	66	C	61	C	59	D	0	2	0
51	VA07W-569	129	28	36	39	5.0	0-16	57	D	51	D	59	D	0	1	30
52	VA08W-622	128	11	34	32	5.0	0-17	70	C	68	C	58	D	10	17	2
53	VA08W-630	129	28	29	3	4.0	0-16	64	C	62	C	68	C	17	19	30
54	VA08W-653	130	41	27	1	6.0	16-0	55	D	59	D	69	C	0	0	30
55	VA08W-709	128	11	34	25	5.0	0-18	62	C	79	B	72	B	5	0	15
56	VA09W-641	126	3	33	21	5.5	0-20	61	C	54	D	60	D	37	24	7
57	VA09W-654	131	49	35	35	2.5	0-15	66	C	51	D	65	C	11	0	30
58	W1104	130	41	31	6	3	0-17	59	D	84	A	65	C	0	0	7
	Mean	129		34		.	.	64		56		55		.	.	54
	LSD (0.05)	3		5		.	.	.		.		.		.	.	13
	CV%	1.1		7.2		.	.	.		.		.		.	.	12

	CULTIVAR/ DESIGNATION	Fhb1	Wuh-1 2DL	Ning 5AS	Ernie 3BSc	Ernie 5AS	H9	H13	1RS tran	Lr34/Yr18	Lr24/Sr24
1	ERNIE	.	.	.	yes	yes	.	.	.	.	.
2	COKER 9835	.	.	.	.	.	.	.	.	.	.
3	BESS	.	.	.	.	.	.	.	.	.	.
4	JAMESTOWN	.	.	.	.	.	.	.	.	.	.
5	LA01164D-94-2	yes	.	.	.	.	.	.	.	.	.
6	03M1539#031	.		.	.	.	yes	.	.	.	.
7	AR 99054-4-1	.	.	.	.	.	.	.	.	.	.
8	ARS03-4736	.	.	.	.	.	.	.	1RS:1AL	.	.
9	ARS05-1234	.	.	.	.	.	.	.	.	.	.
10	LA01141D-98-6-2	.	.	.	.	.	.	.	.	yes	.
11	03M1539#019	.	.	.	.	.	yes	.	1RS:1BL	.	.
12	AR99092-4-1	.	.	.	.	.	.	.	.	.	.
13	AR99102-4-1	.	.	.	.	.	.	.	.	.	.
14	AR99160-1-1-B	.	.	.	yes	.	.	.	.	.	.
15	AR99264-8-1	.	.	.	.	.	.	.	.	.	.
16	AR99311-12-1	.	.	.	.	.	.	.	.	.	.
17	ARGE97-1042-4-5-	.	.	.	.	.	.	.	1RS:1BL	.	.
18	ARGE97-1047-4-2-	het?	.	.	.	.	.	.	1RS:1BL	.	.
19	ARGE97-1048-3-6-	.	yes	.	.	.	.	.	.	.	.
20	ARS04-1267	.	.	.	.	.	.	.	1RS:1AL	.	.
21	ARS05-0005	.	.	.	.	.	.	.	.	.	yes
22	ARS05-0043	.	.	.	.	.	.	.	.	.	yes
23	ARS05-0277	.	.	.	.	.	.	.	1RS:1AL	.	.
24	ARS07 0095	.	.	.	.	het	.	.	1RS:1AL	.	yes
25	ARS07-0203	.	.	.	.	.	.	.	.	.	.
26	GA031188-O15	.	.	.	.	.	.	.	.	.	.
27	GA031188-O16	.	.	.	.	.	.	.	.	.	.
28	GA031188-O17	.	.	.	.	.	.	.	.	.	.
29	GA041243-LE36	.	.	.	.	.	.	yes	.	.	.
30	GA041260-Q19	.	.	.	.	.	.	.	.	.	.
31	GA041271-PL49	.	.	.	.	.	.	.	.	.	.
32	GA041271-Q23	.	.	.	.	.	.	.	.	.	.
33	GA041271-Q24	.	.	.	.	.	.	.	.	.	.
34	LA01141D-98-6-3	.	.	.	.	.	.	.	.	yes	.
35	LA02058E63	yes	yes	.	het?	.	.	.	1RS:1BL	.	.
36	LA02058E97	yes	yes	.	.	.	.	.	1RS:1BL	.	.
37	LA03130E68	.	.	.	.	.	.	.	.	yes	.
38	LA03186E2	.	yes	.	.	.	.	.	.	.	.
39	LA04142C-P5	.	.	.	.	.	.	.	.	.	.
40	M08*8005#	.	.	.	.	.	.	.	.	.	.
41	MD01W233-07-1	.	.	.	.	.	.	.	1RS:1AL	.	yes
42	MD02W135-08-9	.	.	.	.	.	.	.	1RS:1BL, 1RS:1AL	.	.
43	MD03W61-09-1	?	.	.	.	.	.	.	1RS:1BL	.	.
44	MD03W91-09-7	.	.	.	.	.	.	.	1RS:1AL	.	.
45	NC07-21036	.	.	.	.	.	.	.	1RS:1AL	.	yes
46	NC07-23081	.	.	.	.	.	yes	.	1RS:1AL	.	.
47	NC07-23126	.	.	.	.	.	.	.	1RS:1AL	.	yes
48	NC07-23771	.	.	.	.	.	.	.	.	.	.
49	NC07-24445	.	.	.	yes	.	.	.	.	.	.
50	VA06W-580	.	.	.	.	.	.	.	.	.	.
51	VA07W-569	.	.	.	yes?	.	.	.	1RS:1AL	.	.
52	VA08W-622	.	.	.	.	.	.	.	non-1RS	.	.
53	VA08W-630	.	.	.	.	.	.	.	1RS:1AL	.	.
54	VA08W-653	.	.	.	.	.	yes	.		.	.
55	VA08W-709	.	.	.	.	.	.	.	1RS:1BL, 1RS:1AL	.	yes
56	VA09W-641	.	.	.	.	yes	.	.	1RS:1AL	.	.
57	VA09W-654	.	.	.	.	.	.	.	.	.	.
58	W1104	.	.	.	.	yes	.	.	1RS:1BL	.	.

	CULTIVAR/ DESIGNATION	Sr2	Sr36	Lr37/Yr17/Sr28	BVD2/3	Rht-B1b (Rht1)	Rht-D1b (Rht2)	Rht8	Ppd-D1a Insen.	Bx7 OE	Glu-D1	Glu-A1
1	ERNIE	.	het	.	.	yes	.	.	.	.	2+12	Ax1 or null
2	COKER 9835	.	yes	.	.	.	yes	.	yes	.	2+12	Ax2*
3	BESS	.	.	.	.	yes	.	.	het	.	2+12	Ax1 or null
4	JAMESTOWN	.	.	.	.	.	Negative	.	yes	.	2+12	Ax2*
5	LA01164D-94-2	.	het	yes	.	.	yes	.	.	yes	2+12	het
6	03M1539#031	.	.	.	.	yes	het	.	yes	.	2+12	het
7	AR 99054-4-1	.	.	.	.	.	.	.	.	.	2+12	Ax2*
8	ARS03-4736	.	.	.	.	yes	.	.	nd	.	2+12	Ax2*
9	ARS05-1234	.	.	yes	.	yes	.	.	.	.	2+12	Ax1 or null
10	LA01141D-98-6-2	.	.	yes	.	.	yes	.	yes	het	2+12	Ax2*
11	03M1539#019	.	.	yes	.	yes	.	.	.	.	2+12	Ax2*
12	AR99092-4-1	.	.	.	.	.	.	.	yes	.	2+12	Ax2*
13	AR99102-4-1	.	.	.	.	het	.	het	yes	.	5+10	Ax1 or null
14	AR99160-1-1-B	.	.	.	.	.	.	.	.	.	2+12	Ax1 or null
15	AR99264-8-1	.	.	.	.	.	.	.	yes	.	2+12	Ax2*
16	AR99311-12-1	.	.	.	.	.	yes	.	yes	.	2+12	Ax2*
17	ARGE97-1042-4-5-	.	.	.	.	yes	.	.	.	.	2+12	Ax2*
18	ARGE97-1047-4-2-	.	.	.	.	het	.	.	yes	.	het?	Ax2*
19	ARGE97-1048-3-6-	.	.	.	.	yes	.	.	yes	.	2+12	Ax1 or null
20	ARS04-1267	.	.	yes	.	yes	.	.	.	.	5+10	Ax2*
21	ARS05-0005	.	.	.	.	yes	.	.	yes	.	2+12	Ax2*
22	ARS05-0043	.	.	.	.	yes	.	.	yes	.	2+12	Ax1 or null
23	ARS05-0277	.	het	.	.	yes	.	.	.	.	5+10	Ax2*
24	ARS07 0095	.	.	.	.	Unknown	Unknown	.	.	.	5+10	het
25	ARS07-0203	.	yes	yes	.	.	yes	.	yes	.	2+12	Ax1 or null
26	GA031188-O15	.	.	yes	.	.	yes	.	yes	.	2+12	Ax2*
27	GA031188-O16	.	.	yes	.	.	yes	.	yes	.	2+12	Ax2*
28	GA031188-O17	.	.	yes	.	.	yes	.	yes	.	2+12	Ax2*
29	GA041243-LE36	.	.	yes	.	yes	.	.	yes	.	2+12	Ax1 or null
30	GA041260-Q19	.	.	yes	.	.	yes	.	yes	.	2+12	Ax1 or null
31	GA041271-PL49	.	.	yes	.	.	yes	.	.	.	5+10	Ax2*
32	GA041271-Q23	.	.	yes	.	Unknown	Unknown	.	.	.	5+10	Ax2*
33	GA041271-Q24	.	.	yes	.	.	yes	.	.	.	5+10	Ax2*
34	LA01141D-98-6-3	.	.	yes	.	.	yes	.	yes	.	2+12	Ax2*
35	LA02058E63	.	.	yes	.	.	yes	yes	yes	.	het?	Ax1 or null
36	LA02058E97	.	.	yes	.	.	yes	yes	yes	.	het	Ax1 or null
37	LA03130E68	.	yes	.	.	yes	.	.	yes	.	2+12	Ax2*
38	LA03186E2	.	.	.	.	yes	.	.	.	.	2+12	Ax1 or null
39	LA04142C-P5	.	.	.	.	.	.	.	.	.	2+12	Ax2*
40	M08*8005#	.	.	.	.	yes	Unknown	.	yes	.	5+10	Ax2*
41	MD01W233-07-1	.	.	.	.	.	yes	.	.	.	2+12	Ax2*
42	MD02W135-08-9	.	.	.	.	.	yes	.	.	.	2+12	Ax2*
43	MD03W61-09-1	.	.	.	.	.	yes	.	yes	.	2+12	Ax1 or null
44	MD03W91-09-7	.	yes	.	.	.	het	.	yes	.	5+10	Ax2*
45	NC07-21036	.	.	.	.	.	yes	.	.	.	2+12	Ax2*
46	NC07-23081	.	yes	.	.	yes	.	.	yes	.	2+12	Ax2*
47	NC07-23126	.	yes	.	.	yes	.	.	.	.	5+10	Ax2*
48	NC07-23771	.	yes	.	.	.	Unknown	.	.	.	2+12	Ax1 or null
49	NC07-24445	.	yes	.	.	.	yes	yes	yes	.	5+10	Ax1 or null
50	VA06W-580	.	yes	.	.	.	yes	.	yes	.	2+12	Ax2*
51	VA07W-569	.	.	.	.	.	yes	.	.	.	2+12	Ax2*
52	VA08W-622	.	yes	.	.	.	.	.	.	.	2+12	Ax1 or null
53	VA08W-630	.	.	.	.	.	yes	.	.	.	2+12	Ax2*
54	VA08W-653	.	het	.	.	.	yes	.	.	.	2+12	Ax1 or null
55	VA08W-709	.	.	.	.	.	yes	.	.	.	het?	Ax2*
56	VA09W-641	.	.	.	.	.	yes	.	.	.	2+12	het
57	VA09W-654	.	.	.	.	.	.	.	.	.	5+10	Ax1 or null
58	W1104	.	.	.	.	yes	.	.	yes	yes	2+12	Ax2*

APPLICATION OF SINGLE KERNEL NIR TECHNOLOGY FOR EVALUATION OF WHEAT CULTIVARS AND FUNGICIDES FOR FHB MANAGEMENT

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ABSTRACT

DON value of grains and percentage of *Fusarium* damaged kernels (FDK) are often used for the evaluation of cultivars for FHB resistance and also for evaluation of the efficacy of fungicides for control of FHB disease in wheat. The ability of the Single Kernel Near Infra Red (SKNIR) instrument to estimate DON and FDK levels of small bulk samples as a rapid, non-destructive and economical method was tested using 80 wheat samples from a nested trial established to evaluate eight wheat cultivars and five fungicides.

About 50 g of kernels were loaded into SKNIR grain feeder and programmed to estimate single kernel DON levels in 500-kernels per sample. Each sample was sorted into four groups based on estimated DON value and the number of kernels and weight of each group was recorded. The bulk DON level of the 500-kernel sample was computed by using single kernel DON concentration and average weight of kernels in each group. The FDK levels were computed by calculating the number of kernels having DON in 500-kernel sample. The SKNIR estimated bulk DON and FDK values were compared with the visually estimated FDK levels and DON values of representative samples estimated by the standard gas chromatography - electron capture (GC-EC) analytical method.

Compared with the DON values estimated by the standard method, SKNIR estimated the bulk DON level of samples with a mean difference of 4.3 ppm. The DON values of 80% of the samples were estimated within standard DON value ± 5.9 ppm with a mean difference of 2.5 ppm. Likewise, the SKNIR estimated FDK% had a mean difference of 4.9% compared to visual FDK estimates and FDK levels of 80% of the samples were estimated within ± 7.4% with a mean difference of 3.0%.

To assess the sampling error in bulk DON estimation, 3000 kernels from a sample with a DON value of 8.0 ppm was sorted and from this 50 random 500-kernel samples were drawn and DON values were computed. The estimated DON values ranged from 5.4 - 15.8 ppm with a mean of 9.7 ppm and standard deviation of 1.7 ppm. When sampling errors are also taken into account, it seems that SKNIR can estimate the bulk DON value of grain samples fairly well. The SKNIR estimated bulk DON and FDK levels for evaluation of cultivars and fungicide treatments are quite comparable to that of evaluation with visual FDK estimates and standard DON measurements. Ranked single kernel DON values from the SKNIR method also provide additional information on the composition of the bulk DON level and this may be used to assess types of FHB resistance in cultivars. Therefore, the SKNIR technique could be used as a low cost, rapid and nondestructive method for evaluation of cultivars or fungicide treatments for FHB management.

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DEVELOPMENT OF SINGLE KERNEL NIR TECHNOLOGY FOR EVALUATION OF FHB RESISTANCE AND FOR IDENTIFICATION OF REDUCED DON IN HARVESTED WHEAT GRAIN

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ABSTRACT

We have developed a calibration for our Single Kernel Near Infrared (SKNIR) sorter to estimate the DON concentration in single wheat kernels. We used this calibration to estimate DON levels in 500-kernel bulk samples by using estimated DON concentration and weight of single kernels. A preliminary study to evaluate four wheat cultivars with diverse FHB resistance levels using SKNIR DON estimation in 500-kernel bulk samples showed promising results. Using SKNIR, sample DON levels were fairly accurately estimated. The distribution pattern of DON levels in kernel samples among varieties could help geneticists better understand the expression of resistance in varieties. Studies are currently underway to further test this technique, and use it to estimate DON levels to evaluate varieties for resistance and to evaluate the efficacy of fungicides for FHB control in wheat.

We have developed a calibration for the SKNIR to estimate single kernel moisture content, water mass, and weight from samples having sound and *Fusarium* damaged kernels to enable an estimate of certain kernel characteristics on a mass/kernel basis instead of percentage basis. This will be helpful to non-destructively estimate DON and other characteristics of single kernels on a constant moisture basis and to estimate these characteristics in small bulk grain samples more accurately.

We showed that the distribution pattern of DON levels in kernels within artificially inoculated spikes can differ depending on the genotype. Additional experiments are ongoing to estimate DON levels in kernels within artificially inoculated spikes. Use of the SKNIR technique to evaluate disease progression within spikes may help in evaluating for differences in Type II and Type III resistance mechanisms between genotypes.

In a preliminary test, pearl milling and the SKNIR technique was used to evaluate DON levels in kernel bran derived from red and white wheat. Additional tests are planned to develop the SKNIR technique as a method to determine potential differences in the levels of DON accumulation when comparing the bran from near-isogenic red and white seeded lines.

We also plan to study FHB infected kernels and DON using Fourier-transformed mid-infrared spectroscopy and micro-spectroscopy. Such studies are expected to contribute to a better understanding of NIR absorption bands for DON and the distribution of DON within kernels infected with Fusarium.

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MAPPING WHEAT HEAD SCAB RESISTANCE QTL IN MULT757 USING FAMILY-BASED LINKAGE AND ASSOCIATION APPROACH

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ABSTRACT

Breeding for Fusarium head blight (FHB) resistance in wheat is an important objective in breeding programs. Regardless of the number of mapping studies done in the past, progress in finding large effect quantitative trait loci (QTL) is limited. The 3B QTL from 'Sumai 3' is likely the most widely used resistance source in the US. As with other traits in experimental mapping populations, FHB resistance QTL mapping is typically done with specifically designed biparental populations. Some important restrictions associated such an approach include the fact that mapping populations rarely give rise to new cultivars, the time required for population development, and extra steps necessary for validation in multiple genetic backgrounds of interest. Our previous studies have validated the application of using breeding families as mapping populations to solve limitations associated with biparental populations based QTL mapping. The current experiment was conducted to map FHB resistance QTL in wheat genotype *Mult757* (PI 271127) using the family-based linkage and association approach. Eighty-three families of three- or four-way crosses were developed using *Mult757* with 37 susceptible spring wheat genotypes. Previously, validated family-based linkage (pedigree-wide regression and variance component method), as well as association (quantitative transmission disequilibrium), tests were used. A single QTL on chromosome 7B that explained 27 to 32% of total phenotypic variation was identified. This further demonstrates application of family-based approach in plants (does it also demonstrate that a new qtl was found?).

FINE MAPPING OF A REGION ON CHROMOSOME 6H ASSOCIATED WITH DON IN BARLEY

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ABSTRACT

The centromeric region of chromosome 6H has been associated with resistance to Fusarium head blight (FHB), deoxynivalenol (DON) accumulation, kernel discoloration (KD), and grain protein concentration (GPC). Fine mapping this region is important to determine if these QTLs are closely linked or controlled by a single locus and to develop strategies for exploiting genetic resistance. A BC_5 line carrying 17 cM segment encompassing this region, derived from Chevron (resistant with high GPC), in a Lacey (susceptible with moderate GPC) background was used to generate a set of recombinant NILs which were in turn used to fine map this region. To develop the NILs, 1968 F_2 plants generated from a cross between Lacey and the BC_5 line were genotyped with SSR markers and recombinants were advanced to the next generation. Informative recombinants in the F_3 were selected and advanced to produce 269 F_4 plants. We then selected 78 recombinant NILs that represented 20 different recombination classes. These 78 NILs were phenotyped for FHB, DON, KD, and GPC in a total of four field and one greenhouse environments. A fine map for chromosome 6H at this region was constructed using ten simple sequence repeat (SSR) and three single nucleotide polymorphism (SNP) markers. Using these lines we narrowed the region containing the locus associated with DON, KD, and GPC to about 1 cM. We believe this locus is homologous to the *Gpc-B1* locus in wheat which is associated with GPC and senescence. Similar to wheat, we observe that higher GPC is associated with earlier senescence. Our hypothesis is that early senescence shortens the window of time for FHB infection and toxin production by the pathogen resulting in a lower DON.

DEVELOPMENT AND DISTRIBUTION OF MALE-STERILE FACILITATED RECURRENT SELECTION POPULATIONS

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ABSTRACT

Recurrent selection is a breeding procedure with the objective of increasing the frequency of desirable alleles for one or more traits while maintaining a high level of variability in the population. Intermating among selected parents each generation allows recombination to occur thus combining genes from different sources. Male sterility in a self-pollinated species provides a mechanism to easily produce many crosses. Male-sterile recurrent selection in wheat derives its power from recombination of sources of genetic variation for a specific trait and intensity of selection due to large population size that results from many crosses. Progress from selection when recombining genetic sources of FHB resistance is directly related to the amount of genetic variation for the trait in the population and the identification of parents with a high level of expression of the desired trait.

The dominant male-sterile gene was utilized to create recurrent selection populations segregating for FHB resistance because the progenies of the male-sterile plants always segregate 1:1 for sterility and a generation of selfing is not required to obtain true-breeding fertile genotypes. Our objective is to create four populations with FHB resistance adapted to different regions of the eastern U.S.

The male-sterile populations derive from the Idaho Intensive Management Male-Sterile population (PI 573190). They were developed in Wooster, OH beginning in 2006, using elite soft red winter and soft white winter wheat varieties as pollinators. Some were included as sources of FHB resistance and others as sources of adaptation and genes for high yield potential.

Pollinators were planted as mixtures in rows that alternated with rows of male-sterile plants. Seed from the sterile heads are planted, and their sterile offspring are tagged for harvest to repeat the process. Sterile plants are selected; those highly susceptible to FHB are discarded.

In 2009, male-sterile populations were grown in the field at Wooster, OH. From this, four populations were developed in 2009-2010:

1. The early maturity selections from the male-sterile population were planted with pollinator parents for a southern-mid-Atlantic soft red wheat population.

2. Two-thirds of the seed from the mid-maturity selections from the male-sterile population were planted with pollinator parents for an early Midwest soft red wheat population.

3. One-third of the seed from mid-maturity selections from the male-sterile population and some from the late selections were planted with pollinator parents for a late Midwest soft red wheat population.

4. Late maturity selections from the male-sterile population were planted with pollinator parents for a late soft winter wheat population, including white winter wheat genotypes.

In summer 2010, sterile heads were identified and tagged at four different maturity dates. Sterile heads that were very susceptible to *Fusarium graminearium* (Figure 3) were removed on June 14 (early Midwest and mid-Atl.) and June 17 (late Midwest and white). After being harvested and threshed, *Fusarium* damaged kernels were removed by aspiration, removing approximately 50% of the kernels.

A bulk from each population was distributed to cooperating breeding programs in Fall 2010. Cooperating breeding programs have been provided educational materials to assist them in utilizing the populations in their individual breeding programs, continuing cycles of mating and selection for FHB resistance within their target environments.

REPORT ON THE 2009-2010 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERIES

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ABSTRACT

The USWBSI funds the uniform testing of soft winter wheat lines in two tests: the Northern Uniform Winter Wheat Scab Nurseries (NUWWSN and the preliminary NUWWSN (PNUWWSN). In the 2009-2010 season the NUWWSN had 56 entries (plus four checks) from 13 breeders and obtained phenotypic data from 13 locations. The PNUWWSN had 47entries (plus four checks) from nine breeders and obtained phenotypic data from eight locations. The FHB Index valued ranged from 8.1 to 38% in the NUWWSN: 12 entries had an Index value that were not significantly less than the lowest Index value in the test and all 12 had a lower Index value than Truman (the most MR check). The FHB Index valued ranged from 8.0 to 41.5% in the PNUWWSN: 23 entries had an Index value that were not significantly less than the lowest Index value in the test though only two had a lower Index value than Truman. More detailed analysis of the 2009-2010 trials will be presented at the 2010 Forum and a full report will be available on line at http://www.scabusa.org. In addition, we will do analysis of FHB data from past NUWWSN and PNUWWSN to assess trends over time and the prevalence of soft winter wheat lines with good FHB resistance.

GENOMIC SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Resistance to Fusarium Head Blight (FHB) in wheat is a low-heritability trait that is strongly influenced by environment and difficult to evaluate. Although there have been resistance QTL identified in Chinese germplasm that explain up to 30% of the phenotypic variation, there are several native sources of resistance that are currently being mapped and are likely to be controlled by several QTL. Marker-assisted selection (MAS) is an effective approach to pyramiding multiple large-effect genes in a single genotype, however most important agronomic traits are controlled by many genes with effects below levels of statistical significance. The complementary application of MAS for qualitative traits and genomic selection (GS) for quantitative traits, such as FHB resistance, is a promising strategy for increasing the annual rate of genetic gain in breeding programs. This study utilizes data for 252 entries from the 2008-1010 Northern Uniform Winter Wheat Scab Screening Nursery (11-13 locations), Preliminary Northern Uniform Winter Wheat Scab Screening Nursery (8-10 locations), and Uniform Southern FHB Screening Nursery (13 locations) to evaluate GS models for predicting FHB phenotypes. Cross validation of genomic estimated breeding values compared to phenotypic values resulted in correlations ranging from 0.3 to 0.7. Breeding programs are rapidly transitioning to whole genome genotyping and models used to analyze these data will be valuable for selecting FHB resistant genotypes prior to testing. The Northern Cooperative FHB Project has two multi-PI subprojects that will contribute directly to implementing GS in wheat breeding programs. Multi-PI project #4 has as a goal of developing models to implement GS for multiple FHB traits. Multi-PI project #5 has developed male-sterile facilitated recurrent selection populations using FHB resistant germplasm. These projects, combined with the information in this study, set the stage for greatly enhancing the rate of genetic gain from selection for FHB resistance. 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 author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

MILLING AND FLOUR ANALYSIS OF WINTER WHEAT GENOTYPES IN REGIONAL FUSARIUM NURSERIES

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ABSTRACT

We evaluated the milling and flour quality of 117 grain samples from wheat entries in the Northern and Southern Uniform Winter Wheat Scab Nurseries. Samples were provided from uninocculated trials in Warsaw, VA (courtesy of Carl Griffey) for the Southern Nursery and West Lafayette, IN (Courtesy of Herb Ohm) for the Northern Nursery. Data evaluated included grain protein, grain hardness, flour yield, softness equivalent, sucrose solvent retention capacity test and lactic acid solvent retention capacity test. Sequentially selecting for flour yield, softness equivalent and sucrose SRC should identify the best quality genotypes in this study. In the Northern Nursery the best quality genotypes were with better than average ratings for both Fusarium index and FDK were: 03M1539#031, IL06-7550, IL06-14262, and MO071522. In the Southern Nursery, the best quality genotypes (without considering disease ratings) were 03M1539#031, ARS07-0203, GA031188-O15, GA031188-O17, M08*8005#, and VA08W-622. The full report will provide both summaries with disease resistance ratings and soft wheat quality ratings.

COMPARISON OF TWO FUSARIUM HEAD BLIGHT INOCULATION METHODS IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a destructive fungal disease of wheat and barley, causing significant reduction in yield and test weight. Grain may also be contaminated with mycotoxins, posing serious health risks to end users. Developing host plant resistance, a primary control method for FHB, relies on the evaluation of breeding lines using inoculation methods. Various inoculation methods are employed to evaluate wheat breeding lines. The use of an infected grain spawn coupled with irrigation is likely the most common method to screen large numbers of breeding lines. The objective of our study was to evaluate a spray and bag inoculation method by comparing it to a grain spawn mist irrigated method. In the spray and bag method, a macroconidial suspension was used to inoculate a small group of wheat heads in yield plots, and a 1.1L WhirlPak™ bag with six air holes punched in the top of the bag was placed over inoculated heads for 48 h to maintain high relative humidity. If data from the two methods are well correlated, the spray and bag method could be used to evaluate breeding lines for FHB resistance at multiple locations without the establishment of mist irrigation at all sites. These two methods were compared previously in 2008, and results between the two methods were well correlated. In 2010, the spray and bag method was used at two locations in Illinois on a total of 120 lines with two replications. The grain spawn method was evaluated at a single location. Spearman rank order correlation coefficients were obtained using the PROC CORR procedure of SAS 9.2. In 2010, while significant, correlations between the two methods were not as high as observed in 2008. Correlations ranged from 0.22 for incidence to 0.54 for FHB index between the two methods. Of concern were low correlations between locations of the spray and bag method. Correlations with incidence were not significant, and severity (r=.25) and FHB index (r=0.31) were poorly correlated between methods. Incidence was extremely high in one location (X=97.6%) possibly overwhelming genetic resistance and contributing to the weaker correlations between locations. Correlations between the two methods for the most resistant and susceptible lines (determined as the top and bottom 20% of breeding lines for FHB index in the grain spawn method) improved in most cases. Correlation between the two methods for FHB index improved to 0.64. These results indicate that this method is able to determine the most resistant and susceptible lines better than the moderately resistant or moderately susceptible lines. A comparison of breeding lines shared between the top and bottom 10, 20, and 30% of the two methods also supports this conclusion, as half the lines in the top third of each method were shared. Also, only a few lines that were in the bottom third or top third of one method rose or fell to the top or bottom third of the other method. The results of this study indicate the spray and bag method could be used to obtain FHB resistance data in multiple environments. Further work is planned to determine a more suitable inoculum concentration so that genetic resistance is not overwhelmed and a better distinction between resistant and susceptible lines is achieved.

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN A WINTER WHEAT POPULATION

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ABSTRACT

Wheat scab, or Fusarium head blight (FHB), causes devastating losses in wheat worldwide. Host plant resistance is a primary method to reduce the impact of this disease. Obtaining an adequate level of host resistance for this quantitatively inherited trait requires the combination of resistance alleles from different sources. Additionally, to obtain resistance capable of withstanding a disease epidemic it is necessary for a cultivar to possess multiple types of resistance to FHB. Our objective in this study was to identify quantitative trait loci (QTL) for FHB resistance in the soft red winter wheat line IL97-1828. IL97-1828 consistently produces low disease symptoms and low percentage of Fusarium damaged kernels (FDK) under high disease pressure in the field. The resistance within IL97-1828 is considered to be native and independent of widely used resistance sources from Asia and Europe. A population, consisting of 242 recombinant inbred lines, was developed from a cross between IL97-1828 and the FHB susceptible parent Clark. The RIL population was evaluated for disease incidence, severity, FDK percentage, and DON concentration in the field in Urbana, IL in 2009 and 2010, and Wooster, OH in 2010. FHB index and ISK index were also calculated. The population was genotyped using DArT markers by Triticarte Pty. Ltd. and a small set of simple sequence repeat (SSR) markers. A linkage map for the population was constructed using JoinMap 3.0, and composite interval mapping was performed using PLABQTL. Distributions for phenotypic disease measurements were broad and continuous for all measured disease traits. Disease incidence in 2009 was significantly less than that observed for both locations in 2010. Also, FDK percentage was higher in Urbana than in Wooster, OH in 2010. Correlations between environments, while significant, were relatively low ($0.20 < r < 0.54$). Averaged across environments disease measurements were significantly correlated in all cases ($0.31 < r < 0.96$). QTL for resistance to FHB were identified on seven linkage groups that mapped to six different chromosomes (1A, 1B, 1D, 2B, 3B, and 4A). In all cases QTL were minor explaining between 2.9% and 8.7% of the phenotypic variance, respectively. No QTL were detected across all environments, and only three QTL were identified in a single environment. A single region on chromosome 1B was identified for reduction in disease incidence ($0.034 < R^2 < 0.062$). A QTL on 2B was significant for reduction in severity, FHB index, FDK percentage, and ISK index. The QTL on 2B was also identified for DON reduction in both years in Urbana, IL ($0.045 < R^2 < 0.065$). Two QTL on chromosome 3B were not near the 3BS region, and one was likely close to 3BSc and the other near 3BL. A QTL on the long arm of chromosome 3B explained approximately 8% of the phenotypic variance for ISK index at Urbana in 2009. None of these QTL appear to be novel as previous reports have indicated QTL in these regions; however, it is not clear whether they are different alleles at the same loci or different genes in the same region. Our results indicate several regions contributing small effects are important for FHB resistance in IL97-1828.

INTROGRESSION OF TWO MAJOR FHB-RESISTANCE QTLS INTO DURUM AND HARD RED SPRING WHEAT

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ABSTRACT

Identification and deployment of novel sources of resistance to Fusarium head blight (FHB) are crucial in the current effort in fighting against this serious disease in durum and bread wheat. Recently, we identified and mapped a novel major FHB resistance QTL on chromosome arm 5AL and another major QTL on 5AS in the hexaploid wheat accession PI 277012. This accession has been misclassified as cultivated emmer wheat (*Triticum turgidum* subsp. *dicoccum*) in the National Small Grain Collection. PI 277012 is a spring wheat with non-free threshing spikes as well as being morphologically similar to synthetic hexaploid wheat. Thus it is not suitable for direct uses in wheat breeding. The objective of this study was to transfer the two QTLs into durum and hard red spring wheat cultivars adapted to the Northern Great Plains. The accession PI 277012 was crossed with three hard red spring cultivars ('Grandin', 'Reeder', and 'Russ') and three durum cultivars ('Ben', 'Lebsock', and 'Divide'), and the F_1 hybrids were then backcrossed with those cultivars to produce BC_1 seeds. The BC_1F_1 plants were advanced to the BC_1F_4 generation through greenhouse evaluation and selection. The BC_1F_5-derived lines with putative FHB resistance and good agronomic performance were evaluated in field disease nurseries at two locations (Fargo and Langdon, ND). Through this process, a number of hard red spring wheat lines with high levels of FHB resistance and several durum lines with improved levels of FHB resistance were identified. Molecular marker analysis showed that these resistant lines carry at least one of the two major QTLs from PI 277012, indicating that the high level of FHB resistance in PI 277012 can be steadily expressed in different genetic backgrounds.

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OTHER PAPERS

THE U.S. WHEAT AND BARLEY SCAB INITIATIVE'S FHB ALERT SYSTEM

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ABSTRACT

The United States Wheat and Barley Scab Initiative and the curators of the FHB Risk Assessment Tool (based at Penn State University and Kansas State University) have collaborated to provide growers with a live notification system for Fusarium Head Blight (FHB) conditions throughout growing regions in the United States. Users can access maps using the Fusarium Head Blight Risk Assessment Tool (http://www.wheatscab.psu.edu/riskTool_2010.html) to find FHB related field observations and information posted by state specialists in regions of interest. Updates posted to the Fusarium Head Blight Risk Assessment Tool are automatically sent out to the community using various electronic communication methods. These methods include the USWBSI blog (http://scabusa.org/modules/wordpress/), the various subscriber mailing lists hosted by the USWBSI, and a subscription service to receive text messages on cell phones each time new information is posted. The email and/or text message subscription form, as well as additional information about the service, can be found on the USWBSI site at http://scabusa.org/fhb_alert.php.

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2010 FUSARIUM HEAD BLIGHT EPIDEMIC IN OHIO: OUR ROLE IN EXTENSION AND OUTREACH

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ABSTRACT

During the 2010 wheat-growing season, researchers at The Ohio State University continued ongoing efforts to educate growers and others in the wheat industry about the tools and information available to help them assess the risk of a Fusarium head blight (FHB) epidemic in their area of the state and the importance of making informed FHB management decisions. Accomplishing these goals was made easy by the fact that multiple resources are currently in place to provide timely information to our clientele. The available resources include the FHB prediction center, the Crop Observation and Recommendation Network (CORN) Newsletter (a weekly publication by the Ohio State Agronomic Crops Team and Ohio State Extension), and professional relationships with county educators, millers, grain buyers, crop consultants, and local growers. In the early spring, we began promoting use of the FHB prediction center, and prepared a factsheet with detailed information on how to navigate the FHB Risk Assessment Tool and interpret the results. Throughout the anthesis period, we prepared updates for the commentary section of the FHB Risk Assessment Tool and released newsletters that provided interpretations of the risk predictions, information about new tools such as SCAB SMART and SCAB ALERT, and management options and recommendations. The anthesis period lasts approximately 2 to 3 weeks in Ohio where wheat in southern parts of the state reaches this critical growth stage before wheat in northern Ohio. To evaluate the FHB Risk Assessment Tool and provide a quick assessment of FHB levels within selected counties, a field survey was conducted approximately three weeks after anthesis. This coordinated survey has been completed in a fairly uniform manner for the past 9 years. Each year, between 67 and 159 fields were surveyed in 12 to 32 Ohio counties. Within each field, the surveyor (extension educators and graduate students) walked a diagonal through the field and identified ten sites that were approximately 30m apart to assess disease incidence. Each site consists of 0.3m of one row of wheat. Incidence was assessed as the proportion of diseased spikes at each site relative to the total number of spikes examined. In 2010, 145 fields in 32 counties were surveyed and average county incidence ranged from 1.17 to 50.37%. Counties with the highest levels of scab were clustered in the central northwestern part of the state. The FHB Risk Assessment Tool did indicate that these counties were at moderate to high risk for an FHB epidemic during their anthesis periods. The survey results clearly showed that during the 2010 FHB outbreak, the more serious and aggressive managers generally had the best wheat crop. Even in areas where FHB levels were high, some fields with the lowest levels of vomitoxin and highest yields and test weights were those planted with a resistant variety and sprayed with a fungicide application at flowering. Towards the end of the growing season, several newsletters were prepared to provide growers with information on how to harvest and handle grain from FHB-infected fields, sample and test for DON, feed or dispose of contaminated grain, and select moderately resistant varieties for the 2010/2011 growing season.

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