Proceedings of the 2005 National Fusarium Head Blight Forum

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HOST PLANT RESISTANCE AND VARIETY DEVELOPMENT

Chairperson: Paul Murphy

QUANTITATIVE TRAIT LOCI ASSOCIATED WITH REDUCED DEOXYNIVALENOL IN THE SOFT RED WINTER WHEAT 'ERNIE'

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OBJECTIVES

The objectives of this research were to (1) identify QTL associated with low DON in the Missouri Fusarium head blight (FHB) resistant cultivar, Ernie, and (2) determine whether or not they differed from those QTL identified for type II FHB resistance in this cultivar.

INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)] reduces grain yield in wheat (*Triticum aestivum* L.) in many regions of the world. Losses in grain quality can also occur due to contamination with the mycotoxin deoxynivalenol (DON) produced in susceptible wheat varieties. Deoxynivalenol is linked to feed refusal in livestock (Meronuck and Xie, 2000) and causes depression of the immune system, nausea, and vomiting in humans (Prelusky et al., 1992). Genetic resistance is the most cost effective method to reduce quality losses associated with DON contamination in wheat. Breeders believe that selection of lines for low FHB may result in a corresponding reduction of DON in those lines. This association however, has not been well established.

Ernie, a soft red winter wheat developed and released by the University of Missouri, has a moderately high level of type II FHB resistance. In inoculated trials, it also has low DON. Four quantitative trait loci (QTL) associated with type II FHB resistance were identified on chromosomes 2B, 3B, 4B, and 5A using a population of recombinant inbred lines (RIL) derived from cross of Ernie/MO 94-317 (Liu et al., 2005). It is not known, however, whether selection for these QTL in populations derived from Ernie will also result in lower DON levels in resulting genotypes.

MATERIALS AND METHODS

A set of 243 F8 and F9 recombinant inbred lines (RILs) developed from the cross Ernie/MO 94-317 was used for QTL mapping. The experiment was arranged as a randomized complete block design with three replications and grown in the greenhouse in 2002 and 2003. Eight plants/RIL/replication were point inoculated with *F. graminearum*, harvested at maturity, and hand threshed to ensure all diseased kernels were collected. Seed within each replication of each RIL were bulked, ground and analyzed for DON content at Michigan State University. The concentration of DON was quantified using the mycotoxin extraction kit Veratoxin for DON 5/5 (Veratox®). Broad-sense heritability was determined from the analysis of variance as, $h2BS = [ó^2G/(ó^2G + ó^2GxY /Y + ó^2E/RY)]$, where $ó^2G$ is the genetic variance among RILs, $ó^2GxY$ is the variance due to genotype-by-year interaction, $ó^2E$ is the variance due to error, and R and Y are the number of replications, and years, respectively (Nyquist 1991). The minimum number of genes was estimated using Cocherham's (1983) modification of Wright's (1968) formula.

Polymorphisms between Ernie and MO 94-317 were assessed using 64 *Eco*RI/*Mse*I AFLP primer pairs and 420 SSR markers. Both AFLP and SSR polymorphic makers were used to construct the linkage map using Mapmaker, Version 3.0 (Lander et al., 1987) using the Kosambi mapping function. Markers were grouped with a LOD value of 3.0 and distance less than 37 cM. Composite interval mapping was used for the QTL analysis using WINQTLCART (Version 2.0). Permutation tests (Doerge and Churchill, 1996) were used

to determine critical thresholds for significance of each potential QTL at each location. Significance at á=0.05 was determined from the 950th of 1,000 permutations of the data.

RESULTS AND DISCUSSION

Deoxynivalenol data for 2002 and 2003 were continuously distributed but not normally distributed therefore data were log transformed and reanalyzed. Transformed data approximated a normal distribution. Using transformed data, Bartlett's test indicated that the error variances within each year were homogenous (P= 0.05) therefore, data were combined over years and reanalyzed. RILs were considered fixed while years and replications were considered random effects.

Results of the analysis of variance indicated that both genotypic effects (RILs) and genotypic X environment interactions were highly significant (P = 0.0001). Mean DON levels for Ernie (resistant) and MO 94-317 (susceptible) were 4.5 and 89.3 ppm, respectively. Among RILs mean DON levels ranged from 1.5 to 100 ppm.

Broad-sense heritability (68%) indicated that DON levels were genetically controlled and moderately heritable which suggested that lines with low DON levels could be developed in breeding programs. Five genes were determined to condition DON levels in this cross.

A threshold LOD value of 3.5 was used to declare significant QTL. Three QTL were detected which accounted for 32% of the phenotypic variation in DON levels in RILs of this cross. These QTL were located on chromosomes 3B, 4B, and 5A and accounted for 13.2, 6.9, and 11.6% of the phenotypic variation, respectively (Table 1). A fourth QTL on 2B was significant in 2002 (LOD=3.8) but was not significant in 2003 (LOD=1.0) and therefore was not significant (LOD=2.9) in the combined analysis. Based on the sign of additive values, all QTL originated from the resistant parent.

CONCLUSIONS

QTL regions on 3B, 4B, and 5A were consistently associated with low DON in two years of analysis of this trait in the cross Ernie x MO 94-317. All alleles were from the resistant parent Ernie. These QTL were identified in the same regions as those previously identified by Liu et al. (2005) for type II resistance in Ernie. Both the 4B and 5A markers were identical while that on 3B was closely linked to the 3B marker for type II resistance. These data suggest that in Ernie, DON level and type II FHB resistance are not independent, therefore, selection for type II resistance should result in low DON.

ACKNOWLEDGEMENTS

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Table 1. Quantitative trait loci (QTL) associated with reduced DON in recombinant inbred lines of the soft red winter wheat cross Ernie/MO 94-317. Lines were phenotyped for DON levels from experiments conducted in 2002 and 2003. All alleles were from Ernie.

Chromosome location	Marker	QTL peak position	LOD	R^2 (%)	Additive effect
2B	Xgwm276b	120.2	2.9	4.0	-0.088
3B	E8M4_6	123.7	5.3	13.2	-0.142
4B	Xgwm495	0.0	4.7	6.9	-0.102
5A	Xbarc056	44.7	5.0	11.6	-0.133

CIMMYT'S CHALLENGES FOR GLOBAL COMMUNICATION AND GERMPLASM ENHANCEMENT FOR FHB RESISTANCE IN DURUM AND BREAD WHEAT

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OBJECTIVES

CIMMYT's challenge is to identify and validate novel FHB resistance for durum/bread wheat and barley, pyramid the complementary sources of resistance, diversify the resistance gene pool currently utilized by the USWBSI community, and to facilitate the utilization of resistant germplasm through global communication.

INTRODUCTION

Many institutions around the world have devoted substantial resources to combat FHB, and have met a measure of success. However, the global community is facing the threat of imminent epidemics. Unless steps are taken to defeat the disease, this threat will materialize into a much greater problem and as such requires a global response. CIMMYT has adopted a holistic approach to enhance novel FHB resistance among genebank accessions and synthetic wheat derivatives and combine their resistance using systematic screening in multiple environments and genetic characterization by DNA markers. CIMMYT's role in the Global Fusarium Initiative is to provide a platform for international collaboration on Fusarium research, and facilitate information exchange, germplasm enhancement and the development of breeding methods and materials globally. This Global Fusarium Initiative would encourage communication and cooperation among individuals, institutions and governments focusing on this disease. Specific activities will be linked using a web site and on-site forums (http://www.fusarium-net.org). Global G x E meta-data compilation, updated global information, and a global crop information system on FHB data will be a feature of this web site. The Global Fusarium Initiative provides the platform to fight this grave threat which will require all our wisdom and expertise to overcome.

CIMMYT LAUNCHES A NEW CHALLENGE ON FHB RESISTANCE IMPROVEMENT

Evaluation of wheat germplasm for FHB resistance to detect useful genes for various cultivation environments - We aim to acquire potentially novel sources of resistance from global hotspots through our widespread contacts. This will allow us to identify globally stable resistance and will contribute to our understanding of the underlying mechanisms of resistance. Expectations are high that useful resistance genes may be identified during the screening of germplasm accessions and that the effects of Genotype x Environment x Management interactions and the distribution of Fusarium isolates will be better understood. Germplasm will be evaluated first at several FHB hot spot locations in Mexico, and then globally through CIMMYT's International Wheat Improvement Network (including CIMMYT-Turkey). This will ensure that materials are exposed to a range of Fusarium isolates. Validation of newly acquired resistant sources using DNA markers and haplotype evaluation will help identify new resistance genes.

A total of 7,197 spring bread & durum wheat and 2,703 barley entries were evaluated for FHB in the field under natural rainfall and supplementary sprinkler irrigation at CIMMYT's highland research station in Toluca in 2005. In addition, part of the bread wheat material was planted at Patzcuaro in Mexico to

obtain multi environment data. Our FHB shuttle breeding through the exchange of segregating populations with China and Uruguay was also resumed in 2005. A number of F3 segregating bulks have been sent to Wuhan and Nanjing in China for planting in November 2005. These populations combine various scab resistance sources. The same materials have been increased for Uruguay and will be sent in early 2006 for planting in May 2006. Materials selected from these bulks will be selected locally by CIMMYT and local scientists. In some cases, the local/national programs may select a different plant ideotype than CIMMYT scientists for resistance to FHB, levels of the mycotoxin DON, and end use quality attributes. After two or three cycles of selection locally under scab pressure, these materials will be sent to CIMMYT for further development and eventual distribution globally.

We aim to increase FHB resistance using 3 approaches: 1) Acquisition of novel resistance 2) Screening transgressive segregates combining different resistance genes, and 3) Evaluation of advanced, adapted materials with multiple-disease resistance. Figure 1 shows a potential strategy for introgression and pyramiding of genes to enrich FHB resistance in wheat breeding programs. Currently, the effort to combine FHB and Fusarium crown rot (FCR) resistance involves the use of molecular markers to combine the FHB resistance of Sumai 3 with the FCR resistance of a bred wheat line 2.49. A number of derivative materials positive for both markers are currently undergoing field evaluation to test the effectiveness of these combined resistances. In addition, a number of complementary sources of FHB resistance are being combined in crosses. These materials will enter the international shuttle breeding program linking China, Uruguay and Mexico.

CIMMYT efforts and perspective on germplasm enhancement FHB resistance in durum wheat - The most serious challenge in the development of FHB resistant durum wheat is that there is as yet no known effective durum source for resistance. Therefore, it is essential to find novel source of FHB resistance in durum and/or other tetraploid wheat. The resistance sources of these lines would be readily incorporated into elite durum lines in the course of breeding. CIMMYT has adopted a systematic search of the primary gene pool of durum wheat in CIMMYT gene bank containing the largest global collection of wheat and wheat relatives. We are also encouraging global communication to understand the genetic diversity of FHB resistance among primary durum wheat sources with their limit or potential. Establishment of a global platform to facilitate germplasm exchanges and introduction of highly resistant durum wheat germplasm from international programs via CIMMYT's international network will be required. Similarly, we will promote the collaboration with NDSU for diversifying the resistance tetraploid gene pool currently utilized by the USWBSI community to facilitate the utilization of resistant germplasm through the global communication.

An alternative way to diversify FHB resistance of durum wheat is the introduction of resistance factors or QTLs on Dgenome chromosomes. This approach may be more promising than seeking novel sources of resistance, especially if one prefers to avoid the possibility that there are no sources resistance in durum and wild tetraploid wheat because they have been not exposed to FHB in their history. There are several hexaploid and synthetic hexaploid derivatives developed from durum wheat /*Ae. tauschii* crosses which show high FHB resistance. The most resistant hexaploid wheat known and also studied at this moment is Sumai 3 which has the strong resistance QTL on chromosome arm 3BS. Trials to introgress FHB resistance from eight hexaploid wheats crossed with 14 elite durum wheat lines were begun at CIMMYT in the 2004 summer/fall cycle in El Batan. Eight F1 plants produced were top-crossed to six durum elite lines, which were resistant to leaf rust and had good to acceptable quality attributes in the 2005 Obregon nursery, and five of the crosses produced TC1F1 seed. Sixty-five plants of the TC1F1 generation were planted in El Batan and molecularly screened with genotype of DNA markers for the Sumai 3 FHB-QTL region. Twelve plants, representing three crosses, were identified harboring the Sumai 3 FHB-QTL, and back-crossed to the durum elite line used as the recurrent parent in the TC1F1. These lines will be promoted to improve agronomic traits, quality and rust disease resistance in the CIMMYT durum wheat breeding program. Elite candidates of them will be screened for FHB evaluation

after the TC5F1 or BC5F1 generations. Several synthetic wheat of the D genome (genome constitution=AABBDD) showed resistance equal to or higher than that of Sumai 3. These novel sources of resistances were most likely were derived from the D genome. Attempts have been initiated to induce homeologous recombinations between the D genome with A or B genomes using the *ph1* pairing homolog mutant (Figure 2 and 3).

There are two logical reasons for the extreme susceptibility of durum wheat to FHB. One is that durum wheat is inherently lacking in genes conferring resistance. The other is that durum wheat has strong susceptibility factors for FHB (Ban and Watanabe, 2001). Besides systematical screening primary source of durum wheat for resistance, we propose two research strategies for FHB resistance improvement of durum to avoid the risk of only confirming the lack of resistance in the primary gene pool. The first strategy is to find and utilize resistance source in alternative sources in CIMMYT's genebank accessions including durum and other cultivated tetraploid wheats, wild relatives and ancestral species. We have observed that the Type II resistance of *T. monococcum* ranges from 9.4% to 45.7%, and is higher than that of durum wheat. CIMMYT has produced more than 200 lines of synthetic wheat of the A and B genomes (genome constitution=AAAABB and AABBBB), and there are several resistance candidate where the Type II resistance scores are as minimal as 9.5%.

The second strategy is to find and remove of the strong risk factors in durum wheat. The identification of susceptibility factors is important as identification a source of resistance. It is especially true in the case of durum wheat which is quite susceptible to FHB. When the durum-shaped plants in the progenies of Sumai 3/ durum wheat lines are selected, much of the susceptible ideotypes are transmited as well. It may be difficult to produce resistant varieties, even by adding resistance factors, without first eliminating the susceptibility factors. We are making wide crosses to identify FHB risk factors in durum with cytogenetic markers to enhance transgressive segregation for FHB resistance in durum wheat.

Development of Global Fusarium Initiative for collaborative research and FHB holistic operation in CIMMYT – CIMMYT has been conducting a holistic operation to enhance FHB resistance in wheat germplasm through systematic screening in multiple environments. Novel genetic variation is found among CIMMYT's genebank accessions and synthetic wheat derivatives. The FHB research in wheat has been systemized in a simple workflow on four levels: evaluation of resistance in the field (phenotyping); genetic characterization by DNA markers (genotyping); gene discovery; and development of DNA marker assisted selection (MAS) for use in breeding.

FHB is a grave threat that requires an integrated research approach to overcome. CIMMYT intends to develop a global platform for international collaboration on FHB, thereby acting as a facilitator for global information exchange and germplasm enhancement and distribution. We recognize the need to enhance international relationships as a part of each National/International Project/Consortium. CIMMYT will take a more proactive stance to elevate the work of FHB resistance breeding and to raise the profile of this global challenge. New projects recently initiated at CIMMYT could be combined with research efforts elsewhere to focus and organize a worldwide effort to combat the disease.

For this reason, we have developed a Global Fusarium Initiative at CIMMYT which has been supported by the Japanese government since 2004. The challenges and specific activities are based on the new paradigm which arose from the JIRCAS Workshop held in February 2004 in Tsukuba (Ban, 2004). The concept of a Global Fusarium Initiative was proposed and accepted at the 2nd International Symposium on Fusarium Head Blight, incorporating the 8th European Fusarium Seminar, 11-15 December 2004, Florida USA (Van Ginkel and Ban, 2004). A new global collaboration for consensus QTL mapping of FHB resistance in wheat, involving the world's most advanced FHB researchers, will be one of the activities. *Fusarium* fungi, the pathogen of FHB, also causes Fusarium crown rot (FCR) in Australia, Turkey and other places, and is another constraint to global wheat production. We have inte-

grated research and germplasm enhancement for both FHB and FCR under the Global Fusarium Initiative. This initiative will encourage communication and co-operation among individuals, institutions and governments focusing on this disease. We have developed a Global Fusarium Initiative web site with on-site forums (http://www.fusarium-net.org) to facilitate and coordinate our activities in this fight against the dangers of FHB.

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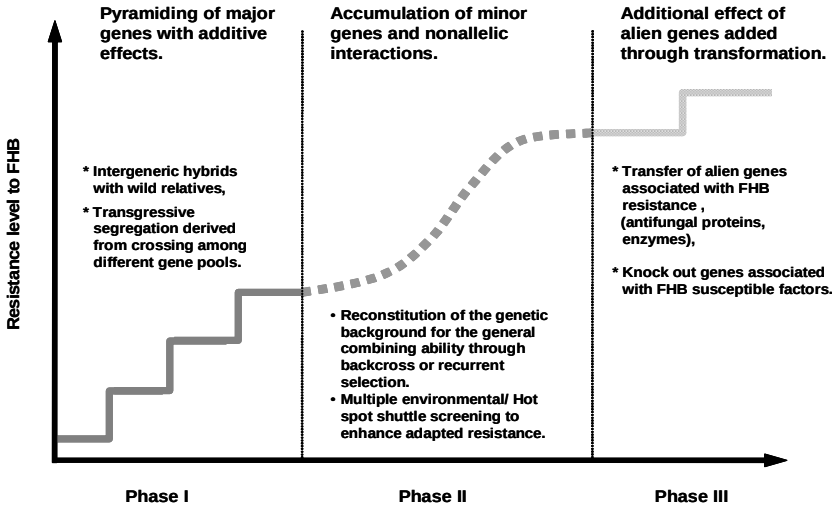


Figure 1. Potential strategy for introgression and pyramiding of genes to enhance FHB resistance in wheat breeding programs.

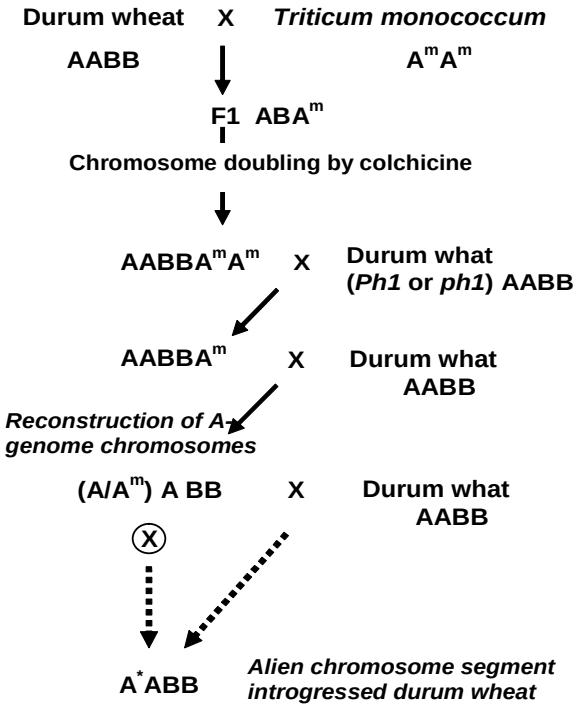
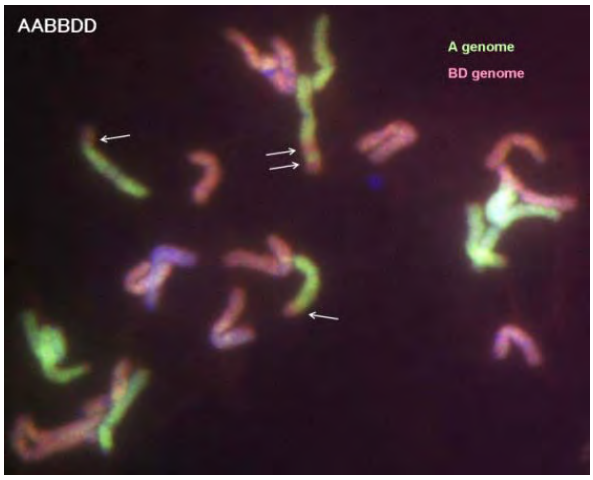


Figure 2. Utilization of A genome ancestor for reconstruction of durum wheat A, B genomes toward FHB improvement.



Superior resistance of FHB can be expected in the D genome of Ae. tauschii. Several synthetic wheat of D genome (durum x Ae. tauschii) in CIMMYT showed resistance equal or higher than that of Sumai 3 (Mujeeb-Kazi and Delgado, 2002). These resistances supposedly reside in D genome and can be transferred into durum by the use of ph1 mutant system.

Figure 3. Translocation of the B and D genome chromosomes into the A genome. The arrows indicate translocation of B or D genome into A genome.

GLOBAL COLLABORATION OF GENETIC STUDIES AND BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a grave threat that we must integrate all our wisdom and expertise to overcome. CIMMYT's role in the **Global Fusarium Initiative** is to provide a platform for international collaboration on *Fusarium* research, and facilitate information exchange, germplasm enhancement and the development of breeding methods and materials globally. This **Global Fusarium Initiative** will encourage communication and cooperation among individuals, institutions and governments focusing on this disease.

The challenges of this initiative are:

- Identification of new sources of resistant germplasm and pre-breeding.
- Delineation of the nature of wheat resistance to FHB and host-pathogen interaction.
- Development of effective cropping systems adjusting pathogen cycle and wheat growth.
- Germplasm sharing and intellectual property rights (IPRs) management.
- Knowledge sharing among the global community .

The specific activities of this initiative are:

- Linking with relevant *Fusarium* initiatives.
- Website and e-News, http://www.fusarium-net.org.
- Global compilation of Genotype x Environment x Management meta-data through new international interactive screening nursery system.
- Up to date global information on FHB epidemics, toxins and resistant breeding.
- Biennial meetings for information sharing and focused discussion.
- Global Crop Information System on FHB.

We aim to acquire potentially novel sources of resistance from global hotspots through our wide-spread contacts. Expectations are high that useful resistance genes may be identified during the screening of germplasm accessions and that the effects of Genotype x Environment x Management interactions and the distribution of *Fusarium* isolates will be better understood. In addition, we are working to develop a compilation/monitoring system for *Fusarium* genetic diversity, pathogenicity, and toxigenicity to further our abilities to control FHB.

IDENTIFYING MARKER-TRAIT ASSOCIATIONS FOR FUSARIUM HEAD BLIGHT USING BREEDING GERMPLASM

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OBJECTIVE

To test the utility of a variance component approach to association mapping in breeding germplasm to identify new and validate existing QTL for Fusarium head blight in barley.

INTRODUCTION

Breeding and genetic research are generally conducted as two separate enterprises. Under the best circumstances they are done in parallel and progress in one informs the other. Integrating gene discovery with breeding has several advantages including using: germplasm most relevant to breeding, large populations over multiple years and environments, and large amounts of breeding data that is not typically used for genetic studies. However, the conflicting procedures used for these two enterprises makes their coordination a challenge. Genetic studies rely on the intensive study of a single carefully chosen population, while breeding research generally allocates resources to study large numbers of progeny from many different populations with limited replication. Association mapping is a general statistical approach to identify genes and marker-trait associations by measuring linkage disequilibrium in populations with complex structure. Genetic studies in animal systems have utilized statistical methods in this area because they are unable to generate large segregating populations as is possible in plant systems (George et al., 2000). Recently, research in plant genetics have begun to use some of these techniques to increase the power of genetic studies (Jannink, 2001).

The USWBSI has provided resources to establish large multi-location Fusarium head blight (FHB) screening nurseries to generate large volumes of FHB disease data. In this study, we use selective genotyping, as described by Wingbermuehle et al. (2004), to identify QTL for FHB using individual breeding populations referred to as Fusarium early generation (FEG) populations. We then use the association mapping approach of George et al. (2000) to identify marker alleles linked to FHB resistance across multiple FEG populations.

MATERIALS AND METHODS

Field evaluations of the FEG lines - The 2004 FEG populations were evaluated at Crookston and St. Paul, MN. The experimental design at each environment was a randomized complete block design with 2 replications. Entries were planted in 1.8 m long single row plots, spaced 30 cm apart. At St. Paul, a macroconidia inoculation technique was used whereas at Crookston a grain-spawn inoculation technique was used (Mesfin et al., 2003). Nurseries were mist irrigated daily to enhance disease. Entries were scored for percent FHB severity by examining 20 random spikes from each plot at Crookston and 10 random spikes from each plot at St. Paul. The number of infected spikelets from each spike were counted and expressed as a percent of the total spikelets present. Heading date was scored as the number of days after planting to 50% emergence from the boot.

Selective genotyping - FEG populations with clear segregation in FHB severity were chosen for further study. The phenotypic extremes for FHB severity, which we refer to as the resistant and susceptible tails of the population, were selected for genotyping analysis. The 12-23 % of the lines with the lowest FHB severity were chosen for the resistant tail. The 11-12% of the lines with the highest FHB severity were chosen for the susceptible tail.

FEG tails t-tests - Forty-eight SSR markers (Beaubien and Smith, 2004; Ramsay et al., 2000; Thiel et al., 2003) were screened on the parents of the chosen FEG populations to test for polymorphisms. For each polymorphic marker in a population, a two-tailed *t*-test comparing the marker allele distribution between the resistant (p_{Res}) and susceptible (p_{Susc}) tails was conducted by calculating:

$$t = \frac{p_{Res} - p_{Susc}}{[p(1-p)/2n_{Res} + p(1-p)/2n_{Susc}]^{1/2}},$$

where n_{Res} is the number of individuals in the resistant tail and n_{Susc} is the number of individuals in the susceptible tail (Bernardo, 2002, p. 294). We used a significance level of $p<0.05$ for our threshold of detection (Snedecor and Cochran, 1980).

Association mapping and Linkage disequilibrium - Associations were detected using a two-stepped variance approach as proposed by George et al. (2000). Genetic variance was estimated using a BLUP approach (Bernardo, 2002, p. 235) (Proc IML, SAS, 2003). Marker-trait associations were detected using a mixed model that accounted for population structure using a coancestry matrix developed from pedigree data. We considered weak associations those with an observed *p*-value between 0.01 and 0.001 and strong associations those with an observed *p*-value of 0.001 or less. Linkage disequilibrium (LD) was calculated as multi-allelic r between markers on the same chromosome (PowerMarker, Liu and Muse, 2005).

RESULTS AND DISCUSSION

Six FEG populations derived from parents representing six sources of FHB resistance (Table 1) were chosen for selective genotyping. Each of these populations showed significant FHB severity segregation as is clear from the population standard deviation and in the difference in mean between the resistant and susceptible tail (Table 1). In some populations both parents had resistance to FHB, but from different resistant sources.

Of the 39 markers that exhibited polymorphism on at least one set of FEG parents, 22 have been evaluated thus far (Table 2). Preliminary LD analysis shows that at distances less than 20 cM, LD is high but it dropped off considerably at distances greater than 20 cM (Figure 1). This indicates that complete genome coverage with gaps less than 20 cM, should be sufficient to detect most marker-trait associations.

Two markers were associated with FHB severity (Table 2). HVM040 [chr. 4(4H)] had a weak association with FHB severity (*p*-value=0.0014, Table 2). HVM040 was also significant based on selective genotyping *t*-tests in three of the four FEG populations for which it was polymorphic (Table 2.). For each of the significant FEG populations, the A allele confers lower FHB severity (data not shown). This allele is traced back to AC Oxbow in the FEG 103 and FEG 112 populations and Atahualpa in the FEG 107 population. HVM054 [chr. 2(2H)] had a strong association with FHB severity (*p*-value=0.0003, Table 2). HVM054 was also significant based on selective genotyping *t*-tests in in all three FEG populations for which it was polymorphic (Table 2), and was associated with a minor FHB QTL in Mesfin et al. (2003). For each of the significant FEG populations, the B allele confers lower FHB severity (data not shown). This allele is traced back to Zhedar1 in the FEG 104 population. It is less clear where the allele originates from in the other FEG populations because the B allele does not match their resistant parent allele source. One possible explanation is that it is expressing the Chevron allele, which was prevalent in our breeding lines even before the FHB program because it is in the pedigree of Peatland. None of the markers were associated with heading date.

In this preliminary study, we were able to detect two FHB marker-trait associations with a minimal set of markers using phenotypic data from breeding trials. We believe that the power of the study will improve as both the number of breeding lines and markers increase. Therefore this approach may serve as an alternative to traditional QTL mapping for FHB QTL and complement existing studies.

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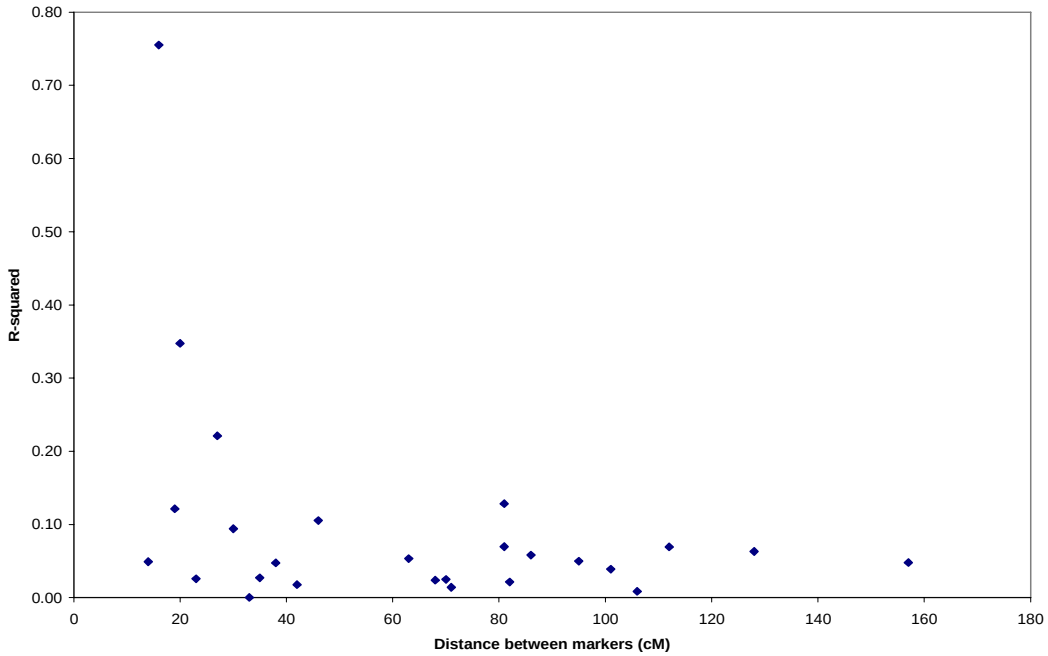

Figure 1. Linkage disequilibrium between markers on the same barley chromosome.

Table 1. FHB severity means for the six selected FEG populations and the resistant and susceptible tails selected from those populations.

FEG Population	Source(s) of resistance	Population Mean (±SD)	Resistant Tail Mean	Susceptible Tail Mean	Parent 1 Mean	Parent 2 Mean
103	AC Oxbow, Atahualpa	9.9±4.6	5.9±2.3	13.0±4.3	5.9	16.3
104	Zhedar1	8.7±4.4	4.4±2.3	15.3±4.5	7.4	16.3
105	Frederickson, PFC88209	7.4±4.5	4.1±2.7	11.8±4.7	5.4	5.6
107	Atahualpa, Frederickson, Harrington	9.9±5.1	6.1±3.2	15.4±4.6	8.2	8.4
112	AC Oxbow, Atahualpa, Zhedar1	10.0±6.4	5.5±4.0	17.0±6.3	6.4	11.0
121	AC Oxbow, Harrington, Zhedar1	5.3±3.4	3.3±1.9	8.5±3.0	3.9	6.1

Table 2. Association analysis and Two-tailed *t*-test results for 22 SSR markers.

		Association analysis *p*-values		Two-tailed *t*-tests by FEG population					
Chr.	Marker	FHB	HD	103	104	105	107	112	121
	EBmac0603	0.5462	0.2312	ns[1]	ns	ns	ns		
1	HVCMA	0.3103	0.8952					ns	
	Bmag0120	0.1868	0.5112			ns	ns	ns	
	Bmac0156	0.6235	0.5637	*p*<0.05		ns	ns	ns	ns
	HVM036	0.1473	0.9225	*p*<0.005				ns	ns
	GBM1052	0.3968	0.91464	*p*<0.025				ns	ns
2	Bmag0140	0.7363	0.0452						ns
	Bmag0125	0.7263	0.8296						ns
	HVM054	**0.0003**	**0.0746**	***p*<0.01**	***p*<0.005**			***p*<0.05**	
	Bmag0749	0.6153	0.0713	ns				ns	
3	Bmag0877	0.1616	0.1185			ns	ns		
	HVM040	**0.0014**	**0.9676**	***p*<0.01**			***p*<0.001**	***p*<0.025**	**ns**
4	EBmac0906	0.683	0.384				ns		
	HVM067	0.0203	0.3791				*p*<0.01		
	HVM043	0.5737	0.634						ns
5	Bmag0718	0.5796	0.4223						ns
	Bmag0579	0.2479	0.9989			ns		ns	*p*<0.05
	Bmag0173	0.2506	0.0239	*p*<0.01	ns	*p*<0.01		ns	
6	Bmac0040	0.112	0.947	ns		ns	ns	*p*<0.05	
	UMB603	0.304	0.6175	ns	ns	ns		ns	ns
7	Bmac0163	0.166	0.7608			ns			
	Bmac0303	0.3464	0.3841			ns	ns		

[1] FEG populations that were polymorphic for a marker but not significant are denoted with "ns." FEG populations that were not polymorphic for a marker have a blank cell.

DETERMINING AND REPORTING THE REACTION OF KANSAS COMMERCIAL WHEAT CULTIVARS TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium Head Blight (FHB) is a serious disease of wheat. The best control for FHB is sowing resistant cultivars. To help Kansas wheat producers select which cultivars to plant, accurate information about their reaction to diseases must be disseminated to them. There are two main Extension publications that are used in Kansas for this purpose. They are *Kansas Performance Tests with Winter Wheat Varieties* and *Wheat Variety Disease and Insect Ratings*. Both are available as hard copy or online (http://kscroptests.agron.ksu.edu/04/04wheat/4w-Disease_Insects.asp and http://www.oznet.ksu.edu/library/plant2/samplers/mf991.asp). The reactions of 28 or 68 cultivars, respectively, to 12 different diseases (including FHB) are reported using a 1-to-9 scale where 1 = highly resistant and 9 = highly susceptible. Forty-seven Kansas winter wheat cultivars have been tested between one and 20 times each in 20 field experiments over a 6-year period. Experimental design for each location/year was a randomized complete block with four replications and plots were single rows, 2.3 m long. Corn grains colonized by *Fusarium graminearum* were applied to the soil surface in three applications about 2 wk apart beginning 4 wk prior to heading (100 g/m^2 total applied). During flowering, plots were sprinkler irrigated (3 min/hr) from 9:00 p.m. until 6:00 a.m. FHB index (% diseased spikelets) was determined for each cultivar between four and six times and averaged. For each experiment/year, index data were transformed to a 1-to-9 scale using linear regression. During the first two years of testing cultivars, an index value of 0% was assigned a Scale Value of "1" and the index value of the susceptible check cultivar with the highest index value in a location/year was assigned a Scale Value of "9." For years three through six, the known Scale Values for reference cultivars within each experiment/year, and their FHB index values, were used to generate the linear model. The models were then used to transform index values for all cultivars in that experiment to Scale Values. A mean Scale Value was calculated for each cultivar (mean of 1-20 experiment/years), rounded to the nearest whole number, and entered in the KSU Extension publications mentioned above. Because both publications are updated every year, Scale Values may be updated as more data become available. Scale Values obtained from these field experiments may be modified based upon observations of cultivars in KSU Extension demonstration plots or producer's fields where FHB naturally occurs. However, no significant disparities have been noted between ratings produced from inoculated nurseries and reactions seen in commercial fields.

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GENE PROFILING STUDIES IN TRICOTHECENE-INFLUENCED BARLEY - *F. GRAMINEARUM* INTERACTION

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ABSTRACT

Fusarium head blight (FHB) of barley is caused by *Fusarium graminearum* (teleomorph *Gibberella zeae*). Trichothecene mycotoxins, produced by the fungus during infection, play a role in virulence. Loss-of-function mutations in the *Tri5* gene, which encodes the first committed enzymatic step in the trichothecene biosynthetic pathway, results in the loss of trichothecene production and reduced virulence. We used the Barley1 and *Fusarium* Affymetrix GeneChips to examine the genetic mechanisms involved in the host and pathogen during trichothecene accumulation. We isolated RNA from spikes of the barley cultivar Morex inoculated with the *Tri5* mutant (non-trichothecene producing), andwild type (trichothecene producing) *Fusarium graminearum* strains and water, and hybridized the RNA to the Barley1 and *Fusarium* GeneChips. Three hundred and thirty seven barley transcripts were identified that were differentially accumulating in wildtype or *Tri5* inoculated plants versus water control inoculated plants. One hundred and twenty three of these 337 barley transcripts, were differentially accumulating in plants inoculated with the wildtype strain versus the plants inoculated with the *Tri5* mutant strain ($P<0.001$), indicating that there are barley genes that are up-regulated specifically during trichothecene accumulation. In the same set of 337 barley transcripts, we also detected 26 that were differentially accumulating in plants inoculated with the *Tri5* mutant strain compared to plants inoculated with the wildtype strain ($P<0.001$), indicating that these barley genes may be down-regulated during trichothecene accumulation. During the same interaction, 603 transcripts were found to be differentially accumulating between the *Tri5* mutant and wildtype *F. graminearum* strains. Seven transcripts showed exclusive accumulation in the *Tri5* mutant *F. graminearum*. Five hundred and ninety three transcripts were up-regulated in wild type compared to the *Tri5* mutant. Three transcripts differentially accumulated in the *Tri5* mutant strain compared to the wildtype strain. Annotations and the functional significance of these differentially accumulating transcripts will be presented.

A COST-EFFECTIVE HIGH THROUGHPUT GENOTYPING METHOD

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ABSTRACT

Although extensive breeding effort has been directed at improving resistance to Fusarium head blight (FHB) in wheat and barley throughout the region, the rate of releasing resistant cultivars is slow. The main challenges lie in the complex inheritance of FHB resistance, and screening of a large number of breeding lines by conventional phenotypic testing. DNA markers have been identified and tagged to a few major resistance genes in both wheat and barley. But widespread application of marker assisted selection in wheat and barley breeding for FHB resistance has been limited up until now. The recent rapid advancement of high throughput platforms and DNA-based diagnostic assay technologies have enabled the Fargo genotyping lab, along with the wheat and barley breeders from Minnesota, to initiate a pilot study to establish a cost-effective working protocol that is amenable to automation. This protocol has been implemented in the breeding programs to enhance wheat and barley breeding efforts in selecting and releasing lines resistant to FHB. A detailed method from sample preparation by the breeders to genotyping data delivery from the Fargo genotyping lab will be presented.

PHENOTYPIC AND GENOTYPIC ANALYSIS OF SCAB RESISTANCE IN SOFT RED WINTER WHEAT GERMPLASM

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ABSTRACT

Effective MAS for FHB resistance depends on knowledge of the genetic relationship of the germplasm to be improved with identified FHB resistance QTLs. This information is lacking for most moderately resistant soft wheat germplasm. In this study, a large set of soft wheat lines and checks were evaluated in inoculated field nurseries at four locations to determine their relative levels of FHB resistance and agronomic performance at each location. The lines were also genotyped at SSR markers from regions of the genome where QTLs for FHB resistance have been identified. These data are being combined with pedigree information to develop a data base on the putative source of FHB resistance alleles in soft wheat germplasm. The analysis will allow breeders to better select parents for crossing in efforts to develop lines having high levels of resistance to scab.

MATERIALS AND METHODS

Breeders from ten states in the eastern US submitted soft wheat lines with moderate to strong resistance from native and/or exotic sources. The 247 lines, including susceptible checks, were grown in screening nurseries at Wooster, OH, Urbana, IL, Lexington, KY and Blacksburg, VA. Data were collected on severity and incidence of disease and a scab index was calculated. Lines were also evaluated in the greenhouse at Lafayette, IN and percent infected florets were recorded.

Genomic DNA was isolated from five plants of each the lines. Two of the plants from which DNA was isolated are being grown in the greenhouse at Raleigh, NC. Seed of these plants will be provided to breeding programs interested in crossing to genotyped individuals. For marker analyses, DNA was also isolated from exotic sources of FHB resistance that were not suited for field evaluation and from lines provided by the University of Missouri not included in the field study. All lines are being genotyped with simple sequence repeat (SSR) markers from regions of the genome where QTLs for resistance have been identified (for review see McCartney et al. 2004).

RESULTS

The mean scab index of lines across locations ranged from 4.1 to 51.7. A large percentage of lines (78%) were classified as having a low rating (<24.3). Twenty-one lines were classified as susceptible (index >32.0) and 36 lines were intermediate or were inconsistent across locations. To date, marker analysis identified the Sumai 3 haplotype at the 3BS QTL region in a small number of lines. These lines had low levels of disease and exotic sources of resistance in the pedigree. Analysis of haplotypes at other QTL regions will be presented.

COMPARISON OF TWO SCAB INOCULATION METHODS IN WHEAT

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ABSTRACT

Fusarium head blight (FHB), or head scab, is a widespread and destructive disease of wheat and barley. Identifying breeding lines with host plant resistance to FHB is an important breeding objective. Many inoculation and evaluation methods are used to identify breeding lines with resistance to FHB. In many cases phenotypic data collected using different inoculation and evaluation methods are poorly correlated. Our objectives in this study were to determine if FHB resistance ratings resulting from two different inoculation methods were highly correlated, and if the same breeding lines with the highest resistance were selected using the two inoculation methods. The two inoculation methods used were a spray-and-bag method using a macroconidial suspension and enhancement of natural infection using infected grain spawn and mist irrigation. Each method was tested in 2005 using a total of 132 lines in three separate experiments. Scab incidence and severity data were collected for both methods, and a FHB index was calculated. The scab data combined with agronomic data were correlated using the PROC CORR procedure of SAS and a significance threshold of a= 0.05. Both scab incidence (r = 0.22) and the FBH index (r = 0.19) were significantly correlated between methods, but with low linear relationships. Analyzing the data using only a subset of the lines with a FHB index in the top 20% and bottom 20% of the grain spawn infected method increased the correlation for both incidence (r = 0.42) and FHB index (r = 0.39). Breeding lines with highest resistance under grain spawn/mist infection did not agree with lines selected as most resistant with the spray and bag infection. For thirty-two (24%) lines the FHB index differed by more than thirty between the two methods. In this study many of the lines selected with one inoculation method would not have been selected with the second method; however, this is preliminary data. It is disconcerting that some of the lines with the highest resistance using one method were not selected using the second method. These results reemphasize the importance of basing selection for FHB resistance on multiple evaluations.

THE ALIEN GENE COULD BE ONE OF THE 'FIGHTERS' AGAINST FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

Fusarium head blight (FHB), caused mainly by the fungus *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schw.) Petch], has been an important disease of wheat worldwide. Epidemics of this disease can result in significant economic losses for wheat growers in terms of yield and quality. Extensive efforts have been made to utilize host resistance to manage this devastating disease. The progress, however, has been limited because of the lack of effective resistance sources and the complex inheritance of the currently identified sources of resistance. This study was initiated to discover novel sources of resistance in the relatives of wheat, an invaluable gene pool for wheat improvement. Fusarium head blight resistance has been identified in a number of relatives of wheat. Resistance in some of the relatives has been transferred to wheat via chromosome manipulation. We have evaluated reaction of 293 lines derived from the crosses of wheat with its relatives to FHB over two greenhouse seasons. Of these 293 derivatives, 66 were susceptible, 153 appeared moderately resistant, and 74 lines exhibited a level of resistance comparable to *Triticum aestivum* cv. Sumai 3, the most widely used source of resistance to FHB. Alien species involved in development of these derivatives include *T. tauschii* (Coss.) Schmal., *Roegneria kamoji* C. Koch, *R. ciliaris* (Trin.) Nevski, *Leymus racemosus* Lam., *Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey, *Th. elongatum* (Host) D.R. Dewey, *Th. junceum* (L.) Love, *Th. intermedium* (Host) Barkworth & D.R. Dewey, *Elymus rectisetus* (Nees) Love et Connor, *Dasypyrum villosa* L., *Secale cereale* L., and oat *(Avena sativa* L.). The wheat-alien species derivatives identified as resistant to FHB include wheat-alien species amphiploids, synthetic hexaploid wheat lines, and wheat-alien species chromosome substitution and translocation lines. These derivatives could serve as novel sources to enhance resistance of wheat to FHB. However, these lines contain varied amounts of alien chromatin in their genomes and cannot be utilized directly in breeding. We have been characterizing chromosome constitutions of these lines using molecular cytogenetic techniques and molecular markers. Meanwhile, we have been eliminating unwanted alien chromatin from their genomes via chromosome manipulation. This will allow for the development of breeder-friendly germplasm lines resistant to FHB and the involvement of alien resistance genes in fighting this destructive disease in wheat.

SEARCHING FOR NOVEL SOURCES OF RESISTANCE TO FHB IN BARLEY

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ABSTRACT

ICARDA has been producing barley with enhanced resistance to Fusarium head blight (FHB) in its program based in Mexico in cooperation with CIMMYT since the early 80's. Its germplasm bank in Syria offers a diverse reservoir of genes that can be explored as possible new sources of resistance for this devastating disease. Barley and wheat were domesticated in the Near East region some 8-10,000 years ago. They have developed different adaptive mechanisms for stress tolerance under farmers' selection. Crop wild relatives represent even richer reservoirs of genes for stress tolerance and adaptation, as their history in the Central and West Asia and North Africa (CWANA) region is much longer and includes periods of a very harsh climate in the Pleistocene Era. The ICARDA/CIMMYT barley breeding program started to research for resistance to FHB, in response to the need of resistance to this disease in the countries of the Andes. In 1986 a total of 5000 barley accessions were screened in Mexico, from those only 23 were found with some level of resistance, which were intensively introgressed into the main program. Resistant sources were shared with programs worldwide, especially after the epidemic outbreaks of the 90's. Collaboration and cooperative research with research groups of advanced research institutions such as the US Wheat & Barley Scab Initiative (USWBSI) is leading the program to make available germplasm sources with enhanced levels of resistance. The environmental conditions present at the Toluca Experiment Station in Mexico are ideal for FHB development and evaluation. Besides Toluca, data available from the US, Canada, China, Ecuador, Brazil and Uruguay were obtained through collaboration with other programs. In recent years the program started a directed comprehensive screening of the gene bank of ICARDA, searching for unique sources of resistance not yet identified by other programs. Preliminary results indicate that new barley sources might be identified at the ICARDA Gene Bank.

HAPLOTYPE SELECTION OF TWO MAJOR QUANTITATIVE TRAIT LOCI FOR IMPROVED FUSARIUM HEAD BLIGHT RESISTANCE IN ELITE WHEAT BACKGROUNDS

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ABSTRACT

This study was conducted to evaluate and validate the presence of two major Fusarium head blight (FHB) resistance QTL on chromosomes 3BS and 5AS in seventy soft red winter (SRW) wheat elite lines. Five haplotypes were characterized among the elite lines on the basis of allelic differences of four marker loci linked to the 3BS QTL and two marker loci linked to the 5AS QTL. Genetic effects of the loci and QTL haplotypes for FHB resistance were analyzed on the basis of disease data collected in both greenhouse (type II resistance) and field experiments (type I and II resistance or field resistance). This study validated the presence of two major QTL on chromosome 3BS and 5AS, and illustrated the utility of SSRs and STS markers in the two QTL regions in selection of FHB resistance in elite wheat backgrounds. Findings of this study also indicate that the 3BS QTL region may be comprised of multiple loci governing FHB resistance. The 3BS QTL1 region, flanked by markers Xbarc133-XSTS142, has a significant effect towards improving both type II and field resistance. The 3BS QTL1 may be unique to Chinese sources. The 3BS QTL2 region, flanked by markers Xgwm493-Xcfd79, has a significant effect towards improving type II resistance but likely has less effect or even a negative effect on field resistance in adapted wheat backgrounds. The 3BS QTL2 is common in both Chinese and native sources. This study confirmed that the 3BS and 5AS QTL have an additive x additive effect towards improving both type II and field resistance. Simultaneous MAS of ideal haplotypes for both QTL likely will be the most effective strategy for improving FHB resistance. The ideal haplotype was comprised of four favorable marker alleles including two (Xbarc133 and XSTS142) on 3BS and two (Xbarc117 and Xbarc56) on 5AS. Selection of desired marker alleles in coupling at each QTL region may be difficult initially if they are derived from different parents, but once combined subsequent selection should be easy and provide a reliable and effective means for incorporating and improving overall FHB resistance in adapted backgrounds. This study also presents and discusses possible strategies for combining FHB resistance with high yield potential through MAS. Elite lines having desirable haplotypes identified in the current study will provide breeding programs with a source of unique and adapted FHB resistant parents and some of the lines also may have potential for release as cultivars.

SCAB SCREENING OF SOFT RED WINTER WHEAT GENOTYPES IN MARYLAND

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ABSTRACT

The 2004/2005 wheat growing season did not present favorable environmental conditions for the development of a scab (*Fusarium graminearum*) epidemic in Western Maryland as in the previous 2 seasons. Seed quality and test weight of wheat were excellent across the mid-Atlantic region. A nursery of soft red winter wheat advanced lines from the Maryland breeding program was grown under field conditions in Salisbury (MD) with misting and artificial inoculation (corn kernel method). The level of scab incidence, severity, percentage of *Fusarium* damaged kernels (FDK), and Deoxynivalenol (DON) were assessed as well as heading date, height and kernel weight. One-hundred and sixty advanced wheat lines were tested in this replicated nursery. The incidence of the disease was fairly uniform across this nursery with significant differences between the susceptible (Coker 9835) and moderately resistant checks (McCormick). There were significant genotypic differences overall for scab incidence, severity, FDK and DON among these genotypes. A small group of advanced lines that included the moderately resistant genotype McCormick showed moderately high levels of resistance to scab with low scab FDK and DON values. On the other hand, there were a large number of genotypes that were very susceptible although variation in the various measures of scab damage was large. The lowest coefficient of variation was observed for incidence (36%) and the largest was observed for FDK (71%). Several lines with low FDK and DON were derived from the cross PION2643/MASSEY*3/ BALKAN//SALUDA. Two of these lines (MV6-82-8 and MV6-82-10) were entered into the Uniform Northern and Southern Scab Nurseries in 2005/2006. These lines do not have any of the chinese or other exotic sources of resistance to scab in their pedigree. It is important, however, to continue to screen adapted advanced lines of soft red winter wheat for even moderate scab resistance within the native soft red winter wheat germplasm. This can be useful for future breeding in combination with other major sources of resistance to scab to reach the goal of developing disease-resistant varieties in the near future that are adapted to the mid-Atlantic region of the USA.

BREEDING EFFORTS TO DEVELOP FUSARIUM HEAD BLIGHT RESISTANT DURUM WHEAT IN NORTH DAKOTA

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ABSTRACT

Durum wheat is one of the major cereal crops in the world and its production in North Dakota accounts for about 75% of the U.S. production. Fusarium head blight (FHB) caused by the fungus *Fusarium graminearum* Schwabe (telomorph *Gibberella zeae* (Schwein.) Petch. has been seriously attacking durum wheat (*Triticum turgidum* L. var. durum) in North Dakota and the surrounding states. FHB has caused a continuous decline in harvested durum acreage and production in ND. Fungicides may reduce the disease but the most environmentally safe and economical way to control the disease is with genetic resistance. Our objectives are in line with the objectives of the US Wheat and Barley Scab Initiative, which are to identify and characterize FHB resistant durum wheat that can be used to develop FHB resistant cultivars/germplasm with good agronomic and quality traits.

To date, we have evaluated a total of 6,000 durum accessions from the world collection at the Academy of Agricultural Sciences, Plant Protection Institute Shanghai, China. None of these accessions were resistant. We are evaluating 1,500 additional accessions at the Department of Plant Protection, Hangzhou, Zhejiang, China. We also have screened material from CIMMYT and ICARDA and identified five Tunisian lines that have a moderate level of Type II resistance to FHB. We are in the process of characterizing the resistance in these lines using segregating pattern and normal distribution for Type II disease severity. We also will be utilizing simple sequence repeat (SSR) markers to identify the FHB QTL in these lines. Two CIMMYT lines have been identified that have 14% Type II disease severity. We have received germplasm from ICARDA for FHB evaluations starting in 2005-06. Our intent is to screen a wide range of durum germplasm until a good source of resistance to FHB is identified.

In previous studies we found that Langdon Triticum dicoccoides 3A substitution line [LDN(DIC-3A)] had a moderate level of Type II resistance. We have developed doubled haploids lines from crossing durum wheat cultivars to the LDN(DIC-3A) line. We have evaluated these lines for Type II resistance using the injection method and the microsatellite marker Xgwm2 and for agronomic and quality traits in preliminary yield trials grown at Prosper and Langdon, ND. Lines that were selected as resistant to FHB did not have acceptable agronomic and quality traits to be released as cultivars. They are being used as parents for second cycle of breeding. Additional lines have been generated by backcrossing this source of resistance to popular durum cultivars ('Ben', 'Lebsock', 'Maire' and 'Plaza'). These lines are now being increased for further evaluation. LDN(DIC-7A) was identified by Drs. James Miller and Robert Stack to have some level of resistance to FHB. We are developing populations by crossing the LDN(DIC-7A) with durum cultivars/experimental lines for breeding purposes.

We have transferred the resistance from the Chinese hexaploid wheat 'Sumai 3' and 'Wangshuibai' to durum wheat. Several populations have been developed from crossing the FHB resistant durum lines with the Sumai 3 and/or Wangshuibai resistance with new ND generations from these populations are being evaluated for Type II resistance using the injection method and the DNA markers Xgwm533, Xgwm493, STS3B-66,

barc133, and barc180. Several lines from these populations will be evaluated as F5:6 lines and subsequent generations for agronomic traits, quality, and disease resistance. Lines that have good level of resistance and possess good agronomic and quality traits will be released as cultivars to the producers. Some of the identified resistant lines will be used as parents in crosses to generate a second cycle of breeding.

ACKNOWLEGDEMENT

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DIGITAL IMAGE ANALYSIS OF PRIMARY LEAF LESIONS ON WHEAT SEEDLINGS OF FRONTANA AND ALSEN INOCULATED WITH *FUSARIUM GRAMINEARUM*

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ABSTRACT

Digital image analyses were conducted on primary leaf lesions of Frontana and Alsen wheat cultivars either 96 or 120 h post-inoculation with macroconidial inoculum of *F. graminearum*. Seedlings were planted in Conetainers in a split-plot design, with cultivars as main-plots and fungal isolates as subplots, and grown to the two-leaf stage in the greenhouse, exactly 14 days post-planting. Primary leaves of seedlings were inoculated separately using five different isolates of *F. graminearum*, obtained from the University of Minnesota Small Grains Pathology Project, St. Paul. Water inoculated primary leaves of seedlings for each cultivar provided control comparisons. Following inoculation, plants were maintained at nearly one hundred percent relative humidity at 23 °C for either 72 or 96 h. Lighting was provided under a 12 h light:dark period while plants were maintained in the incubation chamber. Following incubation, plants were removed to lab benches beneath artificial lighting at temperatures from 21 to 23 °C for another 24 h. Primary leaves were excised at their base near the ligule and placed on a photographic stage. Leaves with lesions were photographed using a high-resolution digital camera. Images were analyzed using the Assess digital image analysis software obtained from the American Phytopathological Society Press. Threshold levels of lesion area were established by setting the hue, saturation, and intensity indices of the program to discriminate lesions of inoculated leaves relative to control leaves to provide differentiation of symptomatic versus healthy appearing leaf area (chlorotic and necrotic tissue relative to healthy green tissue). In preliminary experiments, mean percent lesion area of inoculated leaves of Frontana was 1.9 % and was significantly lower (P=0.05) than for Alsen, which was 4.5 %. No significant differences were observed among isolates (P=0.05) of *F. graminearum* and no significant cultivar by isolate interactions were observed (P=0.05) for percent lesion area assessment. This technique of digital image leaf lesion assessment is being explored to differentiate among susceptible and resistant leaf reactions of segregating wheat populations in a rapid screening of reaction to early infection of wheat.

A RECIPROCAL BACKCROSS MONOSOMIC ANALYSIS OF THE FHB RESISTANT WHEAT CULTIVAR 'FRONTANA'

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ABSTRACT

Fusarium head blight (FHB) caused by the fungal organism *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.)], is a disease of wheat and other small grains which frequently causes a significant reduction in grain yield and quality. Wheat breeders are interested in incorporating alternative sources of resistance into new genotypes as well as identifying genes which function differently from inhibiting fungal spread after infection. The spring wheat cultivar 'Frontana' has been described as exhibiting a mode of FHB resistance which might limit initial infection by the pathogen or degrade the fungal toxin after initial infection. A study was initiated to identify what chromosomes were involved in determining the FHB resistance of Frontana. Frontana was hybridized to a set of 'Chris' spring wheat monosomics, and a backcross reciprocal crossing procedure was followed to produce two sets of disomic lines, one set with critical chromosomes originating from FHB resistant Frontana and the other with critical chromosomes originating from FHB susceptible Chris. The parental genotypes; resistant and susceptible controls; and disomic lines were grown in a RCBD with three replications in two separate greenhouse experiments (GH experiment-1 and GH experiment-2). Disomic lines for Frontana critical chromosomes 3B and 7D were not produced and so were not available for testing. Plants were spray-inoculated with a single *Fusarium* isolate at a concentration of 25,000 spores ml^{-1}. In GH experiment-2, percent disease severity ratings of disomic lines were made one, two, and three weeks after inoculation. After harvesting seed from both experiments, disomic plants were evaluated for resistance by counting tombstone kernels, weighing seed, and analyzing seed samples for deoxynivalinol (DON) content. In GH experiment-1, disomic lines with Frontana chromosomes 2D, 5A, 6A, and 7A had significantly lower percent tombstone kernels, kernels g^{-1} seed, and DON content compared with other disomic Frontana lines; however, all four lines had higher mean values than the Frontana control. In GH experiment-2, disomic lines with Frontana chromosomes 6A and 7A again exhibited significantly lower values for percent tombstone kernels and DON content compared with nearly all of the other disomic Frontana lines as well as the Frontana parent. In GH experiment-2, percent severity ratings for disomic Frontana lines 6A and 7A were similar to the 'Alsen' control at all three weeks and significantly less than the Frontana parent at all three weeks. Results indicate that genes for FHB resistance in Frontana are likely carried on chromosomes 6A and 7A.

EVALUATION OF WHEAT LINES NEAR-ISOGENIC FOR DIVERSE FUSARIUM HEAD BLIGHT RESISTANCE QTLS

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ABSTRACT

The hard red spring wheat (HRSW) production region has suffered large economic losses during the past decade due to Fusarium head blight (FHB) epidemics. To enrich HRSW with novel FHB resistance QTLs, a program to introgress FHB resistance QTL from diverse wheat genotypes and wheat relatives into HRSW in a systematic fashion has been initiated. The project is employing four generations of marker-assisted backcrossing to create HRSW lines near-isogenic for different FHB resistance QTLs (QTL-NILs). These QTL-NILs are being developed in three FHB susceptible HRSW backgrounds (Norm, Wheaton, Apogee). Apogee is a rapid cycling dwarf wheat that allows for the development of unique resources for FHB research. In 2005, FHB resistance evaluations were conducted on the first set of completed QTL-NILs. This included QTL-NIL series in both Norm and Apogee that have either a marker for a FHB resistance QTL on Freedom (a soft red winter wheat) chromosome 2A, or for individual QTLs from Sumai 3 (*Qfhs.ndsu-3BS, Qfhs.ifa-5A*) that are serving as checks. Greenhouse FHB evaluations involved point inoculations to assess the spread of disease symptoms in the spike (type II resistance). Twenty five plants (5 pots with 5 plants per pot) per QTL-NIL were evaluated. Disease spread in Norm was 10.0. In the best Norm-*Qfhs.ndsu-3BS* NIL, disease spread was 4.7, while disease spread was limited to just 4.2 in the best Norm-Freedom 2A NIL. In the Apogee QTL-NIL series, the best *Qfhs.ndsu-3BS* line exhibited a disease spread of 3.4, compared to a disease spread of 9.0 in Apogee. Disease spread in the best Apogee-Freedom 2A NIL was limited to 5.1 spikelets. A replicated FHB field evaluation of the first set of NILs was also conducted in 2005. In the Norm QTL-NIL series, the best *Qfhs.ndsu-3BS* and Freedom 2A NILs exhibited FHB severities of 14.5 and 13.9, respectively. In contrast, Norm exhibited a FHB severity of 27.6. The best Norm-*Qfhs.ifa-5A* NIL exhibited a FHB severity of 18.9. Similarly, Apogee exhibited a FHB severity of 30.2, while the best Apogee-*Qfhs.ndsu-3BS* and Apogee-Freedom 2A NILs exhibited significantly lower FHB severities (10.7 and 11.3, respectively). The best Apogee-*Qfhs.ifa-5A* NIL exhibited a FHB severity of 22.4. These results suggest both that *Qfhs.ndsu-3BS* and the Freedom 2A QTL have each been introgressed into NILs in both Norm and Apogee, and that the Freedom 2A QTL may confer a significant level of FHB resistance to HRSW. Results for *Qfhs-ifa-5A* are more equivocal. Experiments will be repeated to confirm this first round of greenhouse and field evaluations. We are now completing the introgression of two additional QTLs, *Qfhs.ndsu-3AS* from *Triticum dicoccoides*, and a QTL from Frontana located on chromosome 3A. These QTL-NIL series provide prebreeding resources for breeding programs, as well as also genetic stocks for 1) quantifying gene pyramiding, 2) examining the molecular basis of diverse, and 3) exploring biological differences between resistance to initial infection (type I) and type II resistance.

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HIGH RESOLUTION PROFILING OF WHEAT GENES DIFFERENTIALLY EXPRESSED IN RESPONSE TO *FUSARIUM GRAMINEARUM* INFECTION

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ABSTRACT

Fusarium head blight (FHB) of wheat caused by *Fusarium graminearum* (Schw.) affects wheat production worldwide reducing yield and quality. Genome-wide expression profiling of genes altered during host-pathogen interactions may improve our understanding of the mechanism(s) underlying resistance to FHB. A cDNA biochip representing 5664 ESTs, derived from a suppression subtractive hybridization library of wheat-*F. graminearum* interactions, was used for tissue-specific profiling of differentially-expressed genes in response to *F. graminearum* infection. The 93FHB37 wheat line carrying three major resistance QTLs mapped to chromosomes 3BS, 6BS and 5AL was used for the study. Inoculated wheat spikes were dissected into tissues: glume, lemma, palea, ovary, anther and rachis. The monitoring of genes in specific tissues avoided the averaging of expression data that occurs when using an entire spike as a biological sample. Hybridizations were completed using 30 arrays including 5 independent hybridizations for each tissue. Significant analysis of microarrays (SAM) resulted in the identification of transcripts encoding defense and stress related proteins, components of the ethylene and the phenylpropanoid pathways and a member of WRKY transcription factor family. Analysis of variance revealed that about 37% of genes responding to *F. graminearum* showed a significantly different expression pattern among separate floral tissues.

IDENTIFICATION AND INCORPORATION OF FHB RESISTANCE IN WINTER WHEAT: AN OVERVIEW OF PROGRESS AND STRATEGIES

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Since 1990, Fusarium Head Blight (FHB) epidemics have occurred in many of the eastern and Great Plains winter wheat production regions of the U.S. where much of the wheat crop is planted directly into maize residue. Subsequently, severe FHB epidemics resulting in significant losses in grain yield and quality have occurred on an annual basis in one or more of these wheat production regions. Prior to 1990, few winter wheat breeding programs considered it necessary or placed a significant amount of emphasis on the identification and development of cultivars having resistance to FHB. Initially uncertainty prevailed as to whether cultivars having significant resistance to FHB could be derived from existing breeding populations in which parental lines had not been selected on the basis FHB resistance.

PROGRESS AND EFFECTIVENESS OF FHB BREEDING EFFORTS

Much of the information included in this paper and the oral presentation was compiled from responses to a questionnaire sent to colleagues representing 14 public and four private winter wheat breeding programs working on FHB resistance and variety development in the U.S. When asked whether current breeding methods, available technologies, and strategies deployed have been effective in development of FHB resistant cultivars, the responses varied from very effective to not exceedingly effective. A majority of breeding programs indicated that significant progress has been made in development of cultivars having moderate and in a few cases high levels of FHB resistance derived from adapted native sources and/or that FHB resistance has been successfully transferred from exotic sources into more adapted backgrounds. While competitive cultivars having FHB resistance derived from native sources have and will continue to be developed, the FHB resistance level in most but not all of these likely will be inadequate under severe epidemics. To a certain extent the same is true for cultivars having only Type II resistance derived from exotic sources, wherein development of competitive cultivars that are highly resistant to FHB and possess other traits of critical importance has proven more difficult to achieve. However, as breeding programs begin to use more adapted and improved parental lines, having diverse combinations of Type II and other types of resistance derived from exotic sources and adapted lines having native resistance, the number of competitive FHB resistant cultivars will increase dramatically.

PRIMARY FACTORS HINDERING EFFECTIVENESS OF FHB BREEDING

Difficulty in obtaining uniform FHB epidemics of desired intensity and phenotypic data that is consistent and reliable from inoculated and mist-irrigated field nurseries is problematic due to lack of control over environmental conditions, particularly temperature. This is further confounded by the effects of significant genotype by environment interaction and differences in plant structure, height and maturity on FHB phenotypic data, and lack of consistent correlation between FHB incidence, severity and FDK with DON concentration. While significant progress has been made in identifying, mapping, incorporating and enhancing FHB resistance in winter wheat, there has been less success to date in combining high levels of FHB resistance with other traits of critical importance that are ultimately required in successful and competitive cultivars. Deriving lines that are high yielding as well as highly resistant to FHB as well as maintenance of genetic gains in yield that are at least similar to current levels are problematic. However, as one respondent clearly stated "success likely will come through the deployment of a

long term recurrent selection strategy," which is relevant since progress achieved to date in wheat breeding programs has resulted ultimately from long term recurrent selection in which favorable alleles and linkage groups have been established and maintained primarily via phenotypic selection. So the difficulty encountered in initial breeding efforts, which can be considered as pre-breeding, to incorporate high levels of FHB resistance, derived from exotic or non-adapted parental sources, into high yielding wheat backgrounds is not surprising or unexpected. Likewise restoring favorable linkage groups for other quantitative traits, such as adaptation, milling and baking quality and the numerous assortments of major and minor gene loci governing horizontal and general background resistance to a vast array of biotic and abiotic stresses, likely will require several breeding cycles to achieve, which generally is limited to two cycles per year in winter wheat breeding programs. Progress has further been restricted by limited knowledge of the inheritance and diversity of genes conferring FHB resistance among native sources versus known FHB QTL, and lack of molecular markers to apply MAS in populations comprised of native sources of FHB resistance. In addition to the aforementioned problems associated with phenotypic selection, it requires extensive time, resources, and highly-trained personnel to implement. While it is generally agreed upon that high-throughput MAS has the potentially to greatly enhance the effectiveness of breeding for FHB resistance, the capacity to implement this on a broad scale has still not been optimized. There also is need for more PCR-based selectable molecular markers that are reliable, predictive and broadly applicable, such as "gene based" markers. There is need for validation of putative novel FHB resistance QTL in diverse genetic backgrounds and to develop selectable markers for these with emphasis on complementary types of FHB resistance, particularly tolerance to toxin accumulation. There is need to document whether pyramiding diverse QTL conferring primarily Type II resistance will act in a complementary manner and result in an increase in the overall level of FHB resistance as well as providing genetic diversity.

FHB RESISTANCE IDENTIFIED IN NATIVE SOURCES

Upon evaluation of existing adapted winter wheat lines and cultivars in FHB nurseries, several were documented as having moderate levels of FHB resistance, and subsequently referred to as "native" resistance or native sources. The SRW wheat cultivar Freedom, released by Ohio State University in 1991, was among the first released winter wheat cultivars identified with native resistance, and its FHB resistance was subsequently mapped to chromosome 2AS. Shortly thereafter the SRW wheat cultivars COKER 9474 and Ernie, both released in 1994, were identified as having moderately high levels of native FHB resistance. FHB resistance in Ernie was subsequently mapped and reported to be conferred by QTL on chromosomes 2B, 3B, 4B, and 5A. Like many native FHB resistance sources, COKER 9474 has the same allele as Chinese sources for the 3BS QTL marker Xgwm 493. An even higher level of native FHB resistance was identified in the cultivar Truman, released by the University of Missouri in 2003. Since 1994, more than 15 SRW and several HRW wheat cultivars having native FHB resistance have been released and include Foster (1996), Patton, Roane, Hondo and Heyne (1998), Wesley and Goldfield (1999), McCormick and Tribute (2002), Neuse, Truman, INW0304 and IL94-1653 (2003), Cecil and INW0411 (2004), and Bess, NY88046-8138, COKER 9511, and WestBred X00-1079 (2005). Most of these native sources have Type II field resistance, and several also have resistance conferring low FHB incidence (Type I), and reduction in FDK and DON. Winter wheat lines having native FHB resistance and being considered for release during the next three years include eight lines in 2006 (developed by breeding programs in Georgia, Illinois, Indiana, Nebraska, Ohio, and New York), two lines in 2007 (from Ohio and Missouri), and five lines in 2008 (from Kentucky and Ohio). It is apparent that native FHB resistance currently comprises and will continue to provide a base level of FHB resistance in winter wheat cultivars. FHB resistance in only a few native sources has been genetically character-

ized or mapped, and this remains a critical priority if genes in these potentially novel sources of resistance are to be effectively used, selected for and combined with genes from other unique native and exotic sources in cultivar development programs.

INCORPORATION OF FHB QTL FROM ASIAN AND EUROPEAN SOURCES

In an endeavor to incorporate novel FHB resistance and/or to enhance current resistance derived from native sources, many programs initiated efforts using a vast array of breeding methods to incorporate Type II FHB resistance, derived predominantly from a seemingly diverse array of Asian and other sources, into adapted winter wheat backgrounds. Subsequent emphasis has been placed on identifying diverse sources of Type II resistance as well as other unique types of resistance and their incorporation and combination in elite wheat lines. Of the QTL reported for FHB resistance, those located on chromosomes 1B, 2AS, 2B, 2D, 3A, 3BS, 4BL, 5AS, and 7B have been postulated as conferring resistance among current winter wheat cultivars and advanced elite lines. Winter wheat varieties having FHB resistance derived directly from Asian (3BS and 5AS) and/or European (1B and 3A) sources or from diverse combinations of these with native sources includes the cultivars 25R18 (released in 1999), 25R42 (2001), 25R35 and 25R54 (2003), INW0412 (2004) and 25R51 (2005). In addition, six elite wheat lines having FHB resistance derived primarily or partially from exotic sources are being considered for release within the next three years by breeding programs at Purdue, Cornell and Virginia Tech. To date, notably fewer cultivars having FHB resistance derived from exotic sources have been released in comparison to cultivars having native resistance. This is due in part to the time and resources required to incorporate FHB resistance from exotic sources into adapted winter wheat backgrounds, lack of precise and broadly applicable high-throughput PCR-based markers for all known FHB QTL, and difficulties encountered in eliminating undesirable traits and in restoring favorable linkage groups for adaptation, pest resistance, grain yield, and quality.

BACKCROSSING TO INCORPORATE FHB RESISTANCE

Initial backcrossing efforts relying on phenotypic selection for Type II resistance via point inoculation under greenhouse conditions generally were effective in transferring FHB resistance conferred by genes having major effects on reduction of disease spread, and were further expedited upon the availability and use of molecular markers linked to known FHB QTL such as the one on chromosome 3BS. While many of the FHB resistant donor parents were subsequently characterized as having additional QTL (e.g. 5AS, 5DL) conferring significant but lower levels of Type II resistance and/or other types of FHB resistance, many of these QTL were not retained in backcross progeny selected solely on the basis of Type II phenotypic reaction. Subsequent identification and validation of these and other novel QTL and availability of predictive molecular markers to deploy in MAS has and will continue to greatly enhance the ability of breeding programs to further enhance the level of FHB resistance and to reduce linkage drag in future cultivars. In collaboration with the USDA-ARS Genotyping Centers, several programs have developed regional MAS backcrossing populations to rapidly incorporate two or more known FHB resistance QTL into adapted winter wheat backgrounds.

DOUBLE-HAPLOID FHB BREEDING EFFORTS

Doubled haploid breeding was initiated by several programs to accelerate the transfer of FHB resistance into adapted wheat backgrounds. While FHB QTL such as 3BS and 5AS were incorporated into winter wheat backgrounds, most lines lacked other traits of critical importance required for cultivar release, such as resistance to other prevalent diseases and yield potential and, therefore, are best suited for use only as improved FHB resistant parental lines. Most breeding programs have discontinued development of doubled haploid lines as part of their routine cultivar development efforts, as this method requires extensive time and resources to implement and large populations are

required to identify desirable progeny, particularly when non-adapted parents are involved.

BREEDING METHODS AND STRATEGIES DEPLOYED IN DEVELOPING FHB RESISTANT CULTIVARS

In more than half of the winter wheat breeding programs surveyed, parental selection, line screening and selection in all populations includes emphasis on FHB resistance. Breeding programs in regions less prone to FHB epidemics generally develop separate crosses targeting FHB resistance, which are either advanced with their traditional populations to the pure line stage or are advanced separately with selection for FHB resistance. Several programs have independent breeding projects focused specifically on development of FHB resistant cultivars and a few programs also have independent parent building programs. Nearly 85% of the winter wheat breeding programs predominantly use a bulk or modified bulk breeding method with mass selection, while a few of these programs subsequently implement pedigree selection for FHB resistance in target populations. Two breeding programs use a pedigree method predominantly with selection for FHB resistance applied in some or all generations of inbreeding. Most programs begin selection for FHB resistance the first year after deriving pure lines ($F_4 - F_6$) and concurrently with first year yield testing. While most of these programs advance segregating populations to the pure line state without artificially exposing them to FHB epidemics, a few programs advance all or selected target populations in mist-irrigated and/or inoculated (natural or artificial) nurseries and either select and bulk resistant individuals or advance selected progeny using the pedigree method. Several programs using either the pedigree method or applying mass selection for FHB resistance during bulk population advancement select for plump seed via visual selection, sieving or using a gravity table. All programs conduct routine FHB screening of breeding materials, comprised predominantly of pure lines, entries in uniform FHB nurseries and official variety trials, parental lines, and select populations and/or early generation progeny, in 1 to 3 inoculated and mist-irrigated field nurseries. Half of the programs spray FHB field nurseries with conidial spore suspensions and the other half spread *Fusarium* colonized grain, primarily maize, as the primary inoculum source. Programs having FHB nurseries at multiple sites often plant these into maize stubble and rely on natural inoculum and/or epidemics. One program also evaluates Type II resistance in a field nursery using single floret inoculation and another program evaluates FHB resistance of lines via bagging spikes sprayed with a conidial spore suspension. All programs assess incidence, severity, and index in FHB field nurseries, and most assess at least their advanced lines evaluated in these nurseries for percentage of Fusarium Damaged Kernels (FDK), and DON toxin concentration. More than 70% of the programs routinely evaluate FHB resistance of parents, germplasm, advanced lines, and entries in uniform FHB nurseries in greenhouse tests, primarily for Type II resistance using single floret inoculation although a few programs also or only assess Type I resistance using a spike spray-inoculation method. One program also assesses Type II resistance of breeding populations in two greenhouse cycles each year. Several winter wheat breeding programs now conduct routine "in house" MAS or haplotyping of FHB resistance in a diverse assortment of materials and generations, including various stages of backcross progeny development, selection among F_1's derived from 3-way crosses, selection in early generations (F_2 and $F_{3)}$, selection and haplotyping of pure lines (F_4-F_7), and selection throughout the breeding process. A few programs lacking lab support for conducting MAS collaborate with the Genotyping Centers in this endeavor. While most programs applying MAS are routinely using markers for the 3BS QTL and many programs recently have begun using markers for the 5AS and other QTL, availability and use of markers for other validated unique FHB QTL are needed to accelerate progress. Additional information also is needed on haplotypes of FHB resistant parental sources, exotic and native, and for current and new FHB resistant wheat lines to further enhance breeding efforts. Capacity of breeding programs and Genotyping Centers to implement routine MAS on a large scale likely will impact the rate of future success.

STRESS-DIRECTED SELECTION IDENTIFIES LINES OF SPRING WHEAT WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT AND OTHER DISEASES

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ABSTRACT

A protocol of 'Stress-Directed Selection' (SDS, an iterative approach in which populations are subjected to repeated rounds of multiple disease pressures and combined resistances selected) identified spring wheat populations with improved FHB resistance. This approach has already succeeded in identifying lines with superior FHB resistance when one parent of the cross was a known FHB-resistance source. This work examines the application of SDS to populations derived from crosses of elite, FHB-susceptible parents. BC_1F_n progeny of the cross 'Superb'*[2]/ 'CO960293' (spring wheat , winter wheat, respectively) were subjected to SDS and selected under FHB pressure in alternating generations after BC_1 F_4. A small proportion of the lines showed unexpectedly good FHB resistance which was confirmed under controlled conditions. The progeny of these lines were subjected to SDS and about half the population was clearly FHB-resistant while the other half was as susceptible as the recurrent parent. Application of SDS to a selfing population of the breeding line c2652 (highly FHB-susceptible, but being examined as a possible new source of wheat streak mosaic virus resistance) identified lines with improved and apparently stably inherited FHB resistance.

MICROARRAY ANALYSIS OF FUSARIUM HEAD BLIGHT TOXIN DEOXYNIVALENOL (DON) REGULATED GENES OF *ARABIDOPSIS THALIANA*

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OBJECTIVES

This study analyzed gene expression in response to DON in Arabidopsis culture cells using cDNA microarray in order to understand the molecular mechanisms of response to DON activity in the plants and the role of DON in fungal pathogenesis.

INTRODUCTION

Gibberella zeae (Schwein.) Petch. (anamorphs, *Fusarium graminearum* and *F.culmorum*) is the primary producer of DON in cereal grains such as wheat, barley, and corn. The U.S Food and Drug Administration regulation requires that DON levels should not exceed 1ppm and 5 ppm in finished wheat products for human and animal consumption respectively (3). Many genes specifically induced in plants by pathogens and their metabolites play an important role in the defense response of plants. Toxins that interfere with the expression of genes (especially defense related genes) in plants may be considered as virulence factors. A strong correlation was found between *Fusarium* resistance and toxin resistance in the field and laboratory tests of a large number of wheat cultivars (4). The evidence from recent studies indicates that trichothecenes, including DON are involved in plant pathogenesis by enhancing the virulence of plant-pathogenic *Fusarium* species on cereal hosts (6). Deoxynivalenol (DON), a low-molecular-weight inhibitor of protein synthesis binds to the ribosomes and interfers with peptidyltransferase activity (7). Most of our knowledge about the effects of DON on the regulation of gene expression comes from the studies on animals or other microorganisms. DON is known to induces mitogen-activated protein kinases (MAPKs) including stress-activated protein kinases (SAPK/JNK1 and 2), extracellular signal regulated protein kinases (ERK (1/2) and p38MAPK in vitro and in vivo (8). However, little information is available about the activity of DON on the regulation of plant gene expression. DON was reported to inhibit ribosomal gene Rp13 expression in Rice (2). Here, we identified DON regulated Arabidopsis genes and analyzed their functions. Results showed that DON affects the expression of a wide spectrum of genes involved in responses of Arabidopsis plants to many environmental stimuli or stresses.

MATERIALS AND METHODS

DON treatment of Arabidopsis Suspension Cell Cultures and RNA Extraction - The Arabidopsis Columbia-0 suspension cells cultures were grown in Erlenmeyer flasks at room temperature, under ambient light, with constant shaking at 115-rpm in a rotating shaker. DON treatments at different time courses were started using suspension cells 3 d after subculturing, at an approximate cell density of 2 x 105 cells mL–1. Cultures were added with filtered and sterilized DON dissolved in ddH_2O in the amount of 5ug DON /mL. The same volume of sterile ddH_2O was added to the control. RNA extracts from suspension cells collected at 6 and 24 hours after addition of DON were used for microarray analysis. Total RNAs from suspension cells of Columbia wild type treated with DON and water were extracted with Promega RNAgents kit (Cat#Z5110) following the instructions by the manufacturer as recommended by Arabidopsis Functional Genomics Consortium. The RNA was further purified according to Qiagen RNAeasy midi kit protocol (75144).

Probe labeling and cDNA microarrays hybridizations - In each experiment, 100 ug of total RNA from two biological replicates from 6 and 24 hour following

DON treatment was labeled using Klenow Labeling as described (1). The labeling reactions were purified using the QiaQuick PCR cleanup kit (Qiagen). The experimental and control tissues labeled with either Cy3 or Cy5 Fluorescent dye were hybridized to microarray slides (MSU-2_03-00) containing 11,521 element prepared by the Arabidopsis Functional Genomics Consortium as described (1). Each pair of samples from the 6 and 24 hour time points were used in two microarray assays (Technical replicates). However the second replicate (same RNA) were reverse labeled relative to the first one. The slides were scanned to measure the fluorescence corresponding to hybridization intensities using the ScanArray 4000 (Packard BioChip technologies, Billerica MA).

Microarray data analysis - The intensities of the spots were measured using the Scananalyze V2.44 software (http:/rana.lbl.gov/EisenSoftware.htm). An initial normalization for standardizing Cy3 and Cy5 intensities on each of the 4 array was performed with Perl script program. The resulting data were transferred to Excel spread sheet files (Microsoft, Redmond, WA) and imported to microarray analysis software (GeneSpring 7.0; Silicon Genetics, Redwood City, CA, USA). The data were further normalized using per spot (signal channel divided by channel) and per chip (value of each spot divided by the 50 th percentile) intensity dependent normalization (Lowess) function of the GeneSpring 7.0 program. Expression ratios from two repeats were averaged, and genes showing a value above 2.0 or below 0.5 was regarded as induced or repressed, respectively. The Arabidopsis information Resource (TAIR) databases and tools were utilized to update and further analyze the microarray data (www.arabidopsis.org).

RESULTS AND DISCUSSION

The transcript levels of 272 and 480 genes were induced and repressed respectively after the exposure of the suspension cells to DON for 6 hours. Of 272 induced genes, 20 genes displayed minimum of 4 and up to a 25 fold increase in transcript levels. These highly induced transcripts include; a putative steroid sulfotransferase, an F-box family protein, four various types of AtPase proteins, an UDP-glucosyl transferase, an ankyrin repeat family protein, two protein kinase family protein, an ABC transporter family protein, a LEA domain-containing protein, an WWE domain-containing protein and a mitochondrial pentatricopeptide (PPR) repeat-containing protein. The Arabidopsis suspension cells quickly and strongly activated the genes that regulate growth, development and as well as defense response against the phytotoxic action of the toxin. Fourteen genes including a dehydrin family protein, a glutathione S-transferase, two tubulin alpha-2/alpha-4 chain (TUA4–TUA2) protein, an aspartyl protease, a sugar transporter family protein, a delta tonoplast integral transport protein were highly repressed following 6 hour toxin treatment (cut off value of less than 0.2). Exposure of the suspension cells to the toxin for 24 hour resulted in induction of 22 and repression of 35 new genes. The number of genes that remained induced and repressed at both time points were only 4 and 12 respectively.

The distribution of putative functions for 294 induced and 515 repressed genes among functional categories of the Arabidopsis Information Resource (TAIR) showed that they are involved in a broad range of biological processes of plants (Figure 1). However, the functions of large percentage of both induced (72.3 %) and repressed (73 %) genes have still unknown. The genes associated with transcription and transportation related processes were more abundant among induced transcripts than repressed whereas genes associated with electron transport/energy pathways, cell organization/biogenesis, protein metabolism, and stress and abiotic and biotic stimuli response functions were represented at higher proportions among repressed transcripts. Currently, only 7 (2.7 %) of DON induced and 50 (10%) of DON repressed genes can be placed into steps in known metabolic pathways. Interestingly, two DON induced genes are involved in sugar (trehalose) and one in amino acid (cysteine) biosynthesis pathways. Repressed genes catalyze diverse and multiple reactions in a broad range of metabolic pathways in the generation of precursor metabolites and energy (24 %), biosynthesize (36 %) and degradation/ assimilation/utilization (40%) processes of Arabidopsis plants.

DON resulted in induction of 15 and repression of 32 ribosomes and translation related genes that play important roles in protein synthesis. None of the 15 induced genes were ribosomal and none coded for ribosomal proteins however; they were all involved in protein synthesis associated ribosomal and translational activities. On the other hand, 22 out of 35 repressed transcripts were ribosomal genes and coded for ribosomal proteins. 50S, 40S and 30S ribosomal proteins were the most common products of toxin-repressed genes. DON strongly repressed the transcription of ribosomal genes that contributes to the structural integrity of ribosome and impeded the translation and protein synthesis mechanisms. The toxin differentially regulated the transcript levels of the genes that involved in the translation mechanism as translation factors at all levels.

DON induced 32 and repressed 19 genes that code for transcription factors in toxin-treated Arabidopsis culture cells. Most of them were affected within 6 hours of toxin treatment, strongly increasing the possibility that they are part of the regulatory circle that governs toxin response. Various types of 9 Zinc finger and 7 WRKY family protein, 2 AP2, 2 bZip and one disease resistance transcription factor genes constituted the majority of induced transcription regulators. Abundance of these defense related transcription factors suggest that regulatory mechanism controlling the toxin response may be similar to that of the defense response to invading pathogens in plants. Ethylene and auxin responsive protein genes were the most abundant transcription factors among the toxin repressed transcripts. The toxin regulated transcription factors is known to play essential roles extensively in defense response and molecular signaling along with growth and development of plants.

DON toxin caused the induction of 24 and suppression of 48 transcripts categorized as stress response genes by TAIR (Table 1). 14/24 induced transcripts were typical biotic stress genes involved in defense responses to various pathogens. Seven other induced genes are known to be reporters of abiotic stress while three genes function in general stress response. 8/14 defense response genes encode putative disease resistance proteins that all, except one, contains NBS-LRR motif and 2/14 unknown proteins that putatively play role in defense against pathogens. These plant resistance genes (R) play a primary role in detection of pathogen and initiation of specific defense response that includes a type of programmed cell death (apoptosis) known as hypersensitive response (HR) in plants (5). These findings suggest that DON triggers pathogen-like plant defense response and therefore must play an important role in pathogenicity of the fungus. The majority of DON-suppressed stress genes are involved in response to various environmental abiotic stress conditions such as drought, heat, cold, light, toxin and oxidation. Six genes including a pathogenesis-related (PR-1-like) transcript were also known to respond to biotic stresses. Analysis of stress genes shows that DON makes significant contribution to virulence and pathogenicity of the Fusarium head blight pathogen. Real-time quantitative PCR was performed to confirm the results of microarray expression assays for 8 induced and 10 repressed genes. The fold changes in transcript levels from Real-time PCR correlated with those from microarray assays for all the genes tested in regression analyses ($R^2 > 90$).

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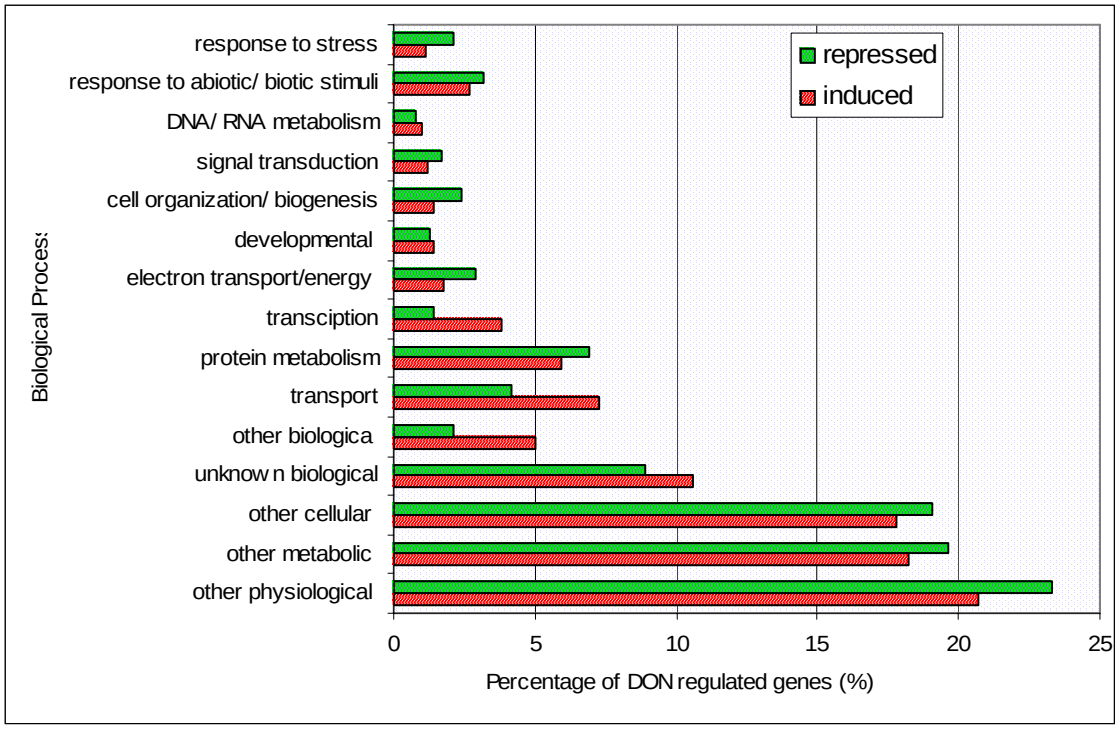

Fig 1. Molecular function and comparative analysis of the DON regulated genes. 294 induced and 515 repressed genes were grouped using the TAIR functional categorization of *Arabidopsis thaliana* genome.

Table 1. Stress (abiotic or biotic) related genes regulated by DON toxin.

Gene (AGI locus)	Description	Expr. [1]	Time (hour)	TAIR [2] Cat.	stress/response [3]
Induced					
At5g44510	disease resistance protein (TIR-NBS-LRR)	2.92	6	S, AB	pathogen/defense-AP
At4g33300	disease resistance protein (CC-NBS-LRR)	2.76	6	S, AB	pathogen/defense-AP
At2g21100	disease resistance-responsive protein	2.71	6	S, AB	pathogen/defense-fungi
At5g46520	disease resistance protein (TIR-NBS-LRR)	2.58	6	S, AB	pathogen/defense-AP
At5g46510	disease resistance protein (TIR-NBS-LRR)	2.55	6	S, AB	pathogen/defense-AP
At5g49140	disease resistance protein (TIR-NBS-LRR)	2.36	6	S, AB	pathogen/defense-AP
At1g72910	disease resistance protein (TIR-NBS-LRR)	2.41	6	AB	pathogen/defense
At3g44630	disease resistance protein (TIR-NBS-LRR)	2.59	6	AB	pathogen/defense
At2g03760*	steroid sulfotransferase-**ST**	25.33	6	AB	pathogen/defense
At3g54420*	class IV chitinase-**ATEP3**	3.03	6	S, AB	pathogen/defense- bacteria
At5g47120*	Bax inhibitor-1 putative-**ATBI-1**	2.01	6	AB	pathogen/defense-AP
At3g13950	expressed protein	3.33	6	S, AB	pathogen/defense
At5g57280	expressed protein	2.73	6	AB	pathogen/defense-bacteria
At1g32230*	WWE domain-containing protein-**RCD-1**	4.11	6	S, AB	pathogen/defense-bacteria-AP
At4g36990*	heat shock factor protein 4-**HSF4**	2.57	6	S, AB	heat
At5g49480*	Na-inducible Ca-binding protein-**ATCP1**	2.40	6	S, AB	salt,
At3g19580*	zinc finger (C2H2 type) protein 2-**AZF2**	2.14	6	S, AB	salt, water deprivation, ABA
At1g66340*	ethylene receptor 1-**EtR1**	2.05	6	S, AB	ethylene
At1g31480*	shoot gravitropism 2-**SgR2**	2.10	6	AB	gravitropism
At3g47340*	asparagine synthetase -**ASN1**	2.78	6	AB	light,sucrose
At5g20250*	raffinose synthase family protein-**DIN10**	3.19	6	AB	light, sucrose
At3g53990	universal stress protein (USP)	2.36	6	S	general stress
At5g01520	zinc finger (C3HC4-type RINg finger)	2.28	6	S	general stress
At1g59870	ABC transporter family protein	2.17	6	S	general stress
repressed					
At4g33680*	aminotransferase class I and II-**AGD2**	0.47	6	S, AB	pathogen/defense-SA
At4g22670	tetratricopeptide repeat(TPR) protein-	0.44	6	S, AB	pathogen/defense
At2g47730*	glutathione S-transferase 6-**ATGSTF8**	0.46	24	AB	pathogen/defense-toxin
At3g16770	AP2 domain-containing protein RAP2.3	0.42	6	S, AB	pathogen/defense, ethylene
At2g46370*	auxin-responsive GH3 family protein-**JAR1**	0.45	6	S, AB	pathogen/defense, auxin, JA
At2g19990*	pathogenesis-related protein-**PR-1-like**	0.25	6	NC	pathogen/defense
At4g15910*	drought-responsive protein-**ATDI21**	0.47	6	S, AB	water deprivation, ABA
At3g63520*	9-cis-epoxycarotenoid dioxygenase-**CCD1**	0.49	6	S, AB	water deprivation
At1g47128*	cysteine proteinase-**RD21A**	0.48	6	S, AB	water deprivation
At1g54410	dehydrin family protein	0.19	6	S, AB	water deprivation-gen.stress
At2g30870*	glutathione S-transferase-**ATGSTF8**	0.28	6	S, AB	water deprivation-toxin
At1g78360*	glutathione S-transferase-**ATGSTF8**	0.31	6	AB	toxin
At2g30860*	glutathione S-transferase-**ATGSTF9**	0.19	6	AB	toxin
At5g64120*	peroxidase	0.41	6	S, AB	oxidative stress
At4g21960	peroxidase 42 (PER42) (P42) (PRXR1)	0.49	24	S,AB	oxidative strees
At5g38000*	NADP-dependent oxidoreductase	0.48	6	S, AB	oxidative stress
At1g08830*	superoxide dismutase-**CSD1**	0.49	6	S, AB	oxidative stress
At1g19570*	dehydroascorbate reductase	0.12	6,24	S, AB	oxidative stress
At2g32120*	heat shock protein 70 family protein	0.47	6	S, AB	heat
At5g59720*	18.1kDa class I heat shock pro-**HSP18.2**	0.46	6	S, AB	heat
At1g54050*	17.4 kDa class III heat shock protein	0.41	6 ,24	S, AB	heat
At5g28540*	luminal binding protein 1 (BiP-1)	0.45	6	S, AB	heat
At5g42020*	luminal binding protein 2 (BiP-2) (BP2)	0.38	6	S, AB	heat
At5g62690*	tubulin beta-2/beta-3 chain-**TUB2**	0.45	6	S, AB	cold
At5g12250*	tubulin beta-6 chain-**TUB6**	0.43	6 ,24	S, AB	cold
At5g23860*	tubulin beta-8 chain-**TUB8**	0.34	6	S	cold
At5g62700*	tubulin beta-2/beta-3 chain-**TUB3**	0.29	6	S	cold
At5g63980*	3'(2'),5'-bisphosphate nucleotidase-**SAL1**	0.43	6	S, AB	cold
At5g09810*	actin 7 / actin 2-**ACT7**	0.44	6	S, AB	ligt, wounding
At4g34190*	stress enhanced protein 1-**SEP1**	0.44	6	AB	light
At1g60950*	ferredoxin, chloroplast (PETF)	0.41	6	AB	light
At3g19820*	cell elongation protein-**DWF-1**	0.36	6	AB	light
At3g07500	far-red impaired responsive protein	0.46	6	AB	light
At3g12610*	DNA-damage-repair/toleration pro-**DRT100**	0.41	6	AB	light, UV, chemical, drugs
At3g43810*	calmodulin-7-**CAM7**	0.34	6	AB	general abiotic-biotic
At5g13740	sugar transporter family protein	0.17	6	AB	general abiotic-biotic
At3g45780*	protein kinase-**PHOT1**	0.46	6	AB	general abiotic-biotic
At1g44575*	photosystem II 22kDa protein-**NPQ4**	0.42	6	AB	general abiotic-biotic
At3g07930	HhH-GPD base excision DNA repair	0.50	24	S	general stress
At2g12730	Mutator-like transposase family	0.49	24	S	general stress
At4g23940	FtsH protease	0.43	6	S	general stress
At3g22880*	meiotic recombination protein-**ATDMC1**	0.42	6	S	general stress
At3g17020	universal stress protein (USP)	0.34	6	S	general stress
At2g47590*	photolyase/blue light photoreceptor-**PHR2**	0.28	6	S	general stress
At1g20340*	plastocyanin (plastocyanin GI)-**DRT112**	0.42	6	AB	chemical
At5g05200	ABC1 family protein	0.46	6	AB	antibiotic
At4g23100*	glutamate-cysteine ligase-**RML1**	0.45	6	AB	cadmium ion
At2g27190*	iron(III)-zinc(II) purple acid phos-**PAP1**	0.41	6	S	phosphate starvation

[1]Fold changes in the transcript levels determined by GeneSpring 7.0 program. The genes with cut off values of above 2.0 and below 0.5 considered induced and repressed, respectively. [2]Functional categories of genes determined by TAIR; S-response to stress, AB-response to abiotic and biotic stimuli. [3]denotes stress that a gene respond. SA: Salycilic acid, JA: jasmonic acid ABA, Abscisic acid. *indicates the genes previously mentioned in the literature as corresponding stress responsive and available at TAIR homepage. Gene models are given in bold uppercase letters.

PROGRESS IN DEVELOPMENT OF RESISTANCE TO FHB IN ROMANIAN WHEAT

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ABSTRACT

Growing wheat cultivars that combine high levels of resistance to FHB (mainly Type II) and low contamination with DON, with other desirable agronomic traits, remains the most reliable strategy for control of scab in both, conventional and organic farming systems in Romania.

The damaging potential of this disease could be considerably high in years and locations characterized by high humidity at anthesis. That is why improvement of resistance to FHB is continuously an objective of major concern in the breeding research of winter wheat carried on in Romania, at ARDI Fundulea and other of its regional breeding centers.

Current goals:

i) Improvement of screening techniques;
ii) Selection of new sources that combine a higher resistance to FHB than that of *Fundulea 201R*, with resistance to other pathogens (e.g.*Tilletia* spp.) and better agronomic traits; and
iii) Introduction of MAS.

Screening - Due to the very complex inheritance of resistance and high genotype x environment interaction, our screening strategy is based on multi environment field experiments (year/location), under artificial inoculation (point/ single head method). For a better characterization of host resistance, at least two selected *Fusarium* isolates and combined pre and post-harvest criteria are used per genotype/year for inoculation and assessment, respectively.

Selection of *Fusarium* isolates according to their high aggressiveness and DON content (if available) is a prerequisite condition for more accuracy of assessment for resistance. A large range of variation for this trait has been reported in local *Fusarium* populations**.**

To avoid the misinterpretation of experimental field data, the classification of entries into groups of genotypes inoculated in the same day is recommended. Between the groups inoculated in different days, the sum of degrees calculated 48 hours before and 20 days post inoculation could be very informative, too.

It is necessary to emphasize also that ring trials for resistance to FHB, based on large national or/and international cooperation like: CIMMYT, USWBSI (Southern and Northern scab nurseries), European Fusarium Ringtest etc, could play a significant role in rationale and accelerated selection for more resistant and adapted winter wheat genotypes.

Selection for resistance. Trials performed in the last years evidentiated that a higher level of resistance to FHB than that identified in Fundulea 201R could be achieved. Contrary to this previous Romanian source of resis-

tance, not related with Chinese FHB resistant lines, the newest advanced lines have a better bread making quality and other desirable traits. Advanced lines as F 00628 G34-1, F 01461G3-2, F 99051G3-3INC2, F 01096G2-2, F01459G4-1 etc are derivatives of crosses bread wheat/ triticale and of bread wheats with complementary levels of resistance to FHB.

Markers assisted selection - The use of microsatellite markers associated with resistance to FHB has to validate in our research, the resistance derived from crosses among Romanian and Asian sources of resistance and to improve the use of MAS in breeding for this trait in winter wheat research. We currently use microsatellite markers associated with QTL's for resistance to FHB, located on chromosomes 3BS (*Xgwm 493, Xgwm 533*), 3A (*Xgwm 674*) and 5A (*Xgwm 304*) are in progress. The microsatellites markers *Xgwm 493* and *Xgwm* 533, linked with the major QTL *Qfhs.ndsu*-3BS from Sumai, have been already identified in some derivatives from crosses Sumai/Romanian lines.

These results represent a good premise for further approaches on resistance to FHB in winter wheat.

QTL MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN NOVEL WHEAT GERMPLASM CJ 9306

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ABSTRACT

Fusarium head blight (FHB or scab) caused by *Fusarium graminearum* is one of the most destructive diseases in wheat and barley. QTL mapping and marker-assisted selection enhance the efficiency of utilizing elite germplasms and breeding resistant cultivars. The objective of this study was to detect the DNA markers associated with the resistance in the novel wheat germplasm CJ 9306, which was developed through multiple-parent crossing and recurrent selection combined with modified pedigree methods with the aid of a dominant male-sterile gene (*Ta1*) at Nanjing Agricultural University, China (Jiang, 1997). A recombinant inbred population with 152 $F_{6:7}$ RILs derived from a cross Veery xCJ 9306 was phenotyped for resistance to fungal spread in greenhouse in 2002 and 2004 by single-floret inoculation. A total of about 680 SSR primer pairs (including *Xbarc, Xgwm* and *Xwmc* primers) were screened for polymorphism between the two parental lines. Polymorphic markers (about 170) were used to genotype the mapping population, and the segregating data were applied to construct a genetic linkage map using JoinMap version 3.0 and referring to a high-density linkage map (Shi and Ward, 2004). Preliminary results suggested three chromosome regions carrying QTLs associated with the resistance to fungal spread. A major QTL on 3BS (*Xgwm493-Xgwm533-2*) explained 40.3% of the phenotypic variation. Two additional QTLs on 5BL (*Xbarc74-Xbarc408*) and 2DL (*Xgwm157-Xgwm539*) explained separately more than 9% and 8% of the phenotypic variation. In total, these three QTLs could explain approximately 58% of the phenotypic variation. The genotyping data are in progress of further analysis.

RESISTANCE TO FUNGAL SPREAD AND DON ACCUMULATION OF *FUSARIUM GRAMINEARUM* IN WHEAT

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OBJECTIVES

1) To characterize the genetic variability of Type II resistance and DON contents in a RIL population derived from Veery/CJ 9306;

2) To estimate the association between the re sistance to fungal spread and DON accumu lation.

INTRODUCTION

Fusarium head blight caused by *Fusarium graminearum* is a world-wide important disease in wheat and barley. It caused devastating losses of crop production and severe toxin contamination of the grains in the North America in the last decade. The production and accumulation of the mycotoxin, deoxynivalenol (DON) in the infected kernels by the fungus is detrimental to the health of livestock and human beings. Characterization of the genetic variability of the resistance and DON accumulation is informative and helpful for development of resistant cultivars.

MATERIALS AND METHODS

A set of 152 recombinant inbred lines (RILs) derived from a cross Veery/CJ 9306 and two parents were evaluated for FHB resistance (Type II) in greenhouse in 2002 and 2004. The RILs were grown in a randomized design with two replications, and for each replication, six plants were planted in two pots. Two parents Veery and CJ 9306 were planted as controls many times at an interval of a week, in order that they could be included in each inoculation to estimate the differences among inoculation dates.

Single-floret inoculation was conducted immediately prior to or after initial anthesis (Jiang et al., 2001). The inoculumn was *F. graminearum* isolate PH-1 (NRRL 31084) for 2002 and a mixture of two isolates PH-1 and WF-1 for 2004. Twelve to fifteen microliters of conidospore suspension (5×10^4 spores/ml, produced by CMC liquid culture) was injected into a central basal floret of the spike with a self-refilling syringe. Six to eight spikes were inoculated per replication. The inoculated spikes were tagged to indicate the date of inoculation and record the symptoms of disease. The inoculated pots were placed in a misting chamber with an auto-mist-irrigation system programmed to deliver 20-second mist at intervals of 6 minutes and a temperature controlling system set at 22-26ºC. After mist-irrigation, pots were transferred to another greenhouse compartment. The number of scabby spikelets (NSS) on the inoculated spikes was visually counted at 5, 9, 13, 17, 21 and 25 days post inoculation (dpi), respectively. On the 25th day, the number of total spikelets and number of infected rachis sections (NIRS) were also estimated, and the percentage of scabby spikelets (PSS) was calculated for each observation. Using PSS data, the area under disease progress curve (AUDPC) was computed. Due to extremely high correlations between NSS or PSS and AUDPC or NIRS based on 2002 data, only NSS and PSS were determined at 21 and 25 dpi in 2004.

After all the plants were ripe, inoculated and non-inoculated spikes for each replication were harvested separately and threshed carefully with a head thresher at lower speed to avoid blowing the scabby or shriveled kernels away. Ten to twelve scabby kernels were randomly taken from the inoculated spikes to serve as a sample for Deoxynivalenol (DON) test. DON extraction and analysis were based on a modified

method of Mirocha et al. (1998). Briefly, seeds were weighed and placed into a 1-dram glass vial capped with a screw cap and extracted by soaking and shaking with 2 ml of acetonitrile/water (84/16 v/v) for 24 hr. The extract was passed through a minicolumn packed with C_{18} and aluminum oxide. One and a half milliliters of the filtrate were placed into a ½-dram glass vial and evaporated to dryness under nitrogen. Twenty-five microliters of TMS reagent (TMSI/TMCS 100:1) were added, and the vial was rotated so that the reagent contacted with all residue in the vial. The vial was placed on a shaker for 10 min, and then 200µl of isooctane were added followed by 200 µl of HPLC water to quench the reaction. After vortex, the upper layer was transferred to a GC vial. Selected ion monitoring (SIM) was used for GC/MS analysis (Shimadzu GCMS-QP2010, Shimadzu Corporation, Kyoto, Japan), with fragment ion (m/z value) of 235.10 as target ion and 259.10 and 422.10 as reference ions.

Statistical analysis was based on replication means for all the inoculated spikes within a replication. One-way ANOVA was computed first for single year data, and then two-way ANOVA based on two-year combined data was conducted to estimate the inter-year effect and genotype × year interaction. For PSS, due to a high consistency between the results of original observed values and arc-sin transformed values, the results based on original data were presented here. Broad-sense heritability was estimated on the basis of ANOVA results. Simple correlation was analyzed among the traits or indications of the resistance.

RESULTS AND DISCUSSION

All the indications of resistance to fugal spread (NSS, PSS, AUDP and NIRS) for the controls/parents were not significantly different for the date of inoculation for each year (data not shown), suggesting a high consistency among the different inoculations due to well-controlled environmental conditions.

One-year ANOVA showed that the differences among RILs were highly significant for all the indications of resistance and DON contents (Table 1). Two-year ANOVA also suggested a significant inter-year difference (F=6.21–13.52, P<0.001) and a significant genotype × year interaction (F=2.78–7.12, P<0.01) besides significant RILs difference (Table 2). In most cases, the averages of RIL population, with large variability, were around the mid-parent values. Frequency distributions were continuous and exhibited two or three peaks, except for NIRS (Figure 1). The results further supported our previous postulation that the resistance was inherited as a qualitative-quantitative trait (data to be published). Transgressive segregation was evident for all the indications, especially in the susceptible direction. Some lines were superior to CJ 9306 for NIRS and DON content.

The estimates of heritability suggested a higher broad-sense heritability for the resistance to FHB spread within the spikes (Tables 1 and 2). Comparatively speaking, the estimates of broad-sense heritability and coefficients of variation for 2004 were larger than those of 2002. Heritabilities based on two-year combined analysis were reduced due to removing genotype × year variance. Among different measures, DON content and NIRS had lower heritabilities and coefficients of variation, indicating that these two measures were more variable than the others.

Correlation analysis showed that there were extremely high correlations between different indications of the resistance to fungal spread (r=0.90-0.98, P<0.001) (Table 3). However, the correlation between DON content and spread resistance was moderate to higher (r=0.66-0.76, P<0.001). In addition, the correlation between years for DON content was obviously lower than for NSS and/or PSS, further indicating that DON accumulation was more easily affected by the environments. Different degrees of relationship between DON accumulation and resistance were addressed in previous studies: lower (Somers et al., 2003; Zhou et al., 2002), moderate (Bai et al., 2001; Mesterházy et al., 1999) and higher (Mesterházy et al., 1999). Clearly, the inconsistencies were attributed to DON sampling and analysis methods as well as the types and numbers of experimental materials.

ACKNOWLEDGEMENT

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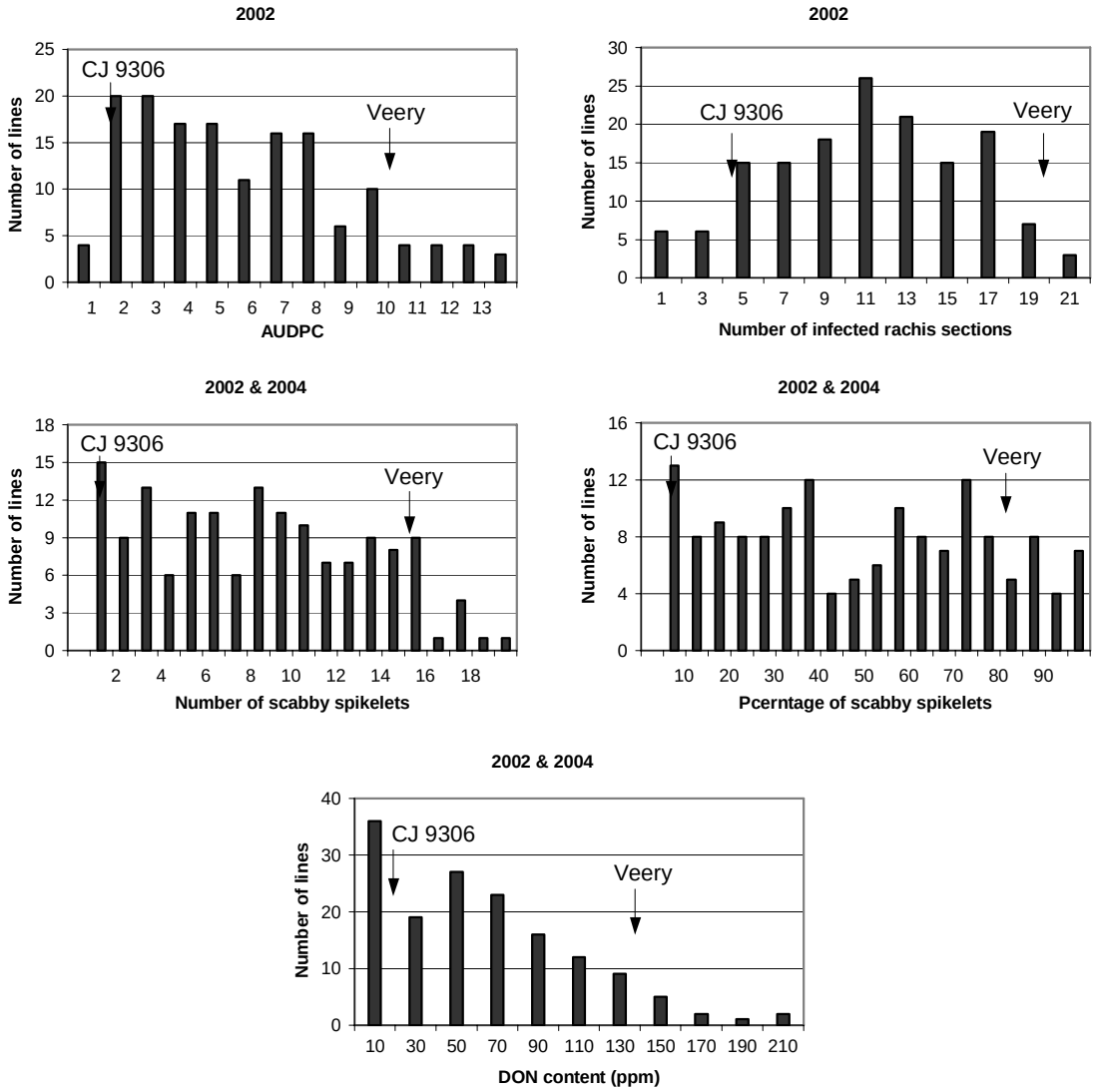

Fig. 1. Frequency distribution of 152 RILs derived from the cross Veery/CJ 9306 for resistance to fugal spread and DON accumulation of *Fusarium graminearum* by single-floret inoculation in greenhouse.

Table 1. Means, variations, *F*-values and estimates of broad-sense heritability of FHB scores and DON content in a 152-RIL population by greenhouse-based single-floret inoculation in two years (2002 and 2004).

Population	Statistics	NSS		PSS (%)		DON content (ppm)		AUDPC	NIRS
		2002	2004	2002	2004	2002	2004	2002	2002
CJ 9306	Mean	1.5±0.2	0.8±0.1	8.3±1.3	4.6±0.5	22.7±3.1	7.7±4.1	1.2±0.2	4.4±1.2
	Range	0.7–2.5	0.5–1.4	4.0–13.9	2.8–7.1	18.5–28.7	0–17.1	0.5–1.7	0.7–9.5
Veery	Mean	16.5±0.5	14.1±0.3	90.2±1.7	76.3±1.4	134.2±15.7	134.1±20.6	9.9±0.6	19.4±0.4
	Range	15.4–19.2	12.3–15.2	83.9–96.2	68.1–81.6	94.7–165.3	84.0–182.9	7.9–12.3	18–21.3
RIL population	Mean	8.7±0.4	8.2±0.4	51.0±2.4	47.4±2.4	65.0±4.4	58.9±4.4	5.4±0.3	11.1±0.4
	Range	0.7–20.2	0.6–20.7	4.0–100	3.4–100	0–265.3	0.2–274.8	0.5–13.8	0.9–22.2
	CV %	58.03	63.58	57.08	62.34	81.62	91.98	60.01	44.32
	F-value	8.04 **	13.99 **	8.13 **	14.95 **	2.98 **	13.13 **	8.79 **	5.60 **
	h_B^2	77.88	86.66	78.10	87.46	49.80	85.85	79.57	69.70

** Significant at the 0.01 probability level on the basis of one-way ANOVA.

Table 2. Means, variations, *F*-values and estimates of broad-sense heritability of the resistance to fugal spread and DON accumulation in a 152 RIL population based on a two-year combined analysis under single-floret inoculation.

Population	Statistics	NSS	PSS (%)	DON content (ppm)
CJ 9306	Mean	1.2±0.2	6.2±0.8	14.1±3.9
Veery	Mean	15.2±0.4	82.8±2.1	134.2±12.0
RIL population	Mean	8.5±0.4	49.2±2.2	62.1±3.8
	Range	1.1–19.5	6.1–100	0.1–235.6
	CV %	56.61	56.02	75.60
	F-value	6.63 **	7.12 **	2.79 **
	h_B^2	67.30	68.74	40.71

** Significant at the 0.01 probability level on the basis of two-way ANOVA.

Table 3. Correlation coefficients between FHB scores and DON content in a 152-RIL population by single-floret inoculation for single year (above diagonal: upper for 2002 and lower for 2004) and two-year combination (below diagonal), and correlation coefficients between years for the same trait (on diagonal and bolded).

	NSS	PSS	DON content	AUDPC	NIRS
NSS	**0.738** ***	0.978 ***	0.670 ***	0.948 ***	0.959 ***
		0.978 ***	0.672 ***		
PSS	0.979 ***	**0.766** ***	0.656 ***	0.967 ***	0.926 ***
			0.698 ***		
DON content	0.763 ***	0.759 ***	**0.448** ***	0.655 ***	0.657 ***
AUDPC					0.895 ***

*** Significant at the 0.001 probability level.

MARKER ASSISTED SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WHEAT FROM DOUBLE HAPLOID POPULATIONS

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ABSTRACT

Fusarium head blight (FHB) is caused by *Fusarium graminearum* Schwabe. The fungus can degenerate the wheat grain tissue and produce deoxynivalenol (DON) which is toxic to both human and animals. Epidemics of FHB can result in severe loss of yield and grain quality. Cultural or/and chemical control of FHB is difficult because infection of FHB occurs during the time of flowering. Chemical control could only be 60-70% effective when applied at the optimum time. Release of FHB resistant cultivars is the most effective option to control incidence of FHB. In the southeast region of the US, resistance to FHB in local adaptive soft red winter wheat is limited. Introduction of resistant genes from exotic sources could enhance the resistance of local adaptive germplasm. A Virginia line AV01W-476 with the most widely used major QTLs in chromosome 2A, 3B and 5A for FHB resistance was used as donor in our program. A total of 47 double haploid individuals were generated from backcross F1 plants induced with maize pollens. Screening with SSR markers indicated the integration of novel FHB resistant QTLs on 3BS and 5AL from donor parents and native adaptive gene pool of ASG2000 and its derivatives. Two double haploid plants from back-cross of VA01W-476/GA98186 and 4 double haploid plants from back-cross of VA01W-476/AGS2485 were identified to have VA01W-476/W14 type QTLs on 3BS and 5A. Further evaluation for agronomic traits is under investigation.

PROGRESS FROM FIVE YEARS OF SELECTING FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN SPRING WHEAT

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ABSTRACT

Some effort aimed at the improvement of resistance to Fusarium Head Blight (FHB) has been practiced within spring wheat breeding programs for many years. With the advent of the US Wheat & Barley Scab Initiative, however, such efforts have become major resource expenditures. As such, it seems worthwhile to periodically monitor progress from such endeavors. In our first attempt to gauge progress, the objective of this project was to determine whether resistance to FHB in a random sample of spring wheat germplasm selected in the upper Midwest has increased from 1998 to 2003. To facilitate such measurement, a test was composed which included 10 varieties that were released to growers between 1998 and 2003, as well as 24 breeding lines that were selected within the same time frame to continue their advancement through their respective breeding program (i.e., advanced to statewide preliminary yield trials). Two additional lines were included in the test as checks. These artificially inoculated tests were grown under mist-irrigation at Brookings, SD and Prosper, ND during the 2004 and 2005 growing seasons. *Fusarium* Head Blight severity data were collected from each four-replication test. Data were subjected to analysis of variance over years and over locations. It was found that entries were significantly different with respect to FHB severity at both locations. Analysis of severity ratings at each location over years revealed that years were significantly different in terms of severity in Brookings, but not at Prosper. The correlation coefficient for overall severity ratings among locations was highly significant ($r=0.7769$; $p=<.0001$). Year of advancement and entry means were used as independent and dependant variables, respectively, to fit a simple regression model. The slope of the simple regression line was not statistically separable from zero ($b=-0.4537$; $p=0.363$). These results suggest that much phenotypic variation for FHB severity is present within this germplasm. At the same time, it appears that the entries sampled from the 5-year time span are still too variable, with respect to FHB severity, to begin monitoring progress in the advancement of FHB resistance. Additional attempts to examine progress in FHB resistance breeding will likely be initiated in the future.

A COMPARISON OF TYPE I AND TYPE II RESISTANCE WITHIN A COLLECTION OF ELITE SPRING WHEAT GENOTYPES

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ABSTRACT

Within the SDSU Spring Wheat breeding program, FHB resistant genotypes are selected based solely on Type I resistance. Implications associated with selecting for only Type I resistance could be negative. Specifically, if independently expressed genes control the two modes of resistance, (as has been suggested) chance alone would dictate whether our selections with high levels of Type I resistance possess similarly high levels of Type II resistance. Little information exists with respect to the genetic control of Type I and Type II resistance. Our objective was to examine associations between Type I and Type II resistance measurements on an elite collection of highly inbred spring wheat germplasm. Two split-plot greenhouse experiments were carried out on seventeen genotypes. Experiment one was conducted on a greenhouse bench where experimental units were a pot of plants. Experiment two consisted of hill plots that were sown into the soil beds of another greenhouse. Two inoculation methods (single floret injection vs. whole head spray) served as the main-plot treatments that were applied to seven replications of the sub-plot treatments (genotypes) within each experiment. Environmental conditions prior to and after inoculations were consistent with our routine procedures that have been optimized for disease expression. Eighteen days after inoculation, the number of diseased spikelets/head, total number of spikelets/head, and disease severity estimates were recorded for plants within each experimental unit. Data collected from the first experiment revealed that genotypic means were significantly different for total number of diseased spikelets in addition to disease incidence and severity values regardless of the inoculation method. Significant tests of Spearman's rank-order correlation suggested that Type I resistance measures were generally similar to those attained from Type II measures, except in the case of diseased spikelets/head. Additional research must be conducted with regard to our current objective, however, it appears as though severity measures collected from spray inoculations, (Type I resistance) for example, provide similar severity data as obtained from point inoculations (Type II resistance). Although it is currently not possible to speculate on a reason for this observation, our findings provide some evidence that Type I and Type II resistances are not completely independent. Results from our second experiment will also be discussed.

IMPROVEMENT OF FHB RESISTANCE OF DURUM WHEAT

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ABSTRACT

The greatest challenge in the development of FHB resistant durum wheat (*Triticum durum*) is the extreme susceptibility of durum wheats to FHB. There are two explanations for this extreme susceptibility: 1) the inherent lack of resistance genes, and 2) the presence of super-susceptibility factors. In light of these two possibilities we are using the following approaches to insure the development of FHB resistant durum wheat. We are systematically screening durum accessions in CIMMYT GeneBank for FHB resistance. In addition, we are screening alternative sources of resistance to incorporate into durum wheat. Multiple alternative sources of resistance exist for the enhancement of durum wheat including:

1) Wild tetraploid wheat having the A and B genomes.
In a preliminary study, we found several resistance lines of *T. dicoccum* and *T. dicoccoides* show ing as few as 7% scabby spikelets when screened for type II resistance.

2) A/B genome of hexaploid wheat.
The transfer of 'Sumai #3' resistance from the 3B chromosome to durum is underway using PCR markers.

3) Ancestral species of the A and B genomes.
We have observed *T. monococcum* accessions having type II resistance responses ranging from 9.4% to 45.7% scabby spikelets. CIMMYT has produced more than 200 lines ofsynthetic wheat of A and B genomes (genome constitution=AAAABB and AABBBB), and there are several Resistance candidates where the type II resistance scores are as minimal as 9.5% scabby spikelets.

4) D genome of hexaploid/synthetic wheat.
Several synthetic (genome constitution AABBDD) wheat incorporating the D genome of *Aegilops tauschii* showed type II resistance equal to or higher than that of Sumai #3. The resistance exhib ited by these synthetic wheats is supposedly residing in the D genome. We crossed synthetic wheats with *ph1* mutant lines to transfer such resistance into durum through recombination between the A and D genomes.

In addition to utilizing alternative sources of resistance, we are removing susceptibility factors from durum wheat. We have generated a cross between a durum wheat and Sumai #3. From this cross, we will develop four kinds of populations: (1) Plants having *T. aestivum* morphology and Sumai #3 resistance(2) Plants having *T. aestivum* morphology and *T. durum* susceptibility, 3) Plants having *T. durum* morphology and Sumai #3 resistance, and 4) plants having *T. durum* morphology and susceptibility. We will create a mapping population from a cross between a resistant durum-like plant and a susceptible durum-like plant to identify and analyze the factors contributing to susceptibility.

EVALUATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

In 2005, Kentucky producers experienced exceptional yields resulting from an extremely favorable environment for wheat production and essentially no FHB pressure. Although there was very little natural disease development throughout the state, the irrigated, scabby-corn inoculated FHB nursery at Lexington, KY produced sufficient disease pressure that breeding lines could be screened for resistance. The difficulty in controlling inoculum level in the irrigated nursery in previous years prompted the addition of an alternative screening method: non-irrigated hill plots inoculated with a conidial spore suspension. Advanced breeding lines were evaluated for resistance in the irrigated FHB nursery by recording severity at 21 days after anthesis. Selected breeding lines from the irrigated FHB nursery were also evaluated in the non-irrigated hill plots. Ten seeds of each line were planted into two non-irrigated hill plots at Lexington, KY. One hill plot was sprayed at anthesis with a conidial suspension (50,000 spores μl^{-1}) until runoff and bagged with a corn shoot glassine bag for 72 hours. The second hill was used as a control and sprayed with water. Twenty-one days after inoculation the hills were rated for disease severity. Disease severity in the nursery ranged from 6 to 93%; the average of the entire nursery was 40%. This is a significant ($P<0.05$) difference from 2004 where resistant and susceptible breeding lines could not be distinguished, the range in disease severity for lines was 17 to 96% and the average severity of the entire nursery was 47%. Breeding lines included in both the irrigated FHB nursery and the non-irrigated hill plots were analyzed to determine if differences existed between average severities and incidence. The non-irrigated hill plots had a significantly ($P<0.05$) higher severity (62%) than the irrigated FHB nursery (46%). However, the irrigated nursery had a significantly ($P<0.05$) higher incidence (64%) than the non-irrigated hill plots (53%). Results from spray-inoculated spikes in the hill plots were promising. Although severity and incidence were not as consistent in the hill plots, modifications to the inoculation procedure and spore concentration can be made to produce more uniform results. This could be useful in testing advanced breeding lines in different locations across Kentucky or in years in which the irrigated nursery produces such high levels of inoculum that differentiation of resistant and susceptible lines is impossible. By simultaneously testing lines in both environments, a better estimation of resistance can be made.

PROFILING THE EXPRESSION OF GENES RELATED TO FHB PATHOGENESIS IN WHEAT WITH AFFYMETRIX GENECHIP WHEAT GENOME ARRAY

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ABSTRACT

FHB-resistant cultivars Sumai 3, Tokai 66 and Abura and FHB-susceptible landrace Y1193-6 were inoculated with *Fusarium graminearum* isolate Fg4 and water (as the mock-inoculated controls). Gene expression in the inoculated and the mock-inoculated samples was profiled with Affymetrix GeneChip Wheat Genome Array 24 hours after inoculation and was verified with Real-Time RT-PCR assay. Comparing the inoculated with their mock-inoculated controls revealed FHB-related gene expressions with a threshold of one fold difference. Some FHB-related gene expressions were cultivar-specific and were associated with FHB resistance. Also observed were *F. graminearum* genes that might only express after infection.

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NEW DNA MARKERS FOR THE CHROMOSOME 3BS FUSARIUM HEAD BLIGHT RESISTANCE QTL IN WHEAT

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ABSTRACT

A major quantitative trait locus (QTL), *Qfhs.ndsu-3BS*, for resistance to Fusarium head blight (FHB) in wheat has been identified and verified by several research groups. The objectives of this study were to construct a fine genetic map of this QTL region and to identify new DNA markers useful for marker-assisted selection (MAS) for this QTL. Two SSR markers (Xgwm533 and Xgwm493) flanking this QTL were used to screen for recombinants in a population of 3156 plants derived from a single F_7 plant heterozygous for the *Qfhs.ndsu-3BS region*. A total of 382 recombinants were identified, and they were genotyped with two more SSR markers and nine STS (sequence-tagged site) markers. A fine genetic map of the *Qfhs.ndsu-3BS* region was constructed and spanned 6.3 cM. Based on replicated evaluations of homozygous recombinant lines for Type II FHB resistance, *Qfhs.ndsu-3BS*, re-designated as *Fhb1*, was placed into a 1.2 cM marker interval flanked by STS3B-189 and STS3B-206. Assuming *Fhb1* is located in the middle of the marker interval, STS3B-256 is within 0.2 cM of *Fhb1* compared to the closest SSR markers, BARC147 and BARC133 that are approximately 0.8 and 1.0 cM from this gene, respectively. Marker STS3B-256 amplifies three loci on chromosomes 3A, 3B and 3D from Chinese Spring. Sumai 3 has a null 3BS allele for this marker. The 75 wheat lines used in our previous haplotype study (*Crop Science* 43:760-766) and 144 parental lines used in the wheat breeding program at the University of Minnesota were genotyped with marker STS3B-256. All wheat lines most likely containing *Fhb1* based on their SSR marker alleles have the null 3B allele. Among the 48 lines with all SSR alleles near *Fhb1* different from Sumai 3, only two lines, Wangshuibai and Ning894013, have the null 3B allele. Surprisingly, four of the seven lines that share only the BARC133 allele with Sumai 3 have the null 3B allele. All the four lines, Nobeoka Bozu, Nyu Bai, Abura, and Tokai 66, were originated from Japan and are well known for FHB resistance. Among the 144 parental lines genotyped with marker STS3B-256, only 20 lines have the null 3B allele, and they all have Sumai 3 or Nyu Bai in their pedigrees. Therefore, we believe the null allele of STS3B-256 is diagnostic for the Sumai 3 allele of *Fhb1* and will be useful in MAS.

MAIN EFFECTS, EPISTASIS AND ENVIRONMENTAL INTERACTIONS OF QTLS ON FUSARIUM HEAD BLIGHT RESISTANCE IN A RECOMBINANT INBRED POPULATION CS-SM3-7ADS /ANNONG 8455

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ABSTRACT

Chinese Spring-Sumai 3 chromosome 7A disomic substitution line (CS-SM3-7ADS) was reported to have a high level of FHB resistance, and an F_7 population of recombinant inbred lines (RILs) derived from the cross between CS-SM3-7ADS and Annong 8455 was evaluated for resistance to Fusarium head blight (FHB) to identify main effects, epistasis and environmental interactions of QTLs on FHB resistance. A molecular linkage map was constructed with 501 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. The map covered a genetic distance of 2546 cM. Ten QTL were identified with significant main effects on the FHB resistance in at least one environment using MapQTL and QTLMapper software. Among them, CS-SM3-7ADS carries FHB-resistance alleles at five QTLs on chromosomes 2D, 3B, 4D and 6A. The QTL on chromosome 3BS has the largest effect on FHB resistance and explained 30.2% of the phenotypic variance when data from two locations were analyzed together. QTL was not detected on chromosome 7A that was from Sumai 3 and therefore the increased FHB resistance in CS-SM3-7ADS may not be due to a major resistance QTL on 7A of Sumai 3, but more likely due to removal of a susceptible QTL on 7A of Chinese Spring. QTLMaper detected nine pairs of additive-by-additive (AA) interactions at 17 loci that explained 26% phenotypic variance. QTL-by-environment (QE) interactions explained about 49% of phenotypic variation, indicating that the expression of the QTLs are significantly affected by the environments and multiple location tests are important for identification of stable QTL.

FRACTIONAL ANALYSIS OF CHROMOSOME 2(2H) FUSARIUM HEAD BLIGHT RESISTANCE QTL

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ABSTRACT

Two quantitative trait loci (QTL) each for lower Fusarium head blight (FHB) severity and plant height, and one major QTL each for deoxynivalenol (DON) accumulation and days to heading were mapped with recombinant inbred lines obtained from a cross between CIho 4196, a two-rowed resistant cultivar, and Foster, a six-rowed susceptible cultivar (Horsley et al., 2005). These loci reside in the barley chr. 2H region flanked by the markers ABG306 and MWG882A (bins 8-10). To date, there are a total of 26 unique loci and 67 markers in this major FHB QTL region on chr. 2. Eighteen markers have been hybridized to the 6x cv. Morex barley BAC library, identifying 131 BAC clones as part of the physical map of the region. Since the region is very large, we are using several different approaches to break down the FHB QTL to target necessary genes for resistance, and separate FHB resistance from undesirable traits to develop genetic and breeding material. Three cleaved amplified polymorphic sequence (CAPS) markers were designed for the major FHB resistance QTL to aid in development of isolines. Seventy-©four lines containing fragments of this region from CIho 4196 in a six-row susceptible cultivar, Morex, background were selected. These lines are being genotyped more extensively and submitted for phenotyping in China. Genotyping data will be presented. A Foster x CIho 4196 inbred recombinant, A171, is of special interest. It has Foster genotype throughout the proximal region of the major FHB QTL through the *Vrs1* locus, resulting in a six-rowed phenotype. However, it maintains low FBH severity, low DON accumulation, is tall, and has a late heading date, traits of CIho 4196. To facilitate development of six-rowed germplasm for breeding and identification of genes that affect these traits, A171 was backcrossed to Morex and CIho 4196. A BC1F2 population was produced summer '05 and will be screened for recombinants with desirable resistance and agronomic traits. A BC2 population was also produced and will be selected with molecular markers for additional recombinants.

In order to develop molecular markers very closely linked to the *Vrs1* locus, we initiated microarray analysis of nine six-rowed *Vrs1* mutants obtained by fast neutron mutagenesis of three two-rowed cultivars. Since these mutants were induced by fast neutrons, they are likely to have very large and possibly overlapping deletions. Such deletions can be quickly identified and genes involved in the deletions used as markers for mapping. Markers closely linked to the *Vrs1* locus are needed to identify crossovers near the *Vrs1* locus. These markers will be used to aid in breeding and identification of FHB resistance genes.

DIALLEL ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a major biotic constraint of winter wheat production and quality in South Dakota. Breeding of resistant varieties is the most efficient approach for combating this problem. This study was performed to investigate genetic control of FHB resistance in selected winter and spring wheat genotypes. A partial diallel mating design included crosses from parents 'Nekota', '2137', 'Harding', 'ND 2710', 'BacUp' and 'Ning 7840'. Both F_1 and F_2 populations were evaluated in the greenhouse, and only F_2 populations were evaluated in the field. In the greenhouse, both F_1 and F_2 populations were artificially point inoculated at anthesis, whereas F_2 crosses evaluated under field conditions were artificially inoculated by a combination of corn spawn spread at jointing stage and inoculum suspension spray at anthesis. Disease index percentage (incidence%*severity%/100) of the crosses was analyzed using Griffing's method 4 and model 1. General combining ability (GCA) was highly significant ($P < 0.01$) in both greenhouse and field environments, but specific combining ability (SCA) was significant ($P < 0.05$) only in the F_2 crosses in the greenhouse. The ratio of combining ability variance components [$2ó^2GCA/(2ó^2GCA+ó^2SCA)$] ranged from 0.66 to 0.89. A high correlation ($r = 0.95$, $P < 0.01$) was observed for disease index between F_2 populations in the greenhouse and field environments. Deoxynivalenol (DON) content was analyzed with GC/ECD in the populations under greenhouse conditions. The DON content ranged from <0.5 to 27.2 ppm. A positive and significant correlation in the F_1 ($r = 0.71$; $P < 0.01$) and the F_2 ($r = 0.84$; $P < 0.001$) populations was found between disease index and DON content. The results showed that both additive and non-additive gene effects were involved in the inheritance of FHB resistance but additive gene effects were more important than non-additive gene effects. A significant and high correlation between disease index and DON content indicates that selecting for low disease index would be useful to indirectly select for low DON content.

EVALUATION OF ELITE HARD RED AND WHITE WINTER WHEAT FOR FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

This study was performed to investigate genetic diversity for FHB resistance in selected advanced winter wheat genotypes. A total of 22 hard red and white winter wheat genotypes representing Crop Performance Testing (CPT) Variety Trial were evaluated for FHB resistance in a mist-irrigated field in 2003 and 2004 and in the greenhouse in 2003. In the field environment, genotypes varied significantly ($P < 0.01$) for flowering date, disease index and percent *Fusarium* damaged kernel (FDK) in both years. Flowering date was correlated with disease index but not with percent FDK. There was no correlation between disease index and percent FDK. This indicated that the disease index and percent FDK should be recorded separately to assess FHB resistance. The disease index and percent FDK varied significantly ($P < 0.01$) between the years. The interaction was also significant ($P < 0.05$) between year and genotype for disease index and percent FDK. Deoxynivalenol (DON) was measured in the genotypes evaluated in the field environment in 2004. The DON content ranged from 21.6 ppm to 52.4 ppm. High DON content demonstrated that these genotypes were not resistant to mycotoxin. Nivalenol accumulation was low (<0.5 ppm) in all the genotypes. The DON content was correlated ($r = 0.6$ and 0.5; $P < 0.05$) with both percent FDK and disease index, respectively. In the greenhouse, genotypes also varied significantly ($P < 0.01$) for disease index. No correlation was observed between disease index in the greenhouse and the field. The interaction effect between year and genotype in the field showed that environment changed ranking of genotypes in the two years. This suggests the need for multiple evaluations of germplasm for disease resistance. The correlations between disease index or FDK and DON content indicated that selecting for low disease index or FDK would also indirectly lead to low DON content. Since there was no correlation between the greenhouse and field environments, disease screening in the field cannot be replaced with greenhouse evaluation.

MOLECULAR CHARACTERIZATION OF WHEAT-ALIEN SPECIES AMPHIPLOIDS AND CHROMOSOME ADDITION LINES RESISTANT TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Four *Triticum aestivum* L. cv. 'Fukuhokomuji (Fuku)'- *Elymus rectisetus* (2n = 6x = 42, genomes StStYYWW) chromosome addition lines (2n = 44), seven *T. aestivum* L. cv. 'Chinese Spring (CS)'- *Thinopyrum junceum* (2n = 6X = 42, genomes $E^bE^bE^bE^bE^eE^e$) amphiploids, and thirteen CS-*Th. junceum* addition lines (2n = 44) were evaluated for Type II resistance to Fusarium head blight (FHB). One Fuku-*E. rectisetus* addition line, A1034, three amphiploids,AJAP3, AJAP4, AJAP7, and two CS-*Th. junceum* addition lines, AJDAj2 and AJDAj3 exhibited resistance comparable to the resistant control 'Sumai 3'. The mean FHB severity of these resistant lines was significantly lower than the susceptible controls CS and Russ, a common spring wheat cultivar. RFLP analysis indicated that the *E. rectisetus* chromosome in three of the four Fuku-*E. rectisetus* addition lines, A1026, A1048, and A1057, belonged to homoeologous group 1. In addition, *Th. junceum* chromosomes belonging to homoeologous groups 1, 2, and 4 were identified through RFLP analysis in the CS-*Th. junceum* addition lines, including AJDAj1, AJDAj2, AJDAj3, AJDAj4, AJDAj8, and HD3515. Characterization of low molecular weight glutenin subunits in the seeds by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) confirmed the RFLP results for the addition lines carrying a group 1 chromosome of *E. rectisetus* or *Th. junceum*. Chromosomes in the resistant amphiploids and addition are being further characterized using fluorescence in situ hybridization (FISH). These resistant lines could serve as a novel source of FHB resistance for wheat breeding. Understanding of their genetic constitutions will enhance the utilization of these resistance sources in the development of wheat varieties resistant to this devastating disease.

THE EFFECT OF GENERAL FIELD SELECTION ON WHEAT MICROSATELLITE ALLELE FREQUENCIES AT FHB RESISTANCE QTLS

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ABSTRACT

The development of FHB resistant cultivars is an important objective of Canadian common wheat breeding programs. The objective of this study was to determine whether selection for agronomic and disease response traits affected microsatellite allele frequencies at FHB resistance quantitative trait loci (QTLs). FHB resistance QTLs, derived from Nyubai, Sumai 3, and Wuhan 1, were backcrossed into three elite western Canadian spring wheat backgrounds, 98B69-L47, BW301, and Kanata, using marker-assisted selection. Eight hundred and one doubled haploid (DH) lines were produced from all possible cross combinations among BC_2F_2 lines derived from these three backgrounds. The DH lines were selected for plant type, plant height, lodging, and time to maturity in New Zealand, and response to leaf rust, stem rust, common bunt, and FHB in nurseries near Swift Current, SK, and Carman, MB. All DH lines were analyzed with microsatellite markers at FHB resistance QTLs on chromosomes 2D, 3BS, 3BSc, 4B, and 5AS. Microsatellite allele frequency changes were not significant at most loci. Selection resulted in a moderate but significant reduction in Nyubai and Sumai 3 alleles at 5AS microsatellite loci. The Wuhan 1 allele at *Xgwm608-2D* was fixed in one population. In general, selection for general field performance and response to leaf and stem rust and common bunt did not adversely affect variation at FHB resistance QTLs in the populations evaluated, except for 5AS.

THE EVALUATION OF FHB RESISTANCE QTLS INTROGRESSED INTO ELITE CANADIAN COMMON WHEAT GERMPLASM

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ABSTRACT

The effects of Fusarium head blight (FHB) resistance quantitative trait loci (QTLs), from Nyubai, Sumai 3, and Wuhan 1, were evaluated in three elite Canadian spring wheat backgrounds, 98B69-L47 (QTLs: 3BS, 3BSc, 4B), BW301 (QTLs: 2D, 3BS, 5AS), and Kanata (QTLs: 3BSc, 5AS). Microsatellite loci, linked to these QTLs, were analyzed to identify homozygous BC_2F_2 individuals. BC_2F_3 and BC_2F_4 lines were evaluated for anthesis date, plant height, FHB incidence, FHB severity, FHB index, Fusarium damaged kernels (FDK), and deoxynivalenol (DON) content in an Ottawa FHB nursery in 2004 and 2005, respectively. In the 98B69-L47 population, the 4B QTL from Wuhan 1 had the largest impact and significantly reduced FHB in 2004. However, this QTL was also significantly associated with increased plant height in both years, which may effect its deployment. In the BW301 population, the 2D QTL from Wuhan 1 decreased FHB in both years while the 5AS QTL from Nyubai decreased FHB in 2004. In the Kanata population, the 3BSc QTL from Sumai 3 was more effective than the 5AS QTL from Sumai 3. The Nyubai 3BS QTL did not have a major effect on FHB in these tests. Overall, there was a general reduction in FHB symptoms as FHB resistance QTLs were introduced into an elite genetic background.

SOURCES OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT: DIVERSITY AND UTILIZATION

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ABSTRACT

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.) which causes Fusarium head blight (FHB), also known as scab, is an increasingly important problem in the north-central region of the United States. Host resistance is considered the most practical and effective means of control but breeding has been hindered by a lack of effective resistance genes. Research funded by the National Wheat and Barley Scab Initiative (USWBSI) has led to the systematic evaluation of spring and winter wheat accessions contained in the National Small Grains Collection at Aberdeen Idaho and to the introduction of resistant germplasm from other programs globally. To date, approximately 15,000 spring and winter wheat accessions have been screened at South Dakota, Minnesota, and Missouri. Additionally, 479 spring and 308 winter wheats carrying putative resistance genes from wheat improvement programs in China, Japan, Argentina, Brazil, Uruguay, Romania, Hungary, and Mexico have been introduced through a collaborative effort between the USWBSI and CIMMYT. Although many good to excellent sources of resistance, particularly type II resistance, have been identified in winter and spring backgrounds no source of complete resistance has been identified and current sources continue to provide only partial resistance. Summarizing information generously provided by US wheat breeders it is clear that most of the FHB resistant germplasm being developed contains some level of type II resistance (reduced spread). Most spring and winter programs use Sumai 3, its derivatives including Ning 7840, or other Asian sources known to carry the 3BS QTL as a major source of type II resistance. The Sumai 3 resistance, particularly when derived from Ning 7840, has good combining ability and can be found in the pedigrees of many promising germplasm lines. More adapted germplasm lines from Virginia, Purdue, and Illinois derived from Ning 7840 are also noted by breeders as good sources of this resistance. Native genotypes that have low incidence, reduced spread, or both, are also widely used. Sources most commonly include: Freedom, Roane, Goldfield, Ernie, and Truman, and, to a lesser extent, McCormick, and Tribute. Promising germplasm combining Asian and native sources of resistance has now been developed in several programs. Some programs are also using sources of resistance identified through germplasm screening programs funded by the USWBSI. Both spring and winter wheat programs are using germplasm from CIMMYT and South America. Winter wheat programs are also using germplasm from Europe (predominantly the Romanian variety F201R). Hindering the incorporation of other sources identified through germplasm screening is the lack of adaptation of these lines. Although many contain very high levels of resistance, landraces in particular, are often late and tall and their direct utilization in main-stream breeding programs will slow the release of highly resistant soft red winter wheat rather than accelerate their release. Significant pre-breeding is needed to exploit these potentially valuable sources of resistance. Also impeding progress is the lack of genetic characterization of these lines. What FHB genes do they carry? How are the genes in these lines related to those in widely used sources from Asia and those characterized as 'native' sources of resistance? Knowledge of these genetic interrelationships is critical to making informed parental choices in programs aimed at developing lines with the multiple sources of resistance. Haplotype and molecular genetic diversity data based on AFLPs for 96 resistant genotypes from Europe, South America, the United States and Asia will be presented in an effort to provide interested breeders with preliminary information on the diversity of FHB alleles in these germplasm resources.

ENHANCING FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT: A GLANCE INTO SUCCESS AND CHALLENGES

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OBJECTIVES

To develop new improved hard red spring wheat (HRSW) cultivar and germplasm that combines resistance to Fusarium head blight (FHB), leaf diseases, and superior grain yield and bread-making quality.

INTRODUCTION

Scab disease in cereal crops has become a very important disease in recent years. The favorable environmental conditions including wet weather during flowering and grain filling and major changes in cropping system (introduction of Maize and minimum tillage) has favored disease development. Since 1993, FHB has caused serious loss of yield and quality in HRSW and durum wheat (*T. turgidum* L.) in the Northern Great Plains of the USA. Recent reports (Nganje et al., 2004) estimate combined direct and secondary economic losses caused by FHB for all crops were at $7.7 billion. Two states, ND and MN, account for about 68% ($5.2 billion) of the total dollar losses. Direct economic losses for wheat only were estimated to $2.492 billion from 1993 trough 2001 (Nganje et al., 2004). *Fusarium graminearum* Schwabe (perfect stage: *Gibberella zeae* (Schw.)Petch) has been the principal pathogen (McMullen et al., 1997; Stack, 2003). The FHB is unpredictable, and crop management generally has been ineffective for control. Chemical treatments can help reduce FHB but may not give an economic return. The only long –term and sustainable solution to FHB appears to be breeding for resistance (Meidaner, 1997). Resistance to FHB in wheat is a character of highly complex inheritance (Stack, 2003). Introducing complex resistance into commercial wheat and maintaining it through successive cycles of crossing to adapted but susceptible parents is a difficult task. This usually, requires continued effort using a reliable and repeatable disease testing procedures. Given the time and resources for that effort, however, there is no practical or theoretical reason why such a complex character such as FHB resistance cannot be added. In fact, grain yield and quality are also complex traits and they have been part and the driving force behind every wheat breeding effort. Using classical breeding techniques and various novel technologies, the NDSU HRSW breeding project aims to (1) develop improved HRSW cultivars and germplasm which combine higher levels of resistance to FHB, superior grain yield, and bread-making quality; and (2) identify, introgress, and pyramid novel FHB resistance from diverse germplasm sources into adapted HRSW germplasm base.

MATERIALS AND METHODS

Adapted FHB resistant parents developed by the NDSU and other breeding programs and the recently developed genotypes (from the scab initiative germplasm effort) are selected for planned matings in the greenhouse crossing blocks. The segregating populations generated from crosses are screened in field nursery. Breeding cycles have now progressed such that many of the new adapted parents have FHB resistance (type II mainly) based on previous field and greenhouse results plus other agronomic and quality parameters. To introduce FHB resistance into HRSW wheat cultivars adapted to ND and neighboring regions, we have combined extensive FHB screening done in an inoculated, irrigated field nursery and intensive testing of elite materials in the greenhouse. To maintain high disease pressure, a field nursery to screen wheat lines for resistance to FHB was established. Plots are elongated hill plots, each a single genotype, randomized within replicates. Throughout the nursery are multiple repeated check lines of known FHB reaction. Beginning at jointing stage *G. zeae*-colonized corn ("grain spawn") was distributed on the ground

throughout the nursery. By the time the earliest genotypes reached anthesis, blue perithecia of *G. zeae* stage were present on the grain spawn. Light mist irrigation was applied on an intermittent cycle for a period of 3 days per week. By 3 to 3.5 weeks postanthesis FHB had developed and was scored visually on 20 individual heads per hill plot using a 0-100% scale (Stack and McMullen, 1996). Grain was harvested from plots and proportion of visually scabby kernels (VSK) was determined. Deoxynivalenol (DON) levels in grain were determined by the NDSU Veterinary Sciences Laboratory using Gas Chromatographic analysis. The intensive greenhouse testing was done using the single spikelet method of FHB inoculation (Stack et al., 1997).

The advanced and elite HRSW lines generated by the breeding program which have FHB resistance are tested in preliminary, advanced, and elite yield trials at 2, 4, and 6 ND locations, respectively. The agronomic data; including grain yield and quality data, pests reactions including FHB due to natural infection, and shattering are generated from these trials. This data is also crucial to decision making on seed increase and eventual release of the new elite lines. Selected spikes/plants from the populations are sent to the winter nurseries in New Zealand to be advanced and selected for some agronomic traits such as height, maturity, shattering, and other plant type.

RESULTS AND DISCUSSIONS

The use of the FHB evaluation methods described above has enabled us to produce consistently very high FHB disease pressure (Table 1). This has facilitated the identification of improved lines. We have tested the FHB response in many lines representing progeny from first, second, third, and fourth breeding cycles. Some first and second cycle progeny showed good FHB resistance but none combined good FHB resistance with the agronomic traits and quality requirements that meet the commercial release. Several advanced cycles derived lines combined those traits however, and were released as a germplasm (Frhoberg et al., 2004; Mergoum et al., 2005) or commercial cultivars (Mergoum et al., 2005; 2006).

"Alsen"- was the first NDSU spring wheat cultivar released with good FHB resistance. It was derived from the three way cross "ND674//ND2710 /ND688". ND 674 and ND688 are two HRSW experimental lines developed by NDSU breeding program with good adaptation to ND wheat growing conditions and good end-use quality. Both lines are derived from 'Glupro' (PI 592759), a HRSW cultivar released in 1995 by NDSU for its very high grain protein content. ND2710 (Frohberg et al., 2004) is a HRSW experimental line developed by NDSU breeding program from a cross involving Sumai 3. Alsen agronomic performance and disease reactions are reported in Table 2. It has a good yield potential in general, especially in eastern ND where scab disease is prevalent. Test weight and lodging resistance are excellent for Alsen and shattering resistance appears satisfactory. Alsen is moderately resistant to predominant Upper Midwest races of leaf rust (caused by *Puccinia recondita* Rob. Ex Desm. f. sp. *Tritici*), resistant to such races of stem rust (caused by *Puccinia graminis* Per.:Pers. f. sp. *tritici* Eriks. & E. Henn), susceptible to tan spot [caused by *Pyrenophora tritici-repentis* (Died.) Drechs], moderately susceptible to the Septoria leaf disease complex [caused mainly by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano] and to common root rot (Tables 2, 4, and 5). Alsen has Fusarium head blight resistance type II expressed as reduced spreading of the disease in the spike (Table 5). Alsen has been planted on about 1 million hectares from 2002 to 2005; representing more than 30% of ND wheat acreages (N.D. Agricultural Statistics Service, USDA. 2002; 2003; 2004; 2005).

"Steele-ND"- is another HRSW wheat cultivar that was released by NDSU in 2004 with moderate FHB resistance (Mergoum et al., 2005). Steele-ND was selected from the cross 'Parshall' (PI 613587)/5/ 'Grandin' (PI 531005)/3/IAS20*4/H567.71// 'Amidon' (PI 527682)/4/ Grandin*2/'Glupro' (PI 592759). Steele-ND average FHB severity was 31.5% comparable to Alsen (28.7%) but significantly ($p<0.01$) lower than the susceptible check 'Reeder' (58.9%) (Table 5). Visual scabby kernels of Steele -ND (26.5 %) was also very low ($p<0.01$) compared to the susceptible check Reeder (37.2%), but slightly higher than Alsen (20.9%). Steele-ND does not include Sumai 3

in its pedigree and the source of resistance is believed to originate from the wheat relative *T. Dicoccoides*. A recombinant inbred lines (RIL) population derived from the cross of ND 735 with Steele-ND was developed for the purpose of mapping the FHB genes involved in Steele-ND. Grain yield of Steele-ND is similar to Reeder and, Parshall, and Alsen (Tables 3 and 4). Steele-ND is resistant to pathotype THBL, the predominant race of leaf rust in the region, and resistant to stem rust (Tables 3 and 5). Steele-ND is moderate resistant to Septoria nodorum and moderately susceptible to tan spot (Table 5).

"Glenn"- is the most recent NDSU HRSW release (2005) with improved FHB resistance compared to Alsen and Steele-ND. Glenn was selected from the progeny of the ND2831//Steele-ND cross. ND2831 is a Sumai 3 derivative line that has scab resistance similar to Alsen. This cross aimed to combine sources of FHB resistance from Alsen and Steele-ND, high yield, excellent quality, and standability into one package. Data collected during the testing period indicate that Glenn provides scab resistance (Table 4) and yield potential superior to Alsen; along with improved lodging, leaf diseases resistance, and equal or slightly better milling and baking quality (Table 4). Glenn has exceptional high grain volume (Table 4), as well as excellent end-use quality for the domestic and export wheat markets. Glenn grain yield is similar to Alsen, Parshall and Reeder, but lower than Steele-ND. Grain volume of Glenn is 811 kg m^{-3}, significantly higher than Alsen, Parshall, and Dapps. Protein content of Glenn (15.8%) is lower than Dapps (16.4%) but similar to Alsen, Parshall, and higher than Reeder (15.4%). Glenn exhibited a high level of resistance to the predominant races in the region of leaf rust and stem rust. It is medium resistant to tan spot and medium susceptible to septoria nodorum (Tables 4 and 5).

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Table 1. FHB index (Incidence x severity) and Tombstone (scabby) kernels in the North Dakota, hard red spring wheat during the 1995-2004 period.

Year	FHB Index[1]	Tombstone Kernels (VSK)[2]	DON[3]
	(Incidence X severity)	%	ppm
1995	3.0 - 56.6	3.3 - 19.6	0.6 - 7.7
1996	3.7 - 80.8	2.3 - 70.6	2.0 - 116.8
1997	36.8 - 100.	15.2 - 60.5	7.8 - 104.4
1998	19.9 - 80.7	1.8 - 95.9	0.6 - 59.7
1999	23.7 - 89.6	11.6 - 92.4	22.4 - 89.8
2000	22.3 - 92.0	13.9 - 82.8	3.2 - 62.7
2001	25.8 - 75.9	5.0 - 47.5	0.7 - 17.1
2002	32.0 - 97.4	30.0 - 92.5	18.8 - 114.
2003	3.4 - 60.6	8.6 - 65.8	-
2004	2.9 - 87.5	14.1 - 68.4	3.2 - 18.7

[1] FHB Iindex: FHB % by visual scoring at 3.5 wk after flowering; [2] VSK: Visually Scabby Kernels - proportion in harvested grain; [3] DON: Vomitoxin determined by GC analysis of harvested grain.

Table 2. Agronomic traits and reaction to FHB and leaf diseases of Alsen and five other hard red spring wheat cultivars in North Dakota , USA, during the 1998-2000 period.

				Reaction[1]					
Variety	Days to heading	Height	Lodging	Leaf rust	Septoria	FHB	Test weight	Protein	Grain yield
	Days	cm	1-9				Kgm^{-3}	%	$Kgha^{-1}$
Butte 86	59	89	1.5	MS	MS	S	757	15.5	3507
Russ	60	89	1.8	R	R	S	743	15.1	3521
Gunner	63	89	1.3	MS	MR	MS	770	14.4	3306
2375	60	84	3.8	S	S	MS	768	14.7	3467
Grandin	62	79	1.9	MS	S	S	759	15.7	3003
Alsen	61	84	0.9	MR	MR	MR	770	15.3	3279

[1]R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible

Table 3. Agronomic traits and reaction to FHB and leaf diseases of Steele-ND and the most grown three major hard red spring wheat cultivars in North Dakota , USA, during the 2000-2003 period.

				Reaction[1]					
Variety	Days to heading	Height	Lodging	Leaf rust	Tan Spot	FHB	Test weight	Protein	Grain yield
	Days	cm	1-9				Kgm^{-3}	%	$Kgha^{-1}$
Steele-ND	61	84	2.7	R	MS/MR	MR/MS	762	15.7	3837
Alsen	59	79	1.9	MR	S	MR	762	16	3689
Parshall	60	89	1.8	MS/S	MS	MS	764	15.8	3716
Reeder	59	79	1.5	S	MR	S	770	15.5	3910

[1]R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible

Table 4. Agronomic traits and reaction to FHB and leaf diseases of Glenn and five most grown hard red spring wheat cultivars in North Dakota, USA, during the 2002-2004 period.

				Reaction[1]					
Variety	Days to heading	Height	Lodging	Leaf rust	Tan Spot	FHB	Test weight	Protein	Grain yield
	Days	cm	1-9				Kgm^{-3}	%	$Kgha^{-1}$
Glenn	65	87	0.7	R	MS/MR	MR/R	806	15.8	4421
Alsen	65	84	1.1	MR/MS	S	MR	770	15.6	4317
Dapps	65	91	1.2	R/MR	MR	MS	772	16.4	4209
Parshall	65	94	1.3	MS/S	MS	MS	768	15.6	4347
Reeder	66	83	0.5	S	MR	S	755	15.4	4519
Steele-ND	66	87	1.9	R/tMR	MS/MR	MR/MS	772	15.6	4552

[1]R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible

Table 5. Fusarium head blight (FHB) severity, Tombstone and Deoxynivalenol toxin levels; leaf rust , stem rust , tan spot, and Septoria nodorum reactions of HRSW cultivars under natural (3 locations) and artificial (7 locations) inoculation in the filed and greenhouse conditions (4 tests) in Fargo, ND from 2001 to 2004.

Genotype	FHB Severity (Field test under natural infection) [1]			FHB Severity (Field test under artificial inoculation)			FHB GH	SR[2]	TS	SN
	SEV (%)	TMB (%)	DON (ppm)	SEV (%)	TMB (%)	DON (ppm)	SEV (%)		(1-5)	(1-5)
Glenn	7.6	0.7	0.4	18.9	16.0	4.0	16.3	R	3	3
Steele-ND	19.3	1.3	0.9	31.5	26.5	5.3	24.6	R	4	3
Alsen	7.0	0.9	0.8	28.7	20.9	4.8	10.8	R	5	5
Reeder	26.2	5.7	1.5	58.9	37.2	10.3	42.0	R	4	4
2398	41.8	5.4	2.0	75.2	51.7	9.9	55.5	R	-	-

[1]SEV=Severity; TMB= Tombstone; and DON= Deoxynivalenol toxin;
[2] SR= Stem rust; TS= Tan spot; SN= Spetoria nodorum; R=Resistant and MS=Moderate susceptible.

"GLENN" HARD RED SPRING WHEAT CULTIVAR: A NEW STEP IN COMBATING FUSARIUM HEAD BLIGHT DISEASE

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OBJECTIVES

To develop new improved hard red spring wheat (HRSW) cultivar that combines novel source of resistance to Fusarium head blight (FHB) disease and superior grain yield and bread-making quality.

INTRODUCTION

It well documented that today, Fusarium head blight (FHB), commonly known as scab, is one of the serious threat to wheat production throughout the world (Schroeder and Christenson, 1963; Bai and Shaner, 1994; McMullen et al., 1997; Stack, 2003). In North America, FHB is caused mainly by *Fusarium graminearum* Schwabe [telomorph *Gibberella zeae* (Schwein.)] (Bai and Shaner, 1994; McMullen et al., 1997). Wheat FHB has been a major disease for hard red spring wheat (HRSW) produced in North Dakota and neighboring states since 1993. Recent estimates (Nganje et al., 2004) showed that the combined direct and secondary economic losses caused by FHB for all crops were at $7.7 billion. North Dakota and MN, account for about 68% ($5.2 billion) of the total dollar losses. Direct economic losses for wheat only were estimated to $2.492 billion from 1993 trough 2001 (Nganje et al., 2004). The use of genetically resistant cultivars is believed to the most efficient and economical method of controlling this disease in HRSW. In 2002, 2003, 2004, and 2005 crop seasons, "Alsen", a moderate FHB resistance cultivar derived from the Chinese source "Sumai 3", released in 2000 by NDSU (with the support of the scab initiative funds) was planted on more than 2.1, 2.4, and 1.9 million acres representing 30.8, 37.4, 28.9, and 23.1% of the 6.8 million acres of ND wheat, respectively (N.D. Agricultural Statistics Service, USDA. 2002; 2003; 2004; 2005). Similarly, "Steele-ND", a new NDSU HRSW cultivar released in 2004 covered as much as 1.4% of ND wheat acreages. The rapid increase in acreage planted to 'Alsen' and Steele-ND indicates the desire of ND wheat growers to produce such HRSW cultivars. However, the level of resistance of both Alsen and Steele-ND needs to be improved in order to better control FHB. Therefore, any new HRSW cultivar with better FHB resistance than the actual grown HRSW cultivars is warranted.

MATERIALS AND METHODS

To introduce FHB resistance into adapted quality wheats in North Dakota, we have combined extensive FHB screening done in an inoculated, irrigated field nursery and intensive testing of elite materials in the greenhouse. To maintain high disease pressure, a field nursery to screen wheat lines for resistance to FHB was established. Plots were elongated hill plots, each a single genotype, randomized within replicates. Throughout the nursery were multiple repeated check lines of known FHB reaction. Beginning at jointing stage *G. zeae*-colonized corn ("grain spawn") was distributed on the ground throughout the nursery. By the time the earliest genotypes reached anthesis, blue perithecia of *G. zeae* stage were present on the grain spawn. Light mist irrigation was applied on an intermittent cycle for a period of 3 days per week. By 3 - 3.5 weeks postanthesis FHB had developed and was scored visually on 20 individual heads per hill plot using a 0-100% scale (Stack and McMullen, 1996). Grain was harvested from plots and proportion of visually scabby kernels (VSK) was determined. Deoxynivalenol (DON) levels in grain were determined by the NDSU Veterinary Sciences Laboratory using Gas Chromatographic analysis. The intensive greenhouse testing was done using the single spikelet method of FHB inoculation (Stack et al., 1997). As wheat

heads reached anthesis, ten heads in each replicate of each line were individually inoculated by placing a 10 ml droplet of *F. graminearum* spore suspension inside a floret near the midpoint of the head. A gentle overhead mist was applied for three nights following inoculation. FHB was scored at 3.5 wk as described above. Levels of tombstone kernels and DON were also determined in harvested grain.

RESULTS AND DISCUSSIONS

"Glenn" is the most recent NDSU HRSW release (2005) with improved FHB resistance compared to Alsen. Glenn was selected from the progeny of the cross: ND2831//Steele-ND. ND2831 is a Sumai3 derivative line that has scab resistance similar to Alsen. This cross aimed to combine sources of FHB resistance from Alsen and Steele-ND, high yield, excellent quality, and standability into one package. Data collected during the testing period indicate that Glenn provides scab resistance and yield potential superior to Alsen; along with improved standability, an improved leaf disease package, and equal or slightly better milling and baking quality. The improved scab resistance and yield advantage of Glenn is especially evident in areas where disease pressure is high (i.e. Eastern North Dakota). Glenn also exhibits exceptionally high test weight. Glenn appears to have the potential to provide producers with an alternative for 'Alsen'.

Reaction to FHB - Glenn was tested for FHB in seven location-years in the FHB nursery grown at Prosper, ND under artificial inoculation using overhead irrigation techniques. It was also evaluated in three environments under natural FHB infection and in four experiments under greenhouse conditions using the spray inoculation. On the basis of seven location-years of testing in the FHB nursery conducted under field conditions, the FHB incidence (Stack et al., 1997) recorded for Glenn (19%) was significantly higher than the most resistant line '2710' (9%) developed by NDSU (Frohberg et al., 2004); but significantly lower than the incidence for the moderately resistant checks Alsen (29%) and Steele-ND (31%); and susceptible checks Reeder (59%) and '2398' (42%). Similarly, on the basis of the three location-years of testing for FHB under natural infection conducted under field conditions, the FHB incidence recorded for Glenn was 8% compared to 2, 7, 19, 26, and 42% scored for 2710, Alsen, Steele-ND, Reeder, and Pioneer 2375, respectively. Under greenhouse conditions, the FHB incidence of Glenn based on four tests was 16% compared to 9, 11, 25, 42, and 56% for 2710, Alsen, Steele-ND, Reeder, and Pioneer 2375, respectively. Glenn was also evaluated for the levels of the trichothecene mycotoxin deoxynivalenol (DON) produced by FHB in three naturally and three artificially infected field tests. Under naturally infected conditions, the DON level of Glenn was 0.4 $\mu g\ g^{-1}$ compared to 0.5, 0.8, 0.9, 1.5, and 2 $\mu g\ g^{-1}$ for 2710, Alsen, Steele-ND, Reeder, and Pioneer 2375, respectively. Under artificial inoculation, the DON level of Glenn (4 $\mu g\ g^{-1}$) was similar to ND 2710 (2.9 $\mu g\ g^{-1}$), Alsen (4.8 $\mu g\ g^{-1}$), and Steele-ND (5.3 $\mu g\ g^{-1}$); but significantly lower that than the DON levels of Pioneer 2375 (7.4 $\mu g\ g^{-1}$), Reeder (10.3 $\mu g\ g^{-1}$), and 2398 (9.9 $\mu g\ g^{-1}$). Alsen was released in 2000 as the first NDSU HRSW cultivar with resistance to FHB from the Chinese 'Sumai 3' (PI 481542) and has been widely grown in the northern plains since 2001. Steele-ND, a NDSU HRSW cultivar released in 2004 (Mergoum et al., 2005b), has resistance to FHB comparable to Alsen but has parentage different from the Chinese Sumai 3. Compared to ND 744, Glenn has similar FHB resistance and agronomic performance. However, ND 744 has harder kernels, lower (10 g kg^{-1} in average) protein content, and grain volume than Glenn. Based on seedling and adult plant screening tests conducted under greenhouse conditions from 2000-2004, Glenn exhibited a high level of resistance to pathotype THBL, the predominant race of leaf rust in the region. Glenn was evaluated from 2000 to 2004 at the USDA-ARS, Cereal Crop Research Unit, Fargo, ND for resistance to stem rust (caused by *Puccinia graminis* Per.:Pers. f. sp. *tritici* Eriks. & E. Henn) and was found to be resistant to pathotypes Pgt-QCCJ, -QTHJ, -RTQQ, -TMLK, -TPMK, and -HPHJ. On a scale of 1 to 5 where 1 is resistant and 5 susceptible, Glenn had average scores of 4 and 3 in reaction to Septoria nodorum [caused by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano] and tan spot [caused by *Pyrenophora tritici-repentis* (Died.) Drechs] compared to 5 and 5 for the susceptible cultivar Alsen and

1 and 1 for the resistant check 'Erik' (PI 476849), respectively.

Agronomic performance and quality parameters - Data from 31 site-years in the ND Variety Trials, grain yield of Glenn (4381 kg ha^{-1}) was similar to 'Alsen' (PI 615543) (4300 kg ha^{-1}), 'Parshall' (PI 613587) (4347 kg ha^{-1}) and 'Reeder' (PI 613586) (4448 kg ha^{-1}), but lower ($P<0.05$) than Steele-ND (4885 kg ha^{-1}). In the same trials grain volume of Glenn was 811 kg m^{-3}, significantly higher ($P<0.05$) than 767, 768, and 776 kg m^{-3} of Alsen, Parshall, and 'Dapps' (PI 633862), respectively. Protein content of Glenn (166 g kg^{-1}) was lower than Dapps (171 g kg^{-1}) but similar to Alsen (163 g kg^{-1}), Parshall (164 g kg^{-1}), and higher than Reeder (161 g kg^{-1}). On the basis of 36 locations of the URN conducted in 2003 and 2004, mean grain yield, grain volume weight, and protein content of Glenn were 4340 kg ha^{-1}, 794 kg m^{-3}, and 154 g kg^{-1}, respectively, compared to Steele-ND (4515 kg ha^{-1}, 775 kg m^{-3}, and 152 g kg^{-1}), 'Pioneer 2375' (4475 kg ha^{-1}, 777 kg m^{-3}, and 143 g kg^{-1}), and 'Verde' (PI 592561) (4461 kg ha^{-1}, 762 kg m^{-3}, and 142 g kg^{-1}). Flour yield for Glenn from 13 trials grown in ND averaged 684 g kg^{-1} compared to 692, 693, and 678 g kg^{-1} for Alsen, Parshall, and Reeder, respectively. Water absorption was 65.9%, significantly higher than Reeder (64.8%), but not different from Alsen (65.1%), and Parshall (65.2%). The mixing tolerance of Glenn (20.9 min) was longer than all of the checks including Reeder (13.9 min), Alsen (16.4 min), and Parshall (17.0 min). Loaf volume was 1103 mL, comparable to Parshall (1090 mL) and Alsen (1076 mL), but superior to Reeder (1015 mL).

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FHB RESISTANCE OF THE USSRW SCREENING NURSERY WITH THE MICRO PLOT METHOD

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ABSTRACT

Forty-eight lines were tested in 2005 for Fusarium head blight (FHB) in wheat. All genotypes were sown in a 5 m^2 plot and inoculated at the same time, 31 May. In heading and flowering the genotypes differed 6 days. We thought, even the earlier genotypes were inoculated several days later than optimal, but the problems arising from the different meteorological conditions cause more problems than the several days delay in inoculation. From earlier experience we know that the window for a high infection is about 7-10 days which is significantly longer than generally accepted. For this reason we think that the comparability of the data is much better than before. This view is supported by the fact that the resistant control Ernie is among the best genotypes and the susceptible Cooker takes on of the last positions.

Four isolates were used in each plot, inoculating three groups of heads consisting of 15-20 heads for each isolate with about 15 ml inoculum. After inoculation the groups of heads were covered by plastic bags to secure the high humidity for the infection. No additional mist irrigation was used as they interfere severely with the symptom development of the genotypes. Also three check bunches were added to enable yield response analysis. FHB severity (% of infected spikelets) were rated 10, 14, 18, 22, and 26 days after inoculation. At full ripening the bunches were harvested, from each group ten average ears were separated for threshing with no wind, thereafter a cleaning were made with no loss of infected grains. Fusarium damaged kernels (FDK) was visually evaluated. In several cases also toxin analysis for DON has been made.

The most resistant genotype had 0.46 % FHB as a mean of five readings. The maximum is 30.69 %. The ratio of Fusarium damaged kernels was between 1 and 68 %, the yield loss spreads between 5.6 and 56 %. The correlation between traits is generally $r = 0.80$, significant at $P = 0.001$. This means that lower FHB data normally mean lower FDK and yield loss. Even so, at 12 % FHB we find FDK data between 3 and 38 %. For this reason an absolute forecast of the FDK values or yield loss for a given genotype is not possible as also resistance types influence the infection process. Therefore all parameters should be evaluated.

We represent the view that the American breeding made significant steps in breeding lines with good or excellent FHB resistance. In several lines, also very high resistance to Septoria tritici leaf spot and other leaf spots has been identified combined in some cases with good quality and yield potential.

Additional data for leaf diseases, quality data, plant height, yielding ability and other data help breeders to evaluate the materials properly.

THE 2004-05 UNIFORM SOUTHERN FUSARIUM HEAD BLIGHT SCREENING NURSERY

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ABSTRACT

Phenotypic estimates of host resistance to Fusarium head blight (FHB) are greatly confounded by genotype x environment interaction effects. Thus, multiple evaluations of genotypes are necessary in order to determine true genetic worth. The objectives of the Uniform Southern FHB Nursery are to provide breeders with a comprehensive set of resistance estimates on advanced generation lines in a timely fashion, and to facilitate the sharing of the best resistant materials throughout the breeding community. The 2004-05 nursery comprised 46 advanced generation breeding lies and two check cultivars, 'Ernie' (partially resistant) and 'Coker 9835' (susceptible). Seven U.S. public programs (Univ. of Arkansas, Univ. of Georgia, Louisiana State Univ., Univ. of Maryland, N.C. State Univ, VA-Tech, and USDA-ARS), two private companies (Agripro and Syngenta) and one Romanian (Fundulea) program submitted entries for evaluation. A comprehensive set of field, greenhouse and laboratory results was submitted by eight U.S., one Romanian and one Hungarian cooperator. A preliminary summary table of means for several important host resistance traits is presented below. Copies of the full report will be available at the 2005 National Fusarium Head Blight Forum and subsequently on line at the USWBSI web site: http://www.scabusa.org/.

UNIFORM SOUTHERN FHB NURSERY MEANS ACROSS LOCATIONS 2004-05

	Cultivar/ Designation	FHB Incidence	RANK	FHB Severity	RANK	FHB Index	RANK	FDK	RANK	ISK	RANK	DON	RANK	G'hse Type II	RANK	Heading Date	RANK	Plant Height	RANK	Qfhs.ndsu-3BS Xgwm533.1 161	Qfhs.ndsu-3BS XBARC133 142	Qfhs.ifa-5A Xgwm293 218	Qfhs.ifa-5A Xgwm156 342
1	ERNIE	42	3	16	5	5.7	3	16	3	24	1	1.7	12	9	3	130	5	32	6	.	X	.	.
2	COKER 9835	80	47	46	47	37.3	46	35	42	56	46	12.1	47	46	43	134	41	62	1	.	.	.	.
3	B006624	60	23	19	9	14.3	18	14	1	31	6	3.9	29	38	38	132	20	37	27	X	.	.	.
4	B990081	51	10	21	12	9.2	8	23	16	31	6	0.8	2	26	25	132	20	41	36	X	.	.	.
5	02JH000014	55	14	16	5	10.1	10	27	24	33	10	2.4	20	9	3	132	20	37	41	X	.	.	.
6	AR97002-2-1	52	11	17	8	7.9	5	24	17	33	10	1.2	6	13	9	130	5	33	21	.	.	X	.
7	AR97002-2-2	45	4	14	2	4.5	2	28	29	29	4	1.2	6	8	2	130	5	30	11	.	.	X	.
8	AR97048-1-1	59	21	26	24	14.5	19	22	14	36	18	4.5	31	18	13	133	33	46	47	X	.	.	.
9	AR97048-4-1	72	42	36	42	27.1	43	31	38	48	41	11.8	46	37	37	134	41	42	47	X	.	.	.
10	AR97048-7-1	70	38	27	27	18.8	31	30	35	44	33	5.9	38	15	11	135	46	49	46	X	.	.	.
11	AR97124-4-1	47	5	19	9	9.5	9	20	8	29	4	2.1	18	24	21	132	20	40	44	.	.	.	.
12	D00*6847-1	58	19	25	23	16.0	25	31	38	42	26	4.3	30	42	41	131	12	37	27	.	.	.	.
13	D00*6874-1	49	9	32	37	15.1	21	30	35	42	26	5.7	36	39	39	130	5	38	36	.	.	.	.
14	D00*6874-9	53	12	30	31	13.9	15	24	17	37	21	3.4	27	41	40	130	5	49	27	.	.	.	.
15	D01*7759	73	44	32	37	21.1	33	28	29	41	23	3.1	25	56	46	132	20	39	41	.	.	.	.
16	D01-7017	64	28	32	37	20.6	32	28	29	44	33	4.7	33	21	16	132	20	45	27	.	.	.	.
17	F92080G-01102	56	16	16	5	8.9	7	24	17	33	10	2.3	19	11	7	134	41	42	21	.	.	.	.
18	F95812G1-1 Fz1	70	38	30	31	24.7	40	26	21	47	39	2.9	23	18	13	133	33	35	11	.	.	.	.
19	F96035G11-2	59	21	15	3	8.2	6	20	8	33	10	2.0	17	14	10	131	12	37	11	.	.	.	.
20	GA951395-3E25	70	38	35	40	21.2	36	27	24	43	31	6.0	39	30	28	133	33	35	11	.	.	.	.
21	GA961171-3E38	64	28	35	40	25.1	41	29	33	47	39	8.0	43	25	23	129	2	44	1	.	.	.	.
22	GA961176-3A48	67	34	30	31	21.1	33	27	24	44	33	6.1	40	23	19	131	12	45	21	.	.	.	.
23	GA961526-3E15	67	34	38	44	25.6	42	38	45	50	42	9.0	45	53	45	132	20	59	21	.	.	.	.
24	GA96229-3A41	77	45	50	48	39.2	48	45	48	60	48	17.6	48	64	47	133	33	65	27	.	.	.	.
25	GA96229-3E39	69	37	42	46	29.8	45	39	46	53	45	7.5	42	69	48	132	20	62	27	.	.	.	.
26	LA9560CA22-1	66	32	30	31	21.6	37	37	44	50	42	4.6	32	36	36	133	33	40	44	.	.	.	.
27	LA97407D-17-4	48	6	24	21	13.2	13	21	13	35	16	1.1	5	32	32	128	1	40	11	.	.	.	.
28	LA97448D-27-4	48	6	26	24	13.6	14	17	4	31	6	2.5	21	50	44	129	2	49	36	.	.	.	.
29	LSU04FHB02	60	23	29	28	17.1	26	20	8	39	22	3.3	26	35	35	130	5	39	41	.	.	.	.
30	M01*1019	72	42	23	16	17.2	27	27	24	42	26	1.8	13	21	16	133	33	43	6	.	.	.	.
31	MV-5-46	71	41	31	36	22.2	39	30	35	45	37	5.5	35	29	26	132	20	48	1	.	.	.	.
32	NC03-11457	67	34	29	28	21.7	38	19	7	42	26	1.4	8	15	11	132	20	37	6	X	X	X	X
33	NC03-11458	54	13	22	14	11.6	11	15	2	31	6	1.5	9	21	16	132	20	39	6	X	X	X	X
34	NC03-11465	37	1	15	3	7.3	4	17	4	25	2	0.9	3	25	23	136	47	47	21	X	X	.	.
35	NC03-11561	55	14	23	16	15.0	20	24	17	34	14	1.5	9	31	30	137	48	34	6	X	X	.	.
36	NC03-11588	77	45	36	42	29.6	44	29	33	51	44	5.8	37	29	26	134	41	53	11	.	.	.	.
37	PI564341	86	48	40	45	37.7	47	40	47	58	47	8.1	44	34	34	132	20	53	1	.	.	.	.
38	PI564385	64	28	26	24	18.7	30	26	21	42	26	6.3	41	24	21	133	33	50	36	.	.	.	.
39	TX96D1073	57	18	23	16	13.9	15	26	21	36	18	1.8	13	31	30	131	12	43	21	.	.	.	.
40	TX98D1170	60	23	22	14	21.1	33	36	43	46	38	4.9	34	10	5	132	20	40	27	.	.	.	.
41	TX98D2423	60	23	20	11	12.9	12	20	8	34	14	1.5	9	30	28	134	41	48	27	.	.	.	.
42	TX99D4478	58	19	24	21	15.1	21	32	40	43	31	3.0	24	20	15	131	12	40	27	.	.	.	.
43	VA01W-310	66	32	29	28	18.4	29	28	29	41	23	1.8	13	44	42	133	33	53	11	.	.	.	.
44	VA04W-433	41	2	10	1	4.1	1	18	6	25	2	0.5	1	10	5	129	2	35	11	X	X	.	.
45	VA04W-503	60	23	30	31	17.8	28	27	24	41	23	3.8	28	32	32	131	12	47	36	.	.	.	.
46	VA04W-547	64	28	23	16	15.3	24	34	41	44	33	2.7	22	23	19	131	12	41	11	.	.	.	.
47	VA04W-608	56	16	23	16	15.1	21	20	8	36	18	1.9	16	11	7	130	5	34	11	.	.	.	.
48	VA04W-628	48	6	21	12	13.9	15	22	14	35	16	0.9	3	6	1	131	12	31	1	.	X	.	.
	Sumai 3																			X	X	X	X
		61		27		17.6		26.4		40		4.1		28		132		36					
		17		10		12.4		15.6		14		.		18		2		2					
		27		42		67.0		52.4		29		.		47.2		1.5		5.4					

FINE MAPPING OF A QTL REGION ASSOCIATED WITH FUSARIUM HEAD BLIGHT, KERNEL DISCOLORATION, GRAIN PROTEIN CONCENTRATION, AND HEADING DATE ON BARLEY CHROMOSOME 6H

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OBJECTIVES

To fine map a quantitative trait loci (QTL) region on chromosome 6H in barley (*Hordeum vulgare* L.) previously found to be associated Fusarium head blight (FHB) resistance, kernel discoloration (KD), grain protein concentration (GPC) and heading date (HD); and to determine if the associations can be attributed to tight linkage or pleiotropic effects of a single locus.

INTRODUCTION

In barley, numerous QTLs have been identified for FHB, KD, GPC, and HD (de la Pena et al., 1999; See et al., 2002; Canci et al., 2003; Canci et al., 2004) on all seven chromosomes. However, most of these QTLs are assigned to regions spanning 10-40 cM and therefore cannot be efficiently used for marker-assisted-selection (MAS). To be useful for MAS, the QTL must be validated and localized to a small region flanked by "breeder friendly" markers. We validated QTL for GPC and KD using two mapping populations derived from the cultivar MNBrite (Canci et al., 2003). MNBrite was developed by the University of Minnesota and carries Chevron alleles that condition KD resistance and high GPC, and is moderately resistant to FHB. Results of this study confirmed that the QTL for KD and GPC are coincident and map between markers *Bmag0807* and *Bmac0218* (26 cM) on chromosome 6H. Other studies have detected FHB and HD QTL in the same marker interval (Canci et al., 2004); suggesting that selection for KD resistance in the development of MNBrite may have resulted in increased FHB resistance. MNBrite is also unacceptably high in GPC due in part to the effects of this region on chromosome 6H. We initiated a fine-mapping study to determine the genetic relationships among FHB, KD, and GPC in this region of chromosome 6H.

In this study, we used substitution mapping approach as described by Peterson et al. (1990) to fine map the coincident QTL region. This approach uses recombinant near isogenic lines (rNILs) for mapping and is a powerful method for distinguishing linkage versus pleiotropy.

MATERIALS AND METHODS

Development of the rNILs - A donor parent from the mapping population described by de la Pena et al. (1999) and carrying the Chevron allele at the target QTL region was crossed with the recurrent elite parent M69. A marker-assisted backcrossing scheme using markers *GBM1021* and *EBmac0602* flanking the target QTL region was used to advance lines to the BC3S4. One of the BC3S4 lines (designated as C113) was backcrossed 3 more times to the recurrent parent Lacey. A population of 1200 F3 plants was then developed and screened with the two flanking markers to identify rNILs. One hundred and twenty-nine rNILs were identified. These were then genotyped using five additional SSR markers previously mapped at the *GBM1021-EBmac0602* interval. A linkage map using genotypic data from the entire population of F3s was constructed using the JoinMap version 2.0 (Stam, 1993). The 129 rNILs were advanced to the F3:5 generation for use in field evaluations.

Field Evaluations of rNILs - The 129 rNILs and parental lines Chevron and Lacey were evaluated in

the summer of 2005 at the University of Minnesota Agricultural Experiment Stations at St. Paul, Morris, and Crookston. Entries at each environment were arranged in a randomized complete block design with 2 replications. They were planted in 2.4 m long single-row plots and spaced at 30 cm apart. To inoculate plots, the macroconidia technique was used at St. Paul while the grain-spawn inoculation technique was used at Morris and Crookston as described by Mesfin et al. (2003). To enhance disease development, nurseries were mist-irrigated until the soft dough stage. Four traits, FHB, KD, GPC, and HD were measured for each plot. Data for HD was not collected at Crookston. The percent FHB severity was measured by counting the number of infected spikelets from each spike using10 randomly selected plants from each plot and the count was expressed as a percent of the total spikelets present. Kernel discoloration was measured using a scale of 1-5 (1 = no discoloration and 5 = most discolored) as described by Miles et al. (1987). Grain protein concentration was measured using the Model 6500 NIR Spectrometer (Foss North America). Heading date was recorded as the number of days from planting to 50% emergence from the boot.

Data Analysis - The Proc GLM (SAS Institute, 2000) procedure was used to conduct analysis of variance. Significant G x E effects were observed for all traits except KD and therefore further analyses were performed on individual environments. Means for rNILs and their parental lines were separated using LSD. The means of rNILs carrying similar marker profiles were averaged and compared to the mean of the rNILs carrying Lacey allele for the entire QTL region (control). A genomic region was then declared to be associated with a trait QTL when the trait mean of the set of rNILs carrying the Chevron allele at that region was significantly different from the susceptible control.

RESULTS AND DISCUSSION

The original chromosome 6H region previously found to contain the coincident QTL of interest mapped within a 34 cM segment. The construction of a fine map using 1200 F3s estimated the size of this region as 11 cM. Marker order in the fine map is consistent with the order in the original map.

There was a significant difference among rNILs for all traits measured except for FHB severity at St. Paul; suggesting that each trait QTL was segregating amongst the rNILs evaluated (Table 1). In Crookston, the mean FHB severity of subNILs carrying the Chevron allele at the entire target QTL region was significantly lower than the mean of subNILs carrying the Lacey allele across the QTL region (Fig. 1). The Chevron allele at this environment reduced FHB severity by over 50%. These results suggest that the QTL for FHB maps within the region. However, we could not precisely map the position of the QTL In Morris, we did not detect significant FHB QTL and we believe this was because we assessed FHB severity at this environment when plants were starting to show signs of senescence. The mean percent GPC for subNILs carrying the Lacey allele at the *Bmag0807-GBM1063* interval (2 cM) was 0.5-0.7% lower compared to the mean percent GPC for subNILs carrying the Chevron allele at the same marker locus in all environments tested. This suggests that the QTL for GPC is positioned at the *Bmag0807-GBM1063* interval. Similarly, the mean KD score for subNILs carrying the Lacey allele at the *Bmag0807-GBM1063* interval were found to be significantly higher than those carrying the Chevron allele; suggesting that the KD QTL maps in the same position. Although the HD QTL was generally localized in the same *Bmag0807-GBM1021* interval in the St. Paul environment, the effect was negligible (only 0.2 d).

It appears that GPC and KD map clearly within a 2 cM interval on chromosome 6H as compared to the 26 cM previously mapped by Canci et al. (2003). However, we cannot determine whether the same locus also controls FHB. Additional data collection in three more locations is planned for next year.

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Table 1. Chevron/Lacey population means and P-values for *Fusarium* head blight (FHB), kernel discoloration (KD), grain protein content (GPC), and heading date (HD).

Trait	Environments	Chevron	Lacey	Population	Population range	p-value[a]
FHB[b]	St. Paul 2005	1.3	12.2	6.5	1.9-17.7	ns
	Crookston 2005	1.4	9.7	8.0	0.3-28.0	<0.0001
	Morris 2005	1.9	14.7	16.8	2.6-30.5	<0.0001
KD[c]	St. Paul 2005	1.0	3.0	3.6	1.0-5.0	<0.0001
	Crookston 2005	1.0	3.8	3.3	2.0-5.0	<0.0001
	Morris 2005	1.0	3.0	3.3	2.0-5.0	<0.0001
GPC[e]	St. Paul 2005	15.1	12.7	12.8	11.3-15.4	<0.0001
	Crookston 2005	15.9	13.0	13.5	11.4-15.8	<0.0001
	Morris 2005	16.2	14.0	13.6	12.2-16.0	<0.0001
HD	St. Paul 2005	54.0	49.5	51.1	49.0-52.0	<0.0001
	Morris 2005	56.0	53.0	53.8	53.0-55.0	<0.0001

[a] Test for significant variation among $F_{3:5}$ rNILs
[b] FHB severity (% of infected kernels)
[c] KD score on a 1-5 scale (1=no discoloration, 5=heavily discolored)
[e] Days to anthesis

		FHB		HD		GPC		KD	
#rNILs		MO	CR	SP	MO	SP	MO	CR	SP	MO
15		18.4	11.9	50.9	53.8	12.5	13.5	13.0	4.0	3.6
58		14.4	8.6	51.0	53.8	12.5	13.1	13.3	3.7	3.5
3		16.2	**4.4***	51.3*	54.0	12.7	13.3	13.5	4.0	3.3
2		22.6	8.3	51.5*	53.5	**13.4***	**15.1***	**14.9***	**3.3***	**2.5***
19		19.7	**4.6***	51.5*	53.9	**13.7***	**14.8***	**14.3***	**2.6***	**2.6***
3		24.7	**5.9***	51.3*	54.0	**13.6***	**15.1***	**14.7***	**2.6***	**2.8***
6		17.0	8.6	50.8	53.8	12.3	13.2	13.0	3.8	3.5

= Lacey allele; = Chevron allele

Fig. 1. Mean effects for Fusarium head blight (FHB) severity, heading date (HD), grain protein concentration (GPC), and kernel discoloration (KD) across rNILs of similar graphical genotypes are shown below. Traits were measured in Minnesota at St. Paul (SP), Morris (MO), and Crookston (CR) in 2005. Asterisks indicate a significant mean difference (P=0.05) from the mean of the rNILs carrying the Lacey allele across the entire region using LSD; ns, not significant.

FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID WHEAT

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ABSTRACT

Host resistance has been considered a cost-efficient and environmentally sound strategy to combat Fusarium head blight (FHB); however, progress in developing FHB-resistant wheat cultivars has been hindered by a lack of effective sources of resistance. Although resistant sources have been identified and utilized in hexaploid wheat (*Triticum aestivum* L., 2n = 6x = 42, genomes AABBDD), sources of resistance in durum wheat (*T. turgidum* ssp. *durum* L., 2n = 4x = 28, genomes AABB) are scarce. The objective of this study is to identify germplasm that may be used to enhance FHB resistance in durum wheat. The plant materials comprised 393 accessions of five cultivated subspecies under *T. turgidum*. These subspecies include cultivated emmer wheat [*T. turgidum* ssp. *dicoccum* (Schrank ex Schübler) Thell.], Persian wheat [*T. turgidum* ssp. *carthlicum* (Nevski) Á. Löve and D. Löve], Polish wheat [*T. turgidum* ssp. *polonicum* (L.) Thell.], oriental wheat [*T. turgidum* ssp. *turanicum* (Jakubz.) Á. Löve and D. Löve], and poulard wheat (*T. turgidum* ssp. *turgidum*). These accessions were evaluated for Type II FHB resistance (resistance to the spread of infection) by single spikelet inoculation over three greenhouse seasons. Approximately eighty accessions showed a level of resistance similar to 'Sumai 3', the Chinese common wheat cultivar considered the standard for FHB resistance. Sixty-seven of the 80 accessions identified as resistant to FHB in the greenhouse were further evaluated for FHB reaction in mist-irrigated field nurseries in two locations (Fargo and Langdon, ND). The grain spawn method of inoculation was used. Eighteen accessions exhibited resistance comparable to Sumai 3 in both locations. These resistant tetraploid wheat accessions represent a novel source of FHB resistance and could be utilized directly in durum wheat breeding. Introgression of FHB resistance from these tetraploid wheat accessions to durum is in progress.

WHEAT CULTIVARS WITH IMPROVED RESISTANCE TO FUSARIUM HEAD BLIGHT FOR EASTERN CANADA

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OBJECTIVES

To determine the criteria required to develop FHB-resistant wheat with low DON for the Canadian grain industry

INTRODUCTION

The reaction of wheat lines to fusarium head blight (FHB) is highly variable and is dependent on flowering dates and daily weather conditions. For this reason, it takes many years and test sites to properly assess the FHB reaction of a candidate line or cultivar. One example is the cultivar AC Napier which ranges from best to worst in artificial inoculation tests established in diverse environments, but nevertheless consistently appears somewhat resistant in commercial fields.

It was concluded that in areas where FHB is often severe, the stability of resistance over years and sites should become a key criterion. However, field symptoms are not good predictors of toxin levels in the harvested grain, and correlations may range from poor to good depending on year and site. A visual inspection of grain gives an estimate of fusarium damaged kernels (FDK) that is somewhat better correlated to toxin levels, but still variable enough to be judged inadequate.

Therefore, in areas where the disease is more prevalent, a toxin measurement is the preferred criterion. Eastern Canada has developed a tradition of judging lines for registration on the basis of both toxin (deoxynivalenol, DON) concentration and symptoms.

MATERIALS AND METHODS

Plot size was different among test sites, ranging from single-row plots to 4-row plots, 5 m long. The replicate number at sites was variable but at least 3. Plots were artificially inoculated using a sprayed suspension of *Fusarium graminearum* Schwabe macroconidia (50,000 conidia/ml), corn inoculum or both. Disease symptoms were assessed either by estimating percentage spikelets infected in the field, or else by more precise counts, by harvesting spikes 21 d after inoculation and counting number of spikelets infected. DON was measured using a competitive ELISA method developed by ECORC. The least square means were computed using in order to pool data.

RESULTS

This double-barrelled approach has paid off with lines with improved FHB reaction for the grain industry. One line to be registered (CRGB-O-623.4) has shown stable resistance across many environments (Fig. 1), similar to its FHB resistant parent Nobeoka Bouzu. It is fit for non-rust areas only, and has medium baking quality. It is a CEROM cultivar, and likely to enter the trade under the name Duo. Field observations indicate that this cultivar combines both type 1 and type 2 resistance mechanisms. Type 1 resistance is postu-

lated to be from Frontana LF 320, and type 2, from Nobeoka Bouzu.

The cultivar Nass, from CLRC, Charlottetown, has shown consistent field resistance, despite having somewhat lower type 2 resistance (Fig. 1). This cultivar combines FHB resistance with high yield, although the bread-making properties are not good enough to make it acceptable for milling. Nevertheless, considering that no other FHB resistant cultivars produce a high yield, the cultivar Nass provides evidence that FHB resistance can be combined with high yield. The cultivar was named in honour of its creator, the late Dr Hans Nass.

CONCLUSIONS

The LS means from a general linear model (Fig. 1) lead to the conclusion that field symptoms (observed on spikes) are not adequate to predict DON levels. The FDK count (not shown) provides additional information, but may still leave questions concerning DON levels. Sometimes, DON is present in grain that looks relatively sound. ELISA is providing a rapid method for DON analysis and giving confidence that breeding for FHB resistance can indeed lead to the desired goal, which is improved food safety.

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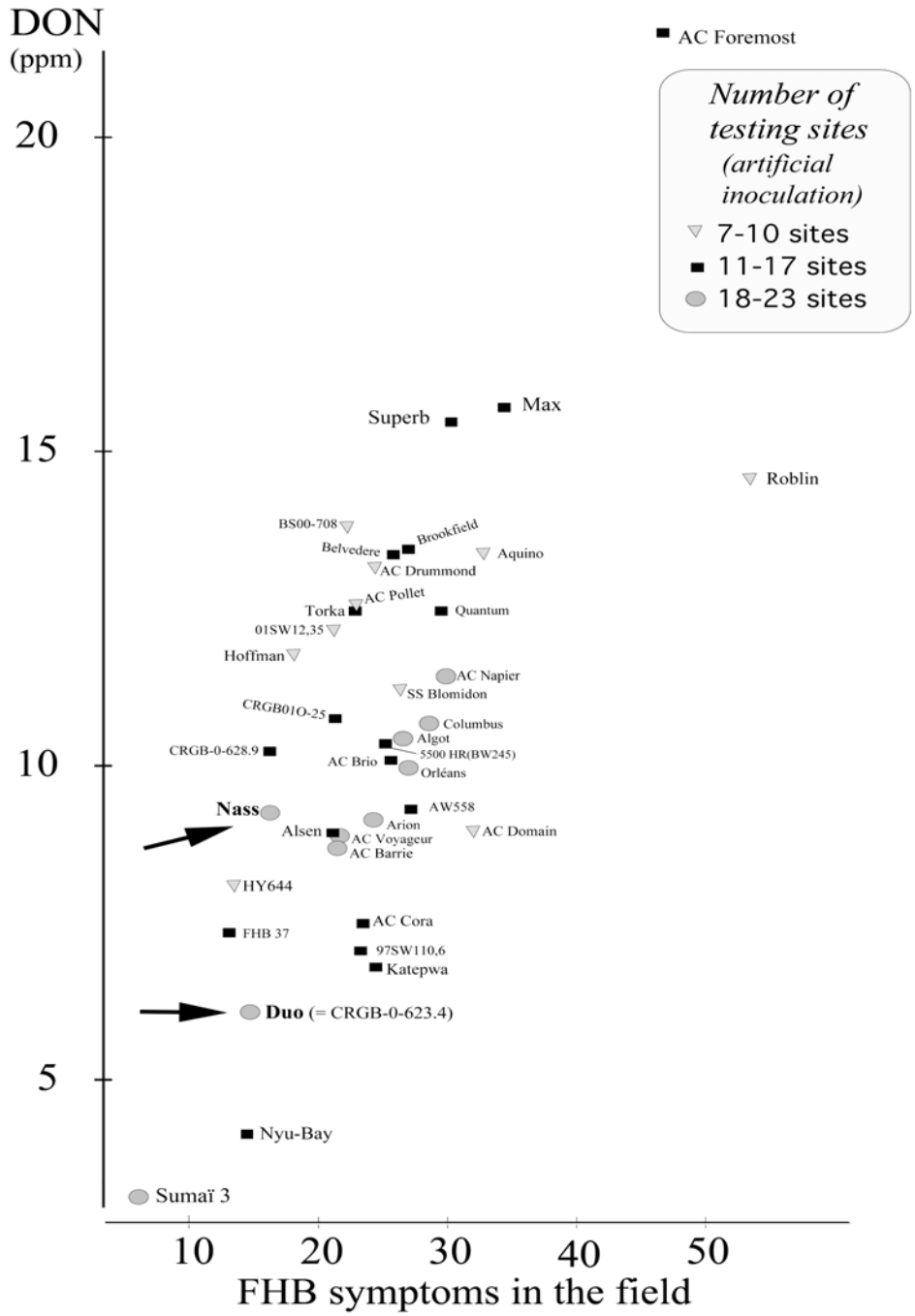

Figure 1. Least square means for fusarium head blight (FHB) symptom severity in the field and deoxynivalenol (DON) data (by ELISA) from multiple test sites and years. The data were obtained from artificial inoculation trials from 1999 to 2004. The sites included Winnipeg, Ottawa, St-Hyacinthe, Quebec City, and Charlottetown.

THE DEVELOPMENT OF SCAB (*FUSARIUM GRAMINEARUM*) RESISTANT VARIETIES OF WHEAT IN NEBRASKA FROM 2001-2005

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ABSTRACT

Fusarium Head Blight (FHB) induced by the fungus *F. graminearum*, affects to varying degrees, approximately one third of Nebraska's yearly wheat crop. The primary objective of the 2005 University of Nebraska breeding program was to select for elite lines of hard red winter wheat which had enhanced agronomic traits and showed resistance to FHB.

In 2005, 125 new crosses were made for FHB resistance using numerous resistance sources primarily from germplasm distributed by Dr. A. McKendry, who is located at the University of Missouri. This germplasm will be advanced to elite line status through modified bulk breeding or backcrossing methods.

A greenhouse screen was used to verify that lines selected from the field screen were truly FHB tolerant and not escapes. The secondary objective was to screen elite hard winter wheat lines in the Regional Germplasm Observation Nursery (RGON). This is a USDA ARS coordinated nursery that screens between 10-30 early generation experimental lines from every public and private wheat breeding program in the Great Plains.

Currently, 95 F2 populations, 44 F3 populations, 1,700 head rows, 98 F5 lines, 57 F6 lines, and 7 F7 lines (3 lines containing some of the Goldfield markers as identified by Dr. Guihua Bai and the Genotyping Center) with diverse sources of FHB tolerance some including Sumai 3 derivatives, are being advanced in the breeding program. Among the most advanced lines, an FHB tolerant line, NE01643 (which had the highest grain yield in the Nebraska State Variety Trial statewide in 2005 and consistently performs well in South Dakota) is tracking for co-release with South Dakota State University in 2006. An additional three lines with high FHB tolerance are also being considered for possible release thereafter. The most interesting of the three lines is NI02425 which has potential for dryland and irrigated production systems. The best lines for FHB tolerance and agronomic performance will be retested in 2006.

MOLECULAR CHARACTERIZATION OF A CHROMOSOME RECOMBINANT CARRYING A FHB RESISTANCE QTL FROM *LOPHOPYRUM PONTICUM*

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ABSTRACT

Robertsonian translocation 7DS.7el$_2$L wheat (*Triticum aestivum L.*) line KS24-2 was characterized as having resistance to Fusarium head blight. However, unwanted linkage drag makes it difficult to be utilized in commercial line development. Our objective is to reduce the amount of 7el$_2$L chromatin, yet retain the FHB resistance. The gene(s) for resistance to FHB is near the distal region of the long arm of 7el$_2$. KS24-2 was crossed to a genetic line of wheat cv. Chinese Spring, in which the *Ph* locus on chromosome 5B was deleted, so that there is induced homeologous pairing and recombination during meiosis in plants that are homozygous for the *Ph* deletion (genotype *phph*). We identified F_2 seedlings that were *phph*, but heterozygous for the long arm using DNA markers. Plants in subsequent generations were genotyped for markers located along 7DL/7el$_2$L and one F_3 derived F_4 plant (line 275-4) lost three *Thinopyrum*-specific markers BE403314, *Xgwm333*, BE406148, which are located in the proximal part of the group 7 chromosome, but retained the 7el$_2$-specific marker loci of the distal half of the 7el$_2$L segment, as revealed with *Xpsr129*, BE445567, and *Xcfa2240*. These three markers were associated by segregation analysis with the FHB resistance. We suggest that the7el$_2$L segment of this wheat line is shorter than that of the donor translocation line KS24-2, but the FHB resistance is retained.

EVALUATION OF BREEDING STRATEGIES FOR ENHANCING FHB RESISTANCE IN BARLEY

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ABSTRACT

Breeding for resistance to Fusarium head blight (FHB) in barley has met significant challenges resulting in relatively slow progress toward the release of new resistant varieties. Among the challenges are the lack of highly resistant sources, quantitative inheritance, large genotype-by-environment interaction, and linkages between resistance genes and undesirable traits. In retrospect, some breeding strategies have proven effective and others less so. Early generation phenotypic selection, particularly on individual plants, has proven to be unsuccessful. Greenhouse screening also has limited utility, primarily since the emphasis in barley is on type I resistance (initial infection) rather than type II resistance (spread in the spike). This is in contrast to wheat where selection for type II resistance in the greenhouse has been fairly effective. On the other hand, large scale screening efforts have identified some promising sources of resistance. All the resistant sources identified to date possess few other attributes necessary for a malting barley adapted to the Upper Midwest. Thus, at least three or more cycles of breeding will be necessary to develop a new malting variety. Extensive field-based testing in inoculated and mist-irrigated nurseries has been the main driver of progress in breeding FHB resistance to date. Breeding programs differ in the allocation of resources to screen for resistance (numbers of breeding lines, replications, and locations). For the first time, in 2005, variety candidates with enhanced FHB resistance were entered into the American Malting Barley Association quality testing program. Numerous quantitative trait loci (QTL) have been identified as potential targets for marker assisted selection (MAS). QTL that appear to have the largest and most consistent effects are linked to heading date, grain protein concentration, or spike morphology. Recent evidence of recombinants breaking some of these undesirable linkages has resulted in the initiation of MAS in collaboration with the USDA genotyping Center at Fargo, ND. Looking forward, some of the greatest gains to be made in FHB resistance in barley will be through the use of breeding data to identify QTL by association mapping. This should permit identification of markers linked to resistance alleles segregating in elite breeding germplasm and significantly expand the use of MAS to develop new malting varieties.

REPORT ON THE 2004-05 PRELIMINARY (PNUWWSN) AND ADVANCED (NUWWSN) NORTHERN UNIFORM WINTER WHEAT SCAB NURSERY

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INTRODUCTION

The PNUWWSN and NUWWSN test the Fusarium Head Blight resistance of primarily soft red winter wheat adapted to the northern areas of North America. There are a few hard and white wheat entries. Each test is conducted in multiple locations with more data collected in the NUWWSN than the PNUWWSN (Table 1). The PNUWWSN had 34 entries and the NUWWSN had 49. For the sake of brevity, this report present data on the FHB traits summarized over locations. Only location means were analyzed and a LSD was calculated using the error mean square after fitting a model with entries and locations. Additional data and analyses, including data by location, can be found in the full report posted on the USWBSI web site or from the corresponding author.

RESULTS

In there was more genotype x location interaction than in past years and ($1-R^2$) was greater than 0.29 for most FHB traits. Based on all FHB traits, the most resistant PNUWWSN entries were Ernie, ILo1-13776, IL01-11934, IL01-6243, and P.99817Rd1-7-5-5-2. The most resistant NUWWSN entries were OH903, OH904, VA04W-474, OH902, IL00-8061, IL00-8530, and P.981517A1-1-5-2.

Table 1. Traits assessed in the 2004-05 PNUWWSN and NUWWSN tests.

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

Table 2. Entries in the 2004-05 PNUWWSN

NAME	PEDIGREE	SOURCE
ERNIE	**Early, moderate resistant check**	
TRUMAN	**Most resistant check**	
FREEDOM	**Late, moderate resistant check**	
PIONEER 2545	**Susceptible check**	
981358C1-4-2-1-3-2	Acc3129/Patterson	IN
99608C1-1-3-4	95172/961331A49/4/INW9811//283-1/INW9811/3/Freedom/Acc3128	IN
99751D8-2-3	INW0123/961331A46/5/INW0123//Acc3128/3/9547B1//Patterson/Ernie	IN
99817RD1-7-5-5-2	9560RB1/92201D5//X117/Acc3128	IN
99840C4-8-3-1	961331A46/92201D5//Acc3128/Patton	IN
D9163	PIONEER_2548/3/C5023=(CHELSEA,SWD/B2141//B5219)	MI
E2001	CAYUGA/RAMROD	MI
E2052	CALEDONIA/PNR XW535	MI
E3005	RAMROD/CALEDONIA	MI
E3009	RAMROD/PIONEER_25R26	MI
IL01-11445	IL87-2834-1 / IL95-678	IL
IL01-11934	IL90-6364 / IL94-1909	IL
IL01-13776	IL94-1653 / IL95-2127	IL
IL01-5550	IL95-3245 / Ernie	IL
IL01-6243	IL90-6364 // IL90-9464 / Ning 7840 /3/ IL94-1909	IL
KY97C-0554-02	VA94-54-549/Roane//Kristy	KY
KY98C-1161-03	Patterson/2540//2552	KY
KY98C-1169-06	Patterson/2568//2552	KY
KY98C-1440-01	VA92-51-12/2540//2552	KY
KY98C-1517-01	Roane/Kristy//2552	KY
OH01-5295	IL87-1917-1/HOPEWELL	OH
OH01-6167	OH530/OH585/OH498/34586-20-1	OH
OH01-6964	5088B-D-32-1/HOPEWELL	OH
OH01-7576	38985-11-2/HOPEWELL	OH
OH01-7653	HOPEWELL/OH601	OH
VA04W-389	Ernie/3/P92823A1-1-2-3-5//Roane/Pion2643,F7	VA
VA04W-569	Roane*2//VR95B717/Roane,BC2F5	VA
VA04W-570	Roane*2//VR95B717/Roane,BC2F5	VA
VA04W-571	Roane*2//VR95B717/Roane,BC2F5	VA
VA04W-592	GA891283LE18//Er-Mai 9/GA891283LE18,BC1F6	VA

Table 3. Entries in the 2004-05 NUWWSN

NAME	PEDIGREE	SOURCE
ERNIE	**Early, moderate resistant check**	
TRUMAN	**Most resistant check**	
FREEDOM	**Late, moderate resistant check**	
PIONEER 2545	**Susceptible check**	
981238A1-11-3W	Ernie//91193D1/X117	IN
981517A1-1-5-2	Goldfield/Acc3128	IN
981542A1-10-4-5-6	Acc3128//Ernie/X117	IN
9824C1-26-2	Ernie/PF9052//INW9811/92162B8	IN
99794RA4-14-10	92201D5/4/9547C1//260-1/92367C2/3/INW9824/92829A1	IN
E0001	CLKS_CREAM/MSU LINE D1277	MI
E2017	(D3913,C4530/AUG)/3/(D0331,B9063/HILLSDALE//C113)	MI
E2042	(D3743,I4360/C5317//FRANKENMUTH)/3/(PIONEER_2555,PNR_W3017/PNR_W521	MI
E2043	(DC076,87F_INTCB_ENT#182/AUG//AUG)/3/(PIONEER_2555,PNR_W3017/PNRW521)	MI
E3012	RAMROD/PIONEER_25W33	MI
IL00-1665	IL91-13114 / Y88-3a // Foster / Pontiac	IL
IL00-8061	P8138I1-16-5-50/Foster//IL93-2489	IL
IL00-8530	IL89-1687 // IL90-6364 / IL93-2489	IL
IL01-15511	IL95-561 / IL95-4154	IL
IL01-5943	IL93-3137 / Roane	IL
KS01HW163-4	Trego/Betty sib	KS
KS950910-8-2	KSU94U284/Karl 92//Custer	KS
KY93C-0378-5-2	VA88-52-69/2510//KY84C-48-1	KY
KY96C-0399-5	2510/2580//2540	KY
KY96C-0769-7-1	2552/Roane	KY
KY97C-0304-16	Kristy/2628//2540	KY
KY97C-0574-01	VA94-54-549/L910097//2552	KY
MV-5-46	91-54-22(71-54-147/CK68-15//IN65309C7-18-2-3-2/FFR555W//93-52-55	MD
NE01643	NE94482 (=ARA/ABILENE//NE86488)/ND89744 x Karl92	NE
NE02465	NE95685 (=MO11785/NE87619//NE88492)	NE
NE02495	Wahoo/AP7601	NE
NE02549	KS940935-125-5-2 x Alliance	NE
NE02588	NE94458 (=GK-SAGVARI/COLT//NE86582) x Jagger	NE
NY91017-8080	U1266-4-11/HARUS	NY
NY91028-7085	HARUS/4/CS/A.CURVIF//GLENN/3/ALD/PVN(m-30)	NY
OH01-75	L910097 / IL87-2834-1	OH
OH01-7664	HOPEWELL/OH601	OH
OH902	ZM10782/FREEDOM//30584-37-2/VA91-54-219	OH
OH903	NING7840/GLORY//OH526	OH
OH904	ZM10782/FREEDOM//30584-37-2/VA91-54-219	OH
RCAT13/18	2737W x Ruby/Frontana #1	ONT
RCAT23/1	ACRON x ENA	ONT
RCATL24	RNA/ACRON/Ruby/Frontana #1	ONT
RCATL28	Ruby/Frontana #1 x ACRON/ EX9806 x ACRON	ONT
RCATL31	Ruby/Frontana #1 x ACRON/ AC RON x SVP72017-17-5-10-1	ONT
VA01W-99	FFR525/93-52-55(MSY*3/BALKAN//SAL),F10	VA
VA04W-439	NING 7840/PION2691//Roane(71-54-147/CK68-15//IN65309C7-18-2-3-2),F8	VA
VA04W-474	ROANE/W14/CK9134,H4	VA
VA04W-561	Roane*2//Futai8944/Roane,BC2F5	VA
VA04W-568	Roane*2//W14/Roane/3/2*Roane,BC4F4	VA

Table 4. Traits means for 2005 PNUWWSN. "l", "h" indicate means that are not significantly different from the lowest (l) or highest (h) mean in a column (LSD (0.05)). These are summed in last columns.

NAME	SEV		INC		IND		IS		GH		KR		PSS		ISK		DON	#l	#h
ERNIE	23.3	l	38.3	l	9.4	l	3.8	l	17.6	l	16.3	l	25.6	l	16.7	l	0.6	8	0
TRUMAN	14.4	l	61.5		7.6	l	4.2		4.8	l	12.0	l	11.1	l	27.3	l	0.6	6	0
FREEDOM	24.7	l	80.1	h	16.8	l	3.0	l	24.0	l	28.6	lh	18.6	l	44.1	h	0.8	6	3
PIONEER 2545	41.1	h	77.0	h	28.0	h	10.3	h	33.9	h	45.6	h	50.9	h	52.0	h	2.4	0	8
981358C1-4-2-1-3-2	27.7	l	48.7	l	11.3	l	4.2		24.9	l	20.8	l	13.2	l	29.6	l	0.1	7	0
99608C1-1-3-4	39.8	h	53.0	l	17.9	l	6.7	h	49.6	h	29.4	lh	37.4	h	37.9		1.6	3	5
99751D8-2-3	18.9	l	52.4	l	9.0	l	4.8		14.9	l	31.4	lh	43.7	h	36.2		0.3	5	2
99817RD1-7-5-5-2	19.0	l	53.1	l	9.8	l	2.9	l	19.7	l	24.7	l	36.3	h	25.4	l	0.2	7	1
99840C4-8-3-1	24.5	l	59.6	l	11.6	l	4.7		19.1	l	25.1	l	21.2	l	32.7	l	0.1	7	0
D9163	40.3	h	78.2	h	26.7		8.7	h	23.9	l	32.8	h	19.0	l	49.1	h	0.9	2	5
E2001	40.9	h	82.7	h	30.6	h	7.9	h	18.0	l	38.1	h	34.0		50.5	h	1.2	1	6
E2052	45.2	h	81.5	h	34.8	h	10.3	h	16.1	l	39.9	h	25.0	l	54.7	h	1.8	2	6
E3005	49.0	h	88.6	h	39.8	h	9.4	h	31.9		47.1	h	40.5	h	60.3	h	0.9	0	7
E3009	30.9		75.0	h	22.7		4.6		16.4	l	37.2	h	33.3		51.1	h	0.4	1	3
IL01-11445	25.1	l	54.1	l	10.8	l	8.8	h	17.7	l	11.9	l	17.7	l	24.0	l	0.3	7	1
IL01-11934	21.5	l	47.0	l	8.1	l	5.7		22.5	l	15.2	l	19.9	l	22.7	l	0.2	7	0
IL01-13776	19.1	l	45.9	l	6.8	l	8.3	h	17.6	l	13.3	l	15.1	l	21.2	l	0.4	7	1
IL01-5550	27.6	l	49.0	l	10.0	l	8.8	h	27.9		19.0	l	34.4		29.8	l	0.4	5	1
IL01-6243	20.7	l	46.3	l	8.4	l	9.2	h	22.8	l	15.3	l	21.4	l	22.0	l	0.6	7	1
KY97C-0554-02	30.2		74.2	h	18.7	l	5.6		20.6	l	25.9	l	24.5	l	41.2		0.9	4	1
KY98C-1161-03	37.6	h	73.6	h	23.2		4.3		32.3	h	33.0	h	32.9		48.1	h	1.1	0	5
KY98C-1169-06	28.9	l	55.1	l	14.8	l	8.4	h	20.9	l	30.4	lh	24.8	l	35.3		1.6	6	2
KY98C-1440-01	34.4	h	69.2	h	22.6		8.5	h	34.5	h	35.9	h	34.4		43.3		1.1	0	5
KY98C-1517-01	27.0	l	63.4		15.6	l	1.8	l	15.7	l	19.3	l	13.2	l	31.0	l	0.3	7	0
OH01-5295	23.8	l	57.5	l	11.6	l	3.5	l	18.1	l	26.7	l	29.7		36.2		0.5	6	0
OH01-6167	37.6	h	70.6	h	22.5		8.5	h	52.9	h	25.8	l	21.4	l	42.2		2.0	2	4
OH01-6964	35.1	h	69.6	h	20.5		3.6	l	32.9	h	28.8	lh	15.6	l	42.1		0.4	3	4
OH01-7576	28.1	l	58.2	l	14.8	l	4.8		43.9	h	20.6	l	14.9	l	32.2	l	0.5	6	1
OH01-7653	35.5	h	70.6	h	22.8		8.4	h	38.6	h	28.6	lh	26.6		42.1		0.1	1	5
VA04W-389	33.4		62.2		18.2	l	3.6	l	18.3	l	28.6	lh	25.0	l	42.7		0.3	5	1
VA04W-569	20.5	l	66.5	h	11.4	l	1.8	l	12.9	l	23.0	l	36.7	h	31.8	l	1.4	6	2
VA04W-570	20.3	l	60.0	l	10.4	l	2.6	l	12.3	l	21.7	l	34.2		26.2	l	0.5	7	0
VA04W-571	20.1	l	67.8	h	11.9	l	1.7	l	15.2	l	24.5	l	37.8	h	31.4	l	0.7	6	2
VA04W-592	21.4	l	49.7	l	8.1	l	6.3	h	15.6	l	22.0	l	28.3		26.8	l	0.2	6	1
AVERAGE	29.0		62.9		16.7		5.9		23.8		26.4		27.0		36.5		0.7	4.5	2.4
MAXIMUM	49.0		88.6		39.8		10.3		52.9		47.1		50.9		60.3		2.4	8	8
MINIMUM	14.4		38.3		6.8		1.7		4.8		11.9		11.1		16.7		0.1	0	0
LSD	17.7		26.9		13.8		2.4		26.3		27.7		14.8		19.6				
CV	38.1		31.8		61.0		49.0		63.9		37.6		37.3		27.1				
# LOCATIONS	5		5		6		1		4		3		1		2		1		

Table 5. Traits means for 2005 NUWWSN. "l", "h" indicate means that are not significantly different from the lowest (l) or highest (h) mean in a column (LSD (0.05)). These are summed in last columns.

NAME	SEV		INC		IND		IS		GH		KR		PSS		ISK		DON		#l	#h
ERNIE	18.5	l	50.8		11.3	l	1.4	l	12.5	l	13.8	l	23.9		31.4	l	7.4	l	7	0
TRUMAN	16.1	l	48.2		9.9	l	3.6		9.0	l	17.3	l	17.4	l	25.9	l	1.7	l	7	0
FREEDOM	21.0		65.0	h	16.1		3.1	l	17.2	l	24.1		29.7		40.3		6.4	l	3	1
PIONEER 2545	39.2	h	72.5	h	32.5	h	8.1	h	27.4	l	40.6	h	41.0	h	59.2	h	8.8	lh	2	8
981238A1-11-3W	19.4	l	50.3		14.0	l	1.4	l	6.8	l	16.4	l	26.0		30.5	l	3.6	l	7	0
981517A1-1-5-2	13.0	l	51.8		11.5	l	1.0	l	5.7	l	18.0	l	16.7	l	30.6	l	1.6	l	8	0
981542A1-10-4-5-6	25.7		64.3	h	21.5		1.6	l	16.2	l	34.2	h	41.0	h	44.4		7.5	l	3	3
9824C1-26-2	19.7	l	55.3		13.6	l	4.7		22.0	l	21.1	l	25.8		31.6	l	1.6	l	6	0
99794RA4-14-10	13.6	l	48.0		10.7	l	1.6	l	12.5	l	20.5	l	26.5		30.7	l	1.2	l	7	0
E0001	23.4		57.7		14.5	l	7.1	h	40.4		22.5	l	18.9	l	40.8		10.1	lh	4	2
E2017	34.1	h	59.9		22.5		7.0	h	24.2	l	26.4		26.4		43.3		9.5	lh	2	3
E2042	24.0		57.8		16.6		5.5		16.2	l	24.0		23.8		44.2		9.0	lh	2	1
E2043	31.3	h	67.5	h	22.9		6.4		26.6	l	34.9	h	31.1	h	54.1	h	13.1	h	1	6
E3012	30.6		66.2	h	24.6	h	4.1		18.0	l	34.5	h	29.8		55.6	h	11.7	lh	2	5
IL00-1665	20.2		54.8		13.5	l	4.6		29.0		20.5	l	22.7	l	32.1		1.2	l	4	0
IL00-8061	16.0	l	47.1		9.7	l	2.9	l	19.7	l	13.1	l	16.6	l	24.9	l	0.8	l	8	0
IL00-8530	18.3	l	53.2		13.5	l	2.9	l	12.0	l	9.6	l	14.4	l	25.7	l	1.0	l	8	0
IL01-15511	22.2		55.7		13.8	l	4.4		27.9	l	16.5	l	19.8	l	34.0		1.4	l	5	0
IL01-5943	15.9	l	51.1		10.6	l	5.8		14.1	l	13.7	l	20.0	l	31.3	l	2.7	l	7	0
KS01HW163-4	41.2	h	70.2	h	34.0	h	6.7		43.7		32.2	h	43.8	h	58.2	h	18.3	h	0	7
KS950910-8-2	28.6		58.2		22.8		8.9	h	54.2	h	29.4	h	32.1	h	48.2	h	4.6	l	1	5
KY93C-0378-5-2	27.1		72.1	h	23.9		2.1	l	33.8		32.1	h	29.3		51.4	h	6.3	l	2	3
KY96C-0399-5	28.1		70.5	h	25.7	h	4.7		25.5	l	33.8	h	32.9	h	55.0	h	9.7	lh	2	6
KY96C-0769-7-1	23.0		64.1	h	18.9		2.1	l	25.0	l	24.0		30.0		46.7	h	4.6	l	3	2
KY97C-0304-16	21.6		63.3	h	20.1		4.6		28.1	l	30.7	h	29.5		46.0	h	4.4	l	2	3
KY97C-0574-01	27.4		62.1	h	21.4		3.1	l	34.4		32.2	h	28.8		45.6	h	3.9	l	2	3
MV-5-46	32.6	h	68.2	h	29.8	h	9.5	h	75.9	h	29.8	h	30.4		49.3	h	5.2	l	1	7
NE01643	23.1		56.7		16.1		7.4	h	26.9	l	32.5	h	26.4		43.3		4.8	l	2	2
NE02465	29.0		56.4		24.5	h	8.5	h	29.2		29.8	h	30.6		43.3		1.5	l	1	3
NE02495	29.2		59.7		22.4		5.6		39.8		27.1		26.9		43.5		4.2	l	1	0
NE02549	28.7		58.8		19.7		7.7	h	60.0	h	22.7	l	32.4	h	43.1		8.4	l	2	3
NE02588	31.9	h	64.0	h	25.4	h	6.5		43.6		34.1	h	37.3	h	56.1	h	8.2	l	1	6
NY91017-8080	26.6		58.0		19.6		6.6		10.5	l	23.6		24.3		45.0		11.8	lh	2	1
NY91028-7085	33.8	h	75.2	h	26.9	h	7.1	h	39.9		27.4	h	32.5	h	54.0	h	20.6	h	0	8
OH01-75	22.2		63.5	h	18.5		6.6		22.0	l	20.7	l	20.0	l	40.6		2.3	l	4	1
OH01-7664	25.0		59.0		18.4		5.7		33.0		20.1	l	25.8		41.4		6.0	l	2	0
OH902	20.3		42.2	l	9.0	l	1.2	l	8.2	l	15.5	l	16.7	l	27.1	l	2.3	l	8	0
OH903	10.0	l	32.1	l	6.2	l	0.9	l	14.9	l	12.4	l	10.6	l	17.8	l	0.8	l	9	0
OH904	17.6	l	35.1	l	6.7	l	3.0	l	9.2	l	13.5	l	14.0	l	22.1	l	1.2	l	9	0
RCAT13/18	32.6	h	61.8	h	23.6		6.2		57.9	h	25.7		27.6		48.0	h	8.3	l	1	4
RCAT23/1	25.4		61.7	h	19.2		4.0		24.2	l	22.3	l	22.6	l	44.9		7.0	l	4	1
RCATL24	27.0		63.7	h	21.7		6.0		12.1	l	16.9	l	20.1	l	40.1		17.3	h	3	2
RCATL28	34.3	h	57.6		22.3		7.5	h	33.7		35.9	h	36.1	h	48.3	h	11.2	lh	1	6
RCATL31	18.7	l	51.5		14.2	l	4.1		25.1	l	15.3	l	20.3	l	35.6		2.6	l	6	0
VA01W-99	24.3		62.5	h	21.3		5.0		38.5		26.6		24.9		42.0		1.7	l	1	1
VA04W-439	18.5	l	57.6		14.1	l	4.9		16.1	l	27.9	h	27.6		39.5		2.1	l	4	1
VA04W-474	11.7	l	48.1		8.7	l	2.3	l	3.7	l	18.1	l	17.9	l	27.5	l	0.8	l	8	0
VA04W-561	20.1	l	57.8		14.9	l	1.9	l	7.7	l	19.1	l	23.7		36.1		1.0	l	6	0
VA04W-568	20.2		60.6		15.7	l	2.6	l	3.5	l	14.6	l	23.5		34.8		1.1	l	5	0
AVERAGE	24.1		58.2		18.1		4.7		25.3		23.8		25.9		40.5		5.8		4	2
MAXIMUM	41.2		75.2		34.0		9.5		75.9		40.6		43.8		59.2		20.6		9	8
MINIMUM	10.0		32.1		6.2		0.9		3.5		9.6		10.6		17.8		0.8		0	0
LSD	10.1		13.8		9.5		2.5		25.4		13.4		12.7		14.1		11.9			
CV	16.7		26.6		61.4		57.3		61.3		39.7		30.2		24.9		127.2			
# LOCATIONS	10		10		11		1		3		4		3		4		3			

SPRING WHEAT LINE TOKAI-66, A SOURCE OF HERITABLE KERNEL RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

The spring wheat line 'Tokai-66' (T-66) (PI#382161) was originally developed in Japan in the late 1960's. We tested T-66 in three years of field trials in our *Fusarium*-inoculated high-disease-pressure FHB nursery. While its visual FHB severity was slightly better than average, the percentage of scabby kernels in harvested grain was consistently among the lowest, as was the level of DON. In greenhouse tests, we included T-66 as one parent in two replicated half-diallele trials. The FHB severity score of T-66 was similar to the other FHB-R parents and the FHB-R checks. The F-1 lines with T-66 as a parent were distributed through the range of FHB severity scores. By comparison, the level of scabby kernels in T-66 was lowest of all parents, and the scabby kernels of F-1's involving T-66 were among the lowest of all the F-1's. Using Griffing's formula for calculating general combining ability (GCA) effects in these trials, the GCA of T-66 for FHB severity was significant, and similar to the two other FHB-R parents. In contrast the GCA of T-66 for scabby kernels was the greatest of any of the parents. T-66 has been used as a parent in the ND spring wheat breeding program and several ND advanced lines which include T-66 in their pedigree are in early field trials. (This poster was presented at the Crop Science Soc. of America meeting on November 6-10, 2005 in Salt Lake City, UT.)

FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY ACCESSIONS FROM THE N. I. VAVILOV INSTITUTE

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ABSTRACT

The deployment of resistant cultivars is one of the best means for combating Fusarium head blight (FHB) in barley (*Hordeum vulgare*). To source additional *Hordeum* germplasm for FHB evaluation, we initiated a cooperative research project with the N. I. Vavilov Institute of Plant Industry (VIR) in St. Petersburg, Russia. The VIR genebank contains over 20,000 barley accessions, comprising 24 *Hordeum* species. Their barley collection is very diverse and contains accessions from regions not represented in the USDA National Small Grains Collection. Since 2003, we evaluated nearly 1100 cultivated (six-/two-rowed as well as winter/spring types) and wild barley accessions from VIR for FHB resistance. These evaluations were made in disease nurseries established in China (Hangzhou) and/or in Minnesota (St. Paul and Crookston). The grain spawn (with ascospores) method of inoculation was used for the nurseries in Hangzhou and Crookston, whereas the foliar spray (using macro-conidia) method was used in the St. Paul nursery. FHB severity assessments were made at the mid-dough stage. From these evaluations, we identified four six-rowed spring-type accessions that exhibited FHB severity levels comparable to Chevron (the six-rowed standard) over multiple locations. The resistant accessions were 15130 and 15133 from Russia and 20731 and 20738 from Afghanistan. Additional screening tests will be made in the field over multiple locations and replicates to confirm the resistance of these accessions and their ability to reduce the accumulation of deoxynivalenol. Moreover, we also will genotype these accessions with molecular markers to determine whether they possess the same alleles as other reported sources of FHB resistance in barley.

COMMON RESISTANCE OF WHEAT TO MEMBERS OF THE *FUSARIUM GRAMINEARUM* SPECIES COMPLEX AND *F. CULMORUM*

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ABSTRACT

Fusarium head blight caused mainly by *Fusarium graminearum* and *F. culmorum* is the most important disease of wheat in Central-Europe. Previous studies clarified that *F. graminearum* is an assemblage of at least 9 geographically separated species (O'Donnell et al., 2004). Among these, *F. graminearum sensu stricto, F. boothii* and *F. vorosii* sp. nov. occur in Hungary (Tóth et al. 2005). Geographical structuring has also been observed in *F. culmorum* (Tóth et al. 2004). Although common resistance of wheat against several *Fusarium* species has been proposed recently (Mesterházy et al., 2005), no data are available in this respect for these recently described species/lineages. In this study, 20 wheat genotypes with highly differing resistance were tested under field conditions by spraying inocula of isolates of eight species of the *F. graminearum* species complex, and 3 *F. culmorum* lineages representing geographically isolated populations in 2003–2004. The severity of Fusarium head blight (FHB), *Fusarium* damaged kernels (FDK), the yield reduction and the deoxynivalenol (DON) contamination were also measured to describe the nature of the resistance. *Fusarium culmorum* isolates were in general more aggressive to wheat than those belonging to the *F. graminearum* species complex. *Fusarium meridionale, F. boothii* and *F. mesoamericanum* were found to be the least pathogenic to wheat. The various wheat genotypes exhibited similar reactions against the different *Fusarium* isolates, indicating that resistance to *F. graminearum sensu stricto* was similar to that for the other species of *F. graminearum sensu lato* examined. This is an important message to breeders as the resistance relates not only to any particular isolate of *F. graminearum*, but similarly to isolates of other *Fusarium* species. This holds true for all the parameters measured. The DON contamination refers only to DON-producing isolates of the *F. graminearum* species complex and *F. culmorum*. Highly significant correlations were found between FHB, FDK, yield loss and DON contamination.

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DEVELOPING A MULTIPLE DISEASE RESISTANCE LINKAGE BLOCK ON WHEAT CHROMOSOME *3BS*

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ABSTRACT

Four wheat (*Triticum aestivum*) fungal resistance genes/QTL have been localized to the region on chromosome 3BS between the markers *Xgwm493* and *Xgwm389*. The durable stem rust (caused by *Puccinia graminis*) resistance gene *Sr2*, Fusarium head blight (caused by *Fusarium graminearum*) resistance QTL *Qfhs.ndsu-3BS*, Septoria tritici blotch (caused by *Mycosphaerella graminicola*) resistance gene *Stb2*, and Stagonospora blotch (caused by *Stagonospora nodorum*) resistance QTL *QSng.sfr-3BS* are all in current cultivars, but the tight repulsion linkage will require recombination events to occur. The initiative to build a 3BS linkage block with all four traits is in progress. Crosses were made between the stem rust resistant parent Ocoroni 86 and the Fusarium head blight resistant cultivar Alsen. Additionally crosses were made between the Septoria tritici blotch resistant parent DH 115 and the Stagonospora blotch resistant cultivar Arina. Recombinant stem rust and Fusarium head blight resistant and susceptible phenotypes were observed in F_2 and verified in F_5 from the cross of Ocoroni 86 x Alsen. The phenotypic marker for *Sr2*, pseudo-black chaff, and DNA markers *Xgwm533* and *Xgwm493* were also used to assist in the selection of *Sr2* and *Qfhs.ndsu-3BS* for recombinant plants. Recombinant plants resistant to both Septoria tritici blotch and Stagonospora blotch from the cross of DH 115 x Arina were observed in F_2 and F_3 and will be verified in the F_4 plants. Respective resistant recombinant plants will be crossed in order to develop plants with resistance to all four diseases.

MAPPING OF QUANTITATIVE TRAIT LOCI FOR FUSARIUM HEAD BLIGHT RESISTANCE IN TWO-ROWED BARLEY

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ABSTRACT

Fusarium head blight (FHB) of barley (*Hordeum vulgare* L.), primarily incited by *Fusarium graminearum*, has been a major production problem in the midwestern part of USA due to deoxynivelenol (DON) contamination of grain. To identify quantitative trait loci for FHB resistance in two-rowed barley, a recombinant inbred line population was developed from the cross ZAU 7/ND16092. ZAU 7 is an early, semidwarf cultivar from Zhejiang University, Hangzhou, China and ND16092 is a line from North Dakota. A single-seed decent population was evaluated for FHB resistance in replicated field experiments over six environments in China and North Dakota. A linkage map of Diversity Array Technology (DArT) and SSR markers was constructed for the population using QTX20. One QTL for FHB resistance was found at the distal region of 2H long arm at all tested locations using both simple interval mapping (SIM) and simplified composite interval mapping (sCIM). The negative effect from ND16092 might be associated with a lax spike gene located in this region of 2H long arm. QTL for FHB resistance were not observed in proximal region of 2H long arm. Four other QTL for FHB resistance were found in one or two environments. Three QTL for DON accumulation, each in a different environment, were identified.

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DISCLAIMER

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

MAPPING QTLS FOR DIFFERENT TYPES OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN WANGSHUIBAI

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, can significantly reduce both grain yield and quality. Growing FHB resistant varieties is an effective means to reduce losses caused by the disease. However, currently used FHB resistance sources are mainly Sumai 3 and its derivatives. Utilization of FHB resistance sources different from Sumai 3 may enrich the genetic pool of FHB resistance sources. Wangshuibai is a FHB resistant Chinese landrace unrelated to Sumai 3. To map QTLs for Type I (initial infection), Type II (spread), and Type III (low mycotoxin deoxynivalenol accumulation) FHB resistance, 139 F_6 derived recombinant inbred lines (RILs) was developed from a cross between FHB resistant Wangshuibai and FHB susceptible Wheaton. More than 1300 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers were analyzed in this population. FHB was evaluated in the greenhouses of both Kansas State University and University of Illinois. Type I resistance was evaluated by spraying about 500 conidiaspores over spike and Type II resistance was evaluated by injecting 1000 conidiaspores into a central floret of a spike. Percentage of symptomatic spikelets in each inoculated spike was calculated at 7th days (Type I) and 21st day (Type II) after inoculation. Five QTLs, located on chromosome 3BS, 4B, 4A, 3A, and 1A for Type I resistance were detected. Seven QTLs, located on chromosome 3BS, 7A, 3D, 3A, 1A, and 5A for Type II resistance were detected. Seven QTLs, located on chromosome 3BS, 7A, 1B, 1A, 5D, and 5A for Type III resistance were detected. These QTLs could jointly explain as much as 24.4% of phenotypic variation for Type I resistance, 60.2% of phenotypic variation for Type II resistance, and 55.6% for Type III resistance. Among these QTLs, five of them involved in more than one type of wheat FHB resistance. Two QTLs located on 3BS (near the distal end of 3BS) and 1A contributed to all of the three types of wheat FHB resistance. Three QTLs located on 3BS (close to the centromere), 7A, and 5A showed effects on both Type II resistance and low DON accumulation. The remaining QTLs only showed effects on one type of FHB resistance. Three QTLs located on 3A, 4A, and 4B showed effects on Type I resistance. Two QTLs located on 3A and 3D showed effects only on Type II resistance. Two QTLs located on 1B and 5D showed effect on Type III resistance. The broad-sense heritabilities estimated in 2003-2005 were 34.2%, 82.1%, and 71% for Type I, Type II, and Type III resistance, respectively. New QTLs for FHB resistance identified in Wangshuibai have potential to be used in developing cultivars with enhanced FHB resistance by pyramiding FHB resistance QTL from different sources.

GENETIC ENGINEERING AND TRANSFORMATION

Chairperson: Ron Skadsen

FUNCTIONAL ANALYSIS OF PUTATIVE GENES FOR FHB-RESISTANCE/SUSCEPTIBILITY IN WHEAT USING RNAI

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ABSTRACT

Fusarium head blight (FHB) significantly reduces grain yield and quality. Identification of genes that govern FHB resistance/susceptibility will facilitate biotechnology-assisted development of superior wheat varieties with FHB resistance. Our study on global expression profiling using microarray and real-time PCR identified three genes that are significantly highly expressed (up-regulated) in the resistant cv. Ning 7840 in comparison with the susceptible cv. Clark. Since these genes were always expressed to a greater extent in the resistant cultivar than in the susceptible cultivar, these could be very important genes for FHB resistance. A cluster of genes with significantly higher expression levels (down-regulated) in the susceptible cultivar Clark relative to the Ning 7840 was also discovered. These genes may facilitate fungal growth in a spike. Further investigation of the functions of these genes may provide useful information for understanding the mechanisms of FHB resistance. To investigate the biological function of these candidate genes, RNAi-mediated gene silencing will be used to knock out their corresponding homologous mRNAs in wheat. Double stranded hairpin RNA molecules corresponding to up-regulated genes will be targeted to resistant cultivars while dsRNA from down-regulated genes will be targeted to susceptible cultivars to create loss-of-function plants. RNAi constructs using pANDA vector are underway.

ACCUMULATION OF TRANSGENE-ENCODED DEFENSE-ASSOCIATED ENZYMES IN TISSUES VULNERABLE TO INITIAL *FUSARIUM* INFECTION

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ABSTRACT

Our goal is to achieve Fusarium Head Blight resistance by employing genetic transformation to introduce new genes into wheat. Of particular interest are anti-*Fusarium* genes that could provide protection in the early stages of infection, thus improving Type I resistance. To achieve this, we have employed an expression vector carrying the barley *Lem1* promoter, which we had previously shown to be active in the outer organs of transgenic wheat florets from anthesis to the soft dough stage of kernel development. Into this vector, we inserted coding regions from three candidate anti-*Fusarium* genes that have been associated with naturally occurring plant defense mechanisms: *Aspergillus* glucose oxidase (*GO*) and barley peroxidases *Prx7* and *Prx8*. GO is an apoplastic enzyme that catalyzes oxidation of β-D-glucose, generating H_2O_2, a compound with multiple functions in plant defense. Induction of the peroxidases Prx7 and Prx8 has been correlated with the appearance of antifungal compounds and papillae structures, respectively, in barley leaves exposed to powdery mildew. We have analyzed several transformed wheat plants for inheritance and expression of the Lem1::PRX and/or Lem1::GO transgenes. Peroxidase and glucose oxidase enzymes were detected *in situ* in the outer tissues of the floret, where they accumulated either in the extracellular space (GO and Prx8) or inside the cells (Prx7). In some of the lines, lignin content was increased in the outer floret tissues. The potential for synergistic effects of the transgene-encoded enzymes in improving host resistance to initial fungal infection and pathogen spread will be discussed.

OVEREXPRESSION OF ANTIFUNGAL PROTEINS INCREASES RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT IN THE FIELD

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ABSTRACT

We are developing and testing transgenic wheat for resistance to Fusarium Head Blight (FHB). Anti-fungal proteins (AFPs), such as ß-1,3-glucanases, thionins, chitinases, thaumatin-like proteins (TLPs), ribosome-inactivating proteins (RIPs) and lipid transfer proteins (LTPs), are thought to inhibit fungal growth via different mechanisms. Transgenic wheat lines over-expressing these AFPs were generated using micro-projectile bombardment of the cultivar 'Bobwhite'. Both single transgenes and combinations of two transgenes were generated, either through co-bombardment or later crossing between transgenic materials. Transgenic materials were screened for Type II resistance in the greenhouse using single floret inoculation. Lines showing reduced severity in comparison with non-transgenic Bobwhite in three to four greenhouse trials were evaluated in field trials in 2004 and/or 2005. Of the seven lines that were evaluated in field trials in both 2004 and 2005 (one a-puro-thionin line, two TLP lines and four ß-1,3-glucanase lines), four showed reduced FHB severity, two showed reduced visually scabby kernels (VSK), and three showed reduced levels of DON (ppm) ($p<0.05$). Of the seventeen additional lines evaluated in the field in 2005 alone, six showed reduced FHB severity, five showed reduced VSK, and two showed reduced levels of DON (ppm) ($p<0.05$). In addition, we developed and tested transgenic wheat carrying LTP, RIP. RIP/TLP, TLP/*TRI101*, ß-1,3-glucanase/*TRI101*, and ß-1,3-glucanase/TLP combinations in the greenhouse using single floret inoculation. Results of these greenhouse screens will be presented.

A RAPID ASSAY SYSTEM FOR TRANSGENES CONFERRING RESISTANCE TO DON

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ABSTRACT

There is considerable interest in using transgenic approaches to enhance resistance to Fusarium Head Blight (FHB). Production of the tricothecene deoxnivalenol (DON) is thought to be a virulence factor for FHB, at least during some stages of infection, and it has been proposed that engineering resistance to DON might make plants less susceptible to infection with FHB. Testing transgenes for efficacy against DON in wheat or barley germplasm is time consuming and labor intensive. It would be extremely useful to be able to assay transgenes for efficacy against DON or FHB, before they are introduced into wheat or barley.

We have developed a rapid and efficient whole-plant system based on the recombinogenic plant *Physcomitrella patens*, an emerging model system for functional genomics. Physcomitrella is sensitive to physiological levels of DON. Toxicity of DON is almost completely abolished in Physcomitrella plants that overexpress the cell death regulator BI-1. These transgenic plants also display almost complete resistance to several necrotrophic pathogens. DON toxicity is also substantially attenuated in transgenic Physcomitrella plants that express a modified ribosomal L3 gene (the L3Ä mutant) or in Physcomitrella plants in which other endogenous pathogen-induced transcripts have been deleted through gene targeting.

These results confirm that the sensitivity to DON is under genetic control in plant cells and demonstrate that sensitivity can be affected at a number of genetic control points. Current efforts are focused on establishing whether genes effective against DON act through similar or independent pathways. Our results form the basis for future studies designed to understand the mechanism of action of mycotoxins. The rapid assay system also provides a means of assessing transgenes for efficacy, prior to their introduction into wheat or barley. The system could also be adapted for medium- to high-throughput screens for novel sources of mycotoxin resistance.

A VIRUS-INDUCED GENE SILENCING SYSTEM FOR THE ANALYSIS OF DISEASE RESISTANCE PATHWAYS IN WHEAT AND BARLEY

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ABSTRACT

Systems for virus-induced gene silencing (VIGS) that can rapidly and efficiently create gene knockout phenotypes, have proven to be very useful tools for the analysis of plant gene function. VIGS is a form of RNA-mediated gene silencing. All forms of RNA-mediated gene silencing involve the production of large amounts of dsRNA that activates a host defense mechanism that results in the degradation of all RNAs with homology to the sequences within the dsRNA. In VIGS, certain RNA viruses are used to produce dsRNAs that trigger the silencing mechanism. If the virus has been engineered to contain sequences from a plant gene of interest, mRNAs from that gene are degraded as well, thus creating knockout phenotype for the chosen gene. Given a validated VIGS system and a 200-500bp fragment from a gene of interest as starting material, it is possible to assemble the VIGS construct, infect plants and observe the knockout phenotype within one month. The first VIGS systems were effective in only a few dicot species however, recently a VIGS system based on Barley stripe mosaic virus has been demonstrated to efficiently trigger VIGS in barley and wheat. The creation of gene knockouts in polyploid plants, such as wheat, is very difficult using conventional mutagenesis strategies because expression of homeoloci mask mutations. However, since VIGS operates through a homology-dependent mechanism, it promises to be particularly useful in polyploids, because mRNAs from homeoloci should be degraded as well, provided they share sufficient sequence homology. This talk will describe the development of the BSMV-VIGS system and demonstrate its utility in the functional analysis of genes required in a range of wheat and barley disease resistance pathways.

ENGINEERING SCAB RESISTANCE IN WHEAT WITH PLANT DEFENSE SIGNALING GENES

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ABSTRACT

Fusarium Head Blight (FHB)/scab is a devastating disease of wheat and barley that severely limits crop productivity and grain quality. We have developed a plant-pathogen system consisting of *Arabidopsis thaliana* and *Fusarium graminearum* to identify genes that are involved in plant defense against *F. graminearum*. We have shown that constitutive expression of the Arabidopsis *NPR1* gene (At*NPR1*) in transgenic Arabidopsis plants enhances resistance to *F. graminearum*. Likewise, the wheat cultivar Bobwhite, which is normally susceptible to scab by *F. graminearum*, when engineered to constitutively express the Arabidopsis *NPR1* gene (At*NPR1*) exhibited heightened resistance to the pathogen. The NPR1 protein is a key regulator of salicylic acid (SA) signaling in plant defense and the activation of systemic acquired resistance (SAR). Resistance against *F. graminearum* in the At*NPR1* expressing wheat plants correlated with a rapid and strong activation of expression of the pathogenesis related, *PR1* gene, in the pathogen-challenged plants. This rapid response of *PR1* expression in the At*NPR1* expressing Bobwhite plants was similar to that of the scab-resistant cultivar, Sumai 3. Application of benzothiadiazole (BTH), a functional analog of SA, also induced *PR1* gene expression faster and to a higher level in Sumai 3 and the At*NPR1* expressing transgenic wheat than in non-transgenic Bobwhite plants, suggesting that a SA/BTH-regulated signaling mechanism regulates plant defense against *F. graminearum*. Indeed, BTH treatment was sufficient to enhance scab resistance in non-transgenic Bobwhite plants. These results suggest that scab resistance in Sumai 3 and the At*NPR1* expressing transgenic plants may result from increased responsiveness of these plants to an endogenous defense signaling molecule. In order to further understand the molecular and physiological basis of scab resistance in these At*NPR1* expressing plants we have carried out microarray studies. We are further developing the Arabidopsis-*F. graminearum* system to rapidly identify other genes and signaling pathways involved in plant defense against *F. graminearum*. In the future some of these genes could be targeted to enhance scab resistance in wheat and barley.

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TRANSGENIC BARLEY CO-EXPRESSING ANTIFUNGAL AND ANTITOXIN GENES

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ABSTRACT

Overexpression of pathogen response proteins in plants could lead to enhanced resistance against diseases and reduce economic losses. Plants expressing combinations of these genes have shown synergistic action against fungal diseases. We have transformed a commercial malting barley cultivar (*Hordeum vulgare* cv. Conlon) to co-express antifungal and antitoxin genes by particle bombardment. In the past, we have produced T_2 homozygous transgenic barley lines that co-express antifungal genes such as thaumatin-like protein (*tlp*) or chitinase (*chi*) genes from rice, and *Tri101*, an antitoxin gene. Backcross lines with these genes are being tested in the field and greenhouse against Fusarium head blight. More than 250 transformed plants carrying antitoxin and antifungal genes have been developed from 72 transformation events including 11 different gene combinations. PCR analysis of T_0 and T_1 progenies from 45 of these events indicated the presence of the transgenes while western blot analysis confirmed expression of the proteins. T_2 homozygous lines have been selected and are currently being tested against FHB.

MODIFICATION OF RIBOSOMAL PROTEIN L3 CONFERS RESISTANCE TO DEOXYNIVALENOL

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ABSTRACT

Wheat and barley scab, also known as Fusarium head blight (FHB) is a devastating disease worldwide, caused mainly by *Fusarium graminearum*. The *Fusarium*-infected grain is contaminated with potent mycotoxins, especially deoxynivalenol (DON), which poses a great threat to human and animal health. DON belongs to the group of trichothecene toxins, which target ribosomal protein L3 at the peptidyltransferase site of eukaryotic ribosomes and inhibit protein synthesis. The goal of our work is to identify mutations in L3 that confer resistance to DON and to determine if FHB resistance can be engineered in transgenic wheat plants by expressing DON resistant L3 genes. We have demonstrated that overexpression of a truncated form of yeast ribosomal protein L3 (L3Δ) in transgenic tobacco plants confers resistance to deoxynivalenol (DON). Expression of the yeast L3Δ also confers resistance to a ribosome inactivating protein, pokeweed antiviral protein (PAP), which binds to L3 to depurinate the ribosomes. Transgenic plants expressing PAP and L3Δ are phenotypically normal and ribosomes are not depurinated in these plants. These results demonstrate that expression of yeast L3Δ leads to *trans*-dominant resistance to PAP and DON, providing evidence that both toxins target L3 by a common mechanism. The goal of our project is to translate the success we had in engineering DON resistance in tobacco to wheat and to generate wheat lines resistant to FHB. In collaboration with Dr. Ann Blechl, we have introduced the yeast L3 genes into wheat and identified transgenic lines containing the maize *Ubiquitin1* promoter and L3Δ, barley *Lem1* promoter and L3Δ, barley *Lem1* promoter and the full length yeast L3. We have used PCR to identify two to five stable transformants per construct and obtained seed from the homozygous lines. The primary wheat transformants and their homozygous progeny are phenotypically normal and fertile. Real time PCR analysis was used to confirm expression of the transgenes in the T2 progeny. The T2 plants, which express the transgenes, are indistinguishable from the non-transformed plants in their growth and morphology. We are in the process of evaluating the transgenic wheat lines for resistance to trichothecenes and FHB.

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ETIOLOGY, EPIDEMIOLOGY AND DISEASE FORECASTING

Chairperson: Ruth Dill-Macky

EFFECT OF HOST RESISTANCE, FUNGICIDE APPLICATION AND INOCULUM LEVELS ON FUSARIUNM HEAD BLIGHT OF WHEAT IN NORTH DAKOTA

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ABSTRACT

Knowledge of host resistance, inoculum levels, and weather conditions favorable for disease development is necessary to optimize a disease forecaster. A group of plant pathologists from five land-grant universities (North Dakota, Ohio, Pennsylvania, Purdue, and South Dakota) have collaborated to develop and improve performance of a disease forecasting system for Fusarium Head Blight (FHB). The main goal of this study was to determine the effect of host resistance, inoculum levels, and fungicide on FHB development in spring wheat. The experiment was conducted at the NDSU Agricultural Experiment Station, Fargo, ND. The experimental design was a split-split plot with three replications; inoculum levels (n = 2), fungicide treatment (1), and cultivars (3) as main plots, sub-plots, and sub-sub plots, respectively. The previous year crop was soybean. The plots with inoculum were created by distributing corn kernels infested with *F. graminearum* in the plots at the 6-leaf stage. Two FHB susceptible cultivars, Argent (hard white spring wheat and early flowering) and Granite (hard red spring wheat and late flowering), and one FHB resistant cultivar, Alsen (hard red spring wheat), were selected and planted on April 29, 2005. Alsen also was planted between main plots and sub-plots at 20 ft wide to serve as buffers. The buffer strips were free of inoculum. Trizole fungicide "Folicur" (@ 4 fl oz/acre) was applied to one sub-plot of each cultivar in each replicate when cultivars Alsen and Argent were at flowering (Feekes GS 10.51-10.52). The *G. zeae* population from each inoculum treatment was monitored daily from Feekes growth stage 8 (early flag leaf emergence) to Feekes GS 11.2 (soft dough) by collecting spores from air, and from Feekes GS 10 (boot stage) to Feekes 11.2 by head washings. Additionally, 90 wheat heads of each cultivar were monitored daily from Feekes scale GS 10 for growth synchrony. The disease incidence (number of infected head/total number of heads examined) and head severity (% of individual infected head) data were recorded in all treatments. FHB incidence was significantly ($P<0.0001$) different among the inoculum levels. The disease incidence and severity ranged from 21 to 51%, and 10 to 32%, respectively. Fungicide application significantly decreased disease severity and increased the seed test weight. The cultivars exhibited significant differences in FHB severity. The disease severity was significantly ($P< 0.0001$) lower (8 to 11%) in the FHB resistant Alsen than in the susceptible Argent (25 to 33%). Both fungicide application and cultivars had little or no effect on the disease incidence. As expected, air samples collected from high inoculum level and low inoculum level plots resulted in high number (range = 20-134) and low number (7-65) of *G. zeae* colony forming units (CFU) in 20 out 21 days of the samples, respectively. The majority (>97%) of the plants began and ended flowering in 3-4 days in both early and late flowering cultivars. These results indicate that favorable weather conditions for FHB, inoculum levels of *G. zeae*, level of host resistance, and fungicide application may have a significant role in disease development. Also, the fungus has a small window of opportunity to infect wheat heads, as the majority of the plants completed flowering within 3-4 days, a crucial stage for infection.

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EFFECTS OF MOISTURE, WHEAT CULTIVAR, AND INFECTION TIMING ON FHB SEVERITY AND DON IN WHEAT

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ABSTRACT

Deoxynivalenol (DON) levels are important both for their health effects and because DON is a pathogenicity factor in cereals. Our knowledge of the epidemiological and host genetic influences governing DON concentrations is incomplete. While anthesis is thought to be the primary period for Fusarium head blight (FHB) infection in wheat, late infections can also lead to DON production. High levels of DON have sometimes been observed in the absence of abundant disease symptoms. The influences of the timing of moisture and the timing of infection on FHB symptoms, *Fusarium* growth, and DON development are not well understood, particularly in relation to cultivar differences. We are investigating these relationships, which are important to the process of forecasting epidemic severity and economic risk. The goal is to improve our understanding of how the duration of moisture and the timing of infection affect disease development, fungal growth, and DON production.

A multi-year field experiment was undertaken in fall 2004 in a misted nursery at Kinston, NC. The experiment had a split-plot design. Whole-plots were four durations of post-anthesis misting: 0, 10, 20, or 30 days. Subplots were seven cultivars, one susceptible to FHB and the others with varying degrees and putative types of moderate resistance. There were two treatments of each cultivar in each irrigation treatment: inoculated and noninoculated. All treatments were replicated three times.

Inoculations of *F. graminearum* spore suspensions were performed either at anthesis with a backpack sprayer on whole plots, or with a spray bottle on individual, funnel-isolated heads that were chosen at random in all noninoculated plots, marked, and protected until inoculation with glassine bags. Late inoculations were performed at 10 or 20 days after anthesis, and late-inoculated heads were compared to those inoculated at anthesis and those never inoculated. Disease incidence and severity were assessed on all plots, omitting the late-inoculated heads. In order to track DON concentrations during grain maturation, heads were selected blindly in all plots in the 30-day-irrigated, backpack-inoculated plots on five occasions starting 2 wks after flowering and continuing at intervals of 7-11 days. Data are being gathered on visual kernel damage, percent infected kernels, and DON concentrations, and also on *F. graminearum* biomass by tissue type (kernel, rachis, or glume) using real-time PCR.

Preliminary Results: In 2004-05, levels of FHB incidence and severity were low, due in part to cool temperatures. Nevertheless, differences in incidence and severity among inoculated cultivars were significant (P d•0.05) both within each irrigation regime and across regimes. Across all cultivars, duration of post-anthesis misting had no significant effect on incidence ($P = 0.859$), nor on severity ($P = 0.124$). Misting duration affected disease severity on NKC 9184 and VA01W99 differently from that on other cultivars ($P = 0.0039$); without those two cultivars, misting duration had a positive effect on severity ($P = 0.024$).

The ratio of FHB incidence to severity was significantly higher for NC Neuse than for the other cultivars (P d•0.05). Lower ratios of incidence to severity support the hypothesis of Type I resistance, while higher ratios support the hypothesis of Type II resistance.

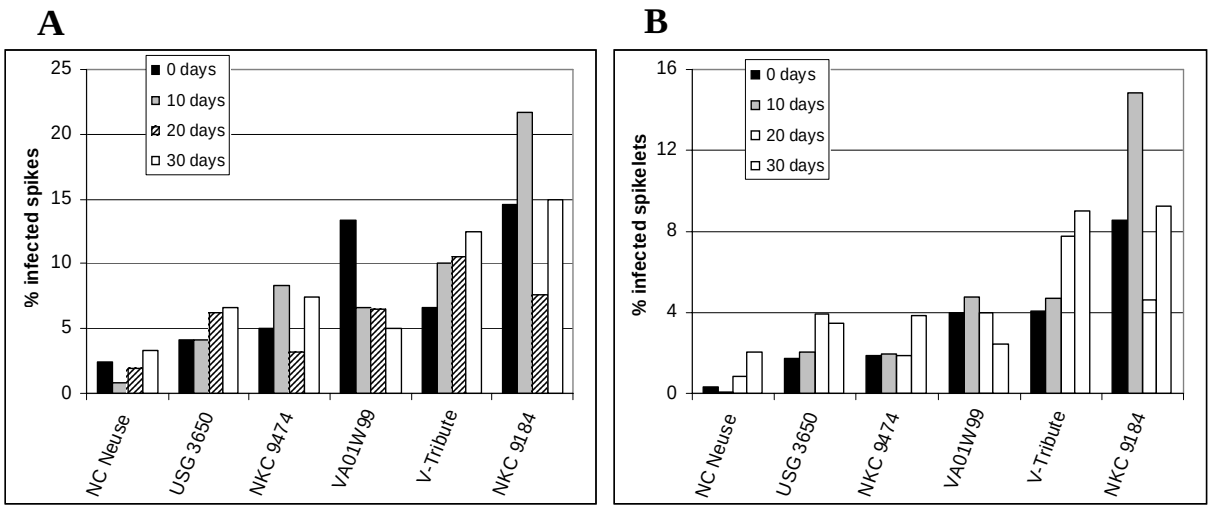

Fig. 1. A. Incidence (% infected spikes) and **B.** severity (% infected spikelets) of FHB in six soft red winter wheat cultivars inoculated with *F. graminearum* spores and subjected to four durations of post-anthesis misting (0, 10, 20 and 30 days). Disease assessments were not available for cultivar Ernie.

FUTURE DIRECTIONS IN THE DEVELOPMENT AND APPLICATION OF RISK ASSESSMENT MODELS FOR FUSARIUM HEAD BLIGHT

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ABSTRACT

A cooperative project to investigate the epidemiology and develop disease forecasting models for Fusarium head blight was established by researchers in IN, ND, OH, PA and SD. This modeling effort is now in its third phase (Phase III) and has demonstrated an iterative progression in model development and deployment. The first generation pre-flowering model used observations of temperature and rainfall from 7-days prior to flowering to predict the probability of a FHB epidemic of greater than 10% field severity. The accuracy of this model was near 70% for data used to develop and validate the model. This experimental model was released for deployment at the state or regional level in 2001-2003. During this period additional information was being collected by members of the cooperative epidemiology effort and used to initiate a second phase (Phase II) of modeling. The resulting model improved accuracy from 70% to near 80% and successfully accounted for potential differences in winter and spring wheat regions. A sub-model for winter wheat also accounted for the presence of corn residue as a local inoculum source. In 2004 the Phase II model was deployed as part of the Fusarium Head Blight Prediction Center (www.wheatscab.psu.edu) that provided daily maps of disease risk for 23 states. We have nearly completed a third phase of model development (Phase III). The Phase III model for spring wheat uses only mean relative humidity for 7 days prior to flowering and a variable describing host resistance to predict epidemics of FHB. This model correctly classified 78% of the cases used to develop and validate the model. The Phase III winter wheat model uses only pre-flowering mean relative humidity and has a prediction accuracy near 70%. The field severity threshold for classifying a case as an epidemic was adjusted from 10 to 2% for this model. These candidate models were evaluated as part of an experimental interface during the 2005 growing season. Additional enhancements to the prediction center including the use of weather forecasts and alternative ways to represent risk over multiple days were also tested as part of the experimental interface. Validation of the Phase III models and other enhancements is ongoing but we anticipate they will be ready for public deployment in 2006. Future goals of the cooperative epidemiology effort include adapting the risk models for use with barley and prediction of the mycotoxin deoxynivalenol.

APPLICATION OF HOTSPOT DETECTION ANALYSIS TO THE PREDICTION OF FUSARIUM HEAD BLIGHT EPIDEMICS

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ABSTRACT

Plant diseases and the pathogens that cause them are distributed spatially at different scales ranging from less than one meter to entire continents. The distribution of disease may result from local pathogen populations or the spread of disease within a geographic region. Locations or regions that have consistently high levels of disease may have characteristics unlike those of the surrounding areas. For example, a particular section of a state may have a climate or cropping practices that are more conducive to pathogen survival, and/or disease development. These highly conducive areas can be called disease hotspots. If patterns in these characteristics can be identified it may provide valuable insights into the factors contributing to disease epidemics and could allow disease prediction models to account for the elevated risk levels in some regions of the country. Hotspot analysis represents a method of searching for consistent spatial patterns in a desired variable. The analysis evaluates three dimensions of patterns: (i) **spatial**, patterns within a landscape; (ii) **temporal**, persistence of high disease levels; and (iii) **spatial-temporal**, addressing the migration of patches of disease or hotspots. FHB is a good candidate for hotspot analysis because of the availability of geo-referenced observations of disease intensity coupled with weather-driven models of disease biology, and information about crop/disease management practices (e.g. tillage, crop rotation). We anticipate that hotspot analysis will allow us to identify areas with consistently higher probability of epidemics given consistent patterns in weather and cultural practices, and determine if these regions have expanded or changed over time. This analysis may also help assess factors that may contribute to the elevated levels of disease within these hotspots and suggest adjustments in crop management practices.

INCORPORATION OF HOST REACTION AND CROP RESIDUE LEVEL INTO PREDICTION MODELS FOR FUSARIUM HEAD BLIGHT

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OBJECTIVES

Develop disease prediction models for Fusarium Head Blight

INTRODUCTION

Fusarium Head Blight is a devastating disease with serious worldwide impacts. Between 1998 and 2000 this disease resulted in more than 800 million dollars in losses for the US wheat and barley industry (Nganje et al., 2004). In the U.S., the disease is primarily caused by the fungus *Fusarium graminearum* (teleomorph: *Gibberella zeae*) (Parry et al, 1995). Several efforts around the world have attempted to forecast the risk for epidemics of FHB, and forecasting models have been proposed for Canada (Hooker et al, 2002), Argentina (Moschini and Fortugno, 1996), China (Zhang and Shang, 1995), USA(De Wolf et al, 2003) and Italy (Rossi et al., 2003). All these models relate the biology of the fungus to environmental conditions using different statistical approaches.

In the US, models developed by our collaborative epidemiology group including researchers in IN, ND, OH, PA, and SD have been deployed on the internet for public use at www.wheatscab.psu.edu. The results of the first phase of this project were published by De Wolf, et al (2003). The following manuscript presents modeling results from phase II and phase III of this development effort.

METHODS

Data collection - Data used to develop the models consisted for observations of disease development and crop phenology from 8 states and multiple wheat production regions in the U.S. The total number of cases available for analysis was 124 and 154 in phase II (2004) and phase III (2005), respectively (Table 1), and comprises a period from 1990-2004. For each of these cases, the observations of disease and crop growth stage were coupled with hourly weather information (hourly temperature, relative humidity, precipitation and dew point temperature), varieties reaction to FHB and the presence of corn residue (a potential local inoculum source).

Variable selection - Hourly observations of temperature, relative humidity, dewpoint temperature and rain fall were used to create candidate variables for use in modeling epidemics. The complexity of the variables ranged from simple summary statistics such as mean temperature to the number of hours of specific weather conditions favorable for certain developmental stages of the pathogen (i.e. perithecia development, or infection) . These variables were calculated to represent 3-, 5-, 7- and 10-day periods prior to flowering. A total of 306 variables were evaluated. Disease was coded as a binary variable (0=no or low disease and 1= severe epidemic). In 2004 (Phase II), cases were considered epidemics if they had a field severity (FHB index) greater than or equal to 10%. In 2005 (Phase III), both 10% and 2% severity threshold were evaluated.

Model development and validation - Variable selection was done by using best subsets. This approach to variable selection helps the modeler identify and eliminated redundant variables, or variables that do not have a strong relationship with the dependent variable. Models describing the relationship between weather variables and FHB epidemics were developed using four modeling approaches: Logistic regression, Classification and Regression Tree (CART), K-Nearest Neighbor discriminant analysis (K-NN), and

Neural Network. In 2005 only the Logistic Regression model approach was used.

In phase II of the analysis (2004), the total data set (n=124) was partitioned into two data sets with one data set (n=86) used for model development. The remaining cases were assigned to a data set used only for model validation. A similar approach was employed in phase III of the analysis (2005), with 108 of the total 154 cases used for model development. However, in phase III we also used a procedure known as 0.632+ Bootstrap. This bootstrap procedure randomly samples the total data set with replacement 200 times and allows for model development and validation on each of the samples. Model fit and accuracy are then based on bootstrap estimates of model parameters, thus minimizing the potential for overfitting the model to a small data set.

Candidate models were selected based on ability to correctly predict epidemics (% accuracy), balance between ability to predict epidemics (% sensitivity) and non-epidemics (% specificity) and measures of model fit. Model errors were evaluated for possible patterns in predictions that might be further explained by additional variables or time periods not currently considered by the models.

RESULTS AND DISCUSSION

Variable selection - The best subsets method of variable selection successfully identified variables related to disease epidemics. In general, temperature variables representing the number of hours that temperature was between 9 and 30°C were selected compared to other representations of temperature, including duration of temperature between 12 and 30°C and 15 and 30°C. Variables summarizing relative humidity were selected over those that used dewpoint temperature to estimate moisture levels. Variables representing the duration of rainfall were selected instead of variables representing summations or frequency of rainfall (number of days with rain). When variables summarizing weather conditions for 3-, 5-, 7- or 10-days periods prior to flowering were considered, only variables from a seven day period were selected by the best subsets analysis. The selected variables (Table 2) are consistent with research results from studies investigating the pathogen reproduction (Dufault et al. 2005). Variables describing crop management types (spring vs winter wheat), or specific production practices (presence of corn residue at a given location or the use of resistant cultivars) were also selected for further model development.

Modeling results Phase II - Among the statistical techniques evaluated, logistic regression and CART had the highest accuracy for all three models (Table 3). A model that used only duration of favorable temperature and humidity for winter wheat without corn residue and additional interaction terms describing interactions between temperature and humidity and hours of rain had the higher prediction accuracy than other models evaluated. For the logistic and CART approaches, this model correctly classified more than 80% of the cases and more than 80% sensitivity and specificity for all pooled cases (training and validation, over both wheat types). Errors of the model appear to be associated with favorable weather conditions during flowering or grain-filling periods of growth that are not considered by the pre-flowering models.

Modeling results Phase III - Logistic regression models were the focus of the phase III analysis, because of their accuracy in the phase II analysis and relative ease of deployment as a simple equation. In this phase of the analysis, we successfully reduced the number of variables used in the models. A candidate model for spring wheat used only mean relative humidity for 7-days prior to flowering and cultivar resistance to FHB as independent variables (Table 4). This model correctly classified 78% of the cases from spring wheat production regions. However, the model has slightly higher sensitivity than specificity indicating that it may overestimate the risk of a FHB epidemic in some years. Bootstrap estimates of model accuracy are similar to the more traditional approach to model validation but have a slightly lower specificity. A model that estimates the risk of a FHB epidemic for winter wheat in fields without corn residue or other local inoculum source was also developed in the phase III analysis. This model uses only mean relative humidity to predict

the risk of epidemic of greater than or equal to 2% field severity with 70% accuracy. Sensitivity and specificity was both 70%. The bootstrap method of model development and validation resulted in models with reduced accuracy and poor fit statistics. Models resulting from this analysis were evaluated during the 2005 growing season and we anticipate that these models will be part of the Fusarium Head Blight Prediction Center in 2006

ACKNOWLEDGEMENTS

This project is a collaborative effort of The Pennsylvania State, Ohio State, South Dakota State, North Dakota State, and Purdue University. Our work would not be possible without the collaboration of Marcia McMullen, Jeffrey Stein, Lawrence Osborne, Gregory Shaner, Ali Shaukat, Tika Adhikari and Laura Sweets.

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Table 1. Data collected for use in Phase II and Phase III model development.

State	Locations	2004 Cases	2005 Cases
Indiana	6	10	23
Kentucky	1	0	2
Michigan	1	0	2
Missouri	3	11	11
North Dakota	6	36	48
Ohio	3	28	33
Pennsylvania	2	11	19
South Dakota	1	10	16

Table 2. Selected variables evaluated for model development.

Variable Name	Meaning	Time Frame
W/S	Wheat type w=winter; s=spring	NA
H1	Mean relative humidity	7 days
R2	Hours of rain	7 days
T3	Temperature 9-30 C	7 days
TH2	Temperature 9-30 C and RH >90%	7 days
Corn	Corn residue	NA
Resistance	Cultivar resistance	NA

Table 3. Phase II model results and comparison between modeling methods.

#	Model	Modeling Method	%Correct	Sensitivity	Specificity
1	T3 H1 R2 W Corn	Classification Tree	0.84	0.76	0.89
		K-Nearest Neighbor	0.83	0.74	0.89
		Logistic Regression	0.76	0.78	0.74
		Neural Network	0.67	0.70	0.65
2	S*H1*T3 W*TH2 W*Corn*H1*T3	Classification Tree	0.82	0.94	0.74
		K-Nearest Neighbor	0.67	0.52	0.77
		Logistic Regression	0.72	0.78	0.68
		Neural Network	0.77	0.76	0.77
3	S*R2*T3 S*H1*T3 W*TH2 W*Corn*H1*T3 W*Corn*R2*T3	Classification Tree	0.88	0.90	0.86
		K-Nearest Neighbor	0.83	0.76	0.88
		Logistic Regression	0.82	0.82	0.82
		Neural Network	0.81	0.76	0.84

Table 4. Phase III model results and comparison between validation methods.

Model	Validation Method	%Correct	Sensitivity	Specificity
S S*Resistance S*H1	Training + Cross-Validation	0.78	0.83	0.72
	.632 Bootstrap	0.78	0.86	0.69
W*H1	Training +Cross-validation	0.70	0.70	0.70

IMPACT OF PREHARVEST MANAGEMENT STRATEGIES IN BARLEY ON FHB, SEED COLONIZATION AND DON

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ABSTRACT

Fusarium Head Blight (FHB) substantially affects the barley crop grown in North Dakota and western Minnesota through reductions in yield, lower test weights and inability of farmers to achieve malting quality barley due to contamination with toxins associated with infection by *Fusarium*. To produce barley with low or no FHB symptoms and DON content, will require an integrated approach that includes use of cultural practices, fungicides, and FHB resistant cultivars. As barley is susceptible from flowering through to harvest, preharvest management which alters the susceptibility of the host or changes environmental conditions to favor the pathogen, could significantly reduce the effectiveness of integrated control methods developed for FHB. Weather conditions often experienced in the upper midwest during barley harvest cause slow and non-uniform crop maturity within a field. In most years feed and malt barley producers use windrowing to accelerate crop maturity and drying. In addition, in feed barley pre-harvest herbicides are sometimes used as desiccants. To test the hypothesis that swathing affects disease under normal or high rainfall conditions, preharvest treatments in Fargo in 2004 and 2005 were a factorial combination of irrigated or unirrigated, swathed or straight combined and the cultivars Robust or Stander. In Fargo, barley flowers and ripens in July and in 2004 Fargo experienced a near average mean daily July temperature of 20^0C and total July precipitation of 97mm, but in 2005 it was hotter and drier with a mean daily July temperature of 22^0C and total July precipitation of 40mm. There was no statistical interaction between any combination of irrigation treatment, cultivar or preharvest treatment. In 2004 irrigation significantly increased DON and the percentage of kernels colonized by *Fusarium*, but did not affect visual symptoms on grain. In 2005, a season with lower disease levels, irrigation had no effect on DON, colonized kernels or visual symptoms. In 2004, straight combined barley had more than double the DON of swathed barley and significantly more visual symptoms on the grain. In contrast in 2005, harvest treatment had no effect on DON or visual symptoms on the grain but swathing slightly increased *Fusarium* infected kernels. Instances of iatrogenic disease associated with pesticides are common. Uneven herbicide application, coupled with various effects of an herbicide on the host and/or a pathogen, is likely to be the cause of an increase in disease. To test the hypothesis that preharvest desiccants were affecting disease, treatments in Fargo in 2004 included glyphosate, metsulfuron or 2-4-D at recommended and twice recommended rates on Robust barley applied at the soft dough stage. Treatments in 2005 at Fargo were a factorial combination of the cultivars Robust or Conlon with recommended or twice recommended rates of dicamba, carfentrazone, 2-4-D, metsulfuron, paraquat or glyphosate. Treatments in 2005 at Minot were a factorial combination of the cultivars Stellar, Tradition, Drummond, Excel, Robust, Divide, Eslick or Conlon with recommended rates of dicamba, carfentrazone, 2-4-D, metsulfuron, paraquat or glyphosate. In none of the three preharvest desiccant experiments was DON in harvested grain significantly affected by the application of herbicide and in 2005 at Fargo and Minot, where more than one cultivar was tested in each experiment, there was no significant interaction between cultivar and desiccant herbicide.

EFFECT OF CORN RESIDUE LEVEL ON THE INCIDENCE OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Corn residue is considered to be an important source of inoculum for Fusarium head blight (FHB); however, the quantitative risk of producing wheat in fields with large amounts of corn residue remains undetermined. Experiments were conducted in IN, ND, OH, and PA during the 2003 and 2004 growing seasons to evaluate the effect of corn residue level or other in-field inoculum source on the incidence of FHB. The experiment was a split-split-plot design with three replications at each location. Treatments included three levels of corn residue (approximately 0, 14 and 80% ground cover) as the main plot factor, two planting dates (normal for the location and 14 days after normal planting date) as the sub-plot factor, and three FHB susceptible cultivars ('Hopewell', 'Patterson' and 'Elkhart') as the sub-sub plot factor in IN, OH and PA. The protocol varied at the ND location, where *Gibberella zeae*-colonized corn kernels were used to establish the main plots, and two susceptible cultivars ('Norm' and 'Grandin') were used as the sub-sub plots; however, the two planting date sub-plot remained consistent. Incidence of FHB varied between years and locations, and current analysis considers each location and year separately. The highest mean incidence was 66.8% for OH-2003, and lowest was 4.0% for OH-2004. Three-way interactions between residue, planting date and cultivar were not significant ($P > 0.05$) in all but one of the locations and years (OH-2003), indicating that cultivars generally responded similarly within planting date and residue levels. Two-way interactions between planting date and cultivar on disease incidence were significant in five out of eight cases. This variation likely resulted from differential effects of planting dates on timing of cultivar flowering, and corresponding weather events conducive to infection around flowering. There was only one case (ND-2004) of an interaction between planting date and residue level on disease incidence. This indicates that the effects of residue levels on disease incidence were similar regardless of planting date in nearly all cases. The interaction between residue and cultivar on disease incidence was not significant in all cases, which suggests that changes in disease incidence in response to the residue level were similar among cultivars. The effect of residue level on FHB incidence was significant ($P < 0.05$) in five out of the eight cases; however the response was not consistent. For example, incidence was significantly lower in plots with no corn residue than plots with either the 14% or 80% residue levels, but disease incidence was not significantly different between the 14% and 80% residue levels in the PA-2003 case. In contrast, incidence was similar at the 0% and 14% residue levels, however, incidence at both of these levels of residue were significantly lower than disease incidence at the 80% residue level for the OH-2004 case. Cases with no significant effect of residue level had disease incidence nearly twice that of the cases where residue was significant. This observation suggests that the effects of local inoculum are variable for disease development and depend on local environment.

EFFECTS OF MOISTURE DURING AND AFTER ANTHESIS ON THE DEVELOPMENT OF FUSARIUM HEAD BLIGHT OF WHEAT AND MYCOTOXIN PRODUCTION

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OBJECTIVES

The objective of this study was to evaluate the potential relationship of moisture during the grain filling growth stages with the development of disease symptoms and DON accumulation

INTRODUCTION

Fusarium damaged grain is commonly contaminated with the mycotoxin deoxynivalenol (DON) (Parry et al. 1995; McMullen et al. 1997). In general, DON contamination is positively correlated with visual ratings of Fusarium head blight (FHB) intensity with the strongest relationship between field severity and Fusarium damaged kernels (Paul et al. 2005). However, lots of asymptomatic grain with greater than 2.0 ppm DON were reported in recent FHB epidemics.

MATERIALS AND METHODS

The role of moisture during and after anthesis was evaluated in a wheat field environment located at the Penn State Plant Pathology Research Farm located near State College, PA. The experimental was a split-plot design with moisture timing as the main plot and cultivar as the sub plot. Treatments included (i) supplemental moisture at anthesis and dry grain filling; (ii) dry during anthesis and supplemental moisture during grain fill; (iii) supplemental moisture during both anthesis and grain fill; and (iv) ambient moisture levels. The sub plots consisted of three soft red winter wheat cultivars. Two of these cultivars were FHB susceptible ('Hopewell' and 'Patterson') and the third ('Valor') was moderately resistant. The different moisture timings were achieved by either excluding with a mobile roof activated by rainfall or by adding moisture with supplemental mist irrigation. All plots were inoculated at the stem elongation stage of growth with corn kernels colonized with *Gibberella zeae*, and subjected to appropriate moisture treatment. The plots were evaluated for disease incidence and severity during the mid-dough growth stage. The harvested grain was evaluated for symptoms of disease and DON levels were assessed by HPLC.

RESULTS AND DISCUSSION

In 2004, disease incidence, disease severity, and DON concentration varied from 16 to 100% (mean 72.3%), 1.5 to 99.8% (mean 43.5%), and 4.9 to 29.4 ppm (mean 14.6 ppm) respectively (Figures 1 and 2). In 2005, development of disease was less than the previous year. Disease incidence ranged from 0 to 28% (mean 8.8%), and disease severity and DON concentration ranged from 0 to 8.6% (mean 2.6%) and 0 to 4.5 ppm (mean 0.9 ppm), respectively (Figures 1 and 2).

In general, treatments that provided misting during the anthesis resulted in significantly higher (*P d"* 0.05) disease intensity (incidence and severity) and DON concentration. An interaction between treatment and cultivar on disease intensity was also observed in 2004. More specifically, for treatments that received supplemental moisture only during the grain-fill, susceptible variety ('Patterson') resulted in larger increases of disease intensity compared with the moderately resistant variety ('Valor'). All varieties resulted in a similar degree of disease intensity with other treatments. No interaction between treatment and cultivar for disease intensity was identified in 2005, and plots that received supplemental moisture only during the grain-fill resulted in low disease intensity (Figure 1) and were not significantly different from the treatment without misting.

In both years, plants that received supplemental moisture only during grain-fill resulted in DON concentration that was relatively low (Figure 2) and not significantly different ($P \leq 0.05$) from the results of the treatment without misting.

In 2005, there were no significant interactions between treatment and cultivar for disease intensity, but a significant ($P \leq 0.05$) interaction between treatment and cultivar on DON production was identified. In this year, the variety 'Hopewell' had significantly higher levels of DON when supplemental moisture was applied only during anthesis than did the other combinations of variety and moisture treatment. Plots that received supplemental moisture during both anthesis and grain-fill produced different responses in disease and DON over two years (Figures 1 and 2). In 2004, both disease intensity and DON concentration were significantly higher than plots that received only ambient moisture, and results were not significantly different from plots that received moisture at either only anthesis or grain-fill stages of growth. In 2005, although disease intensity was significantly higher than plots that received only ambient moisture, it was significantly lower than in plots that received moisture only at anthesis. DON concentration was also low in the grain harvested from plots that had received moisture during both anthesis and grain filling growth stages and were not significantly different DON levels observed in plots that received only ambient moisture.

Plants that did not receive misting (ambient treatment) tended to have low disease development in both years (Figures 1 and 2); however, in some cases, high levels of DON were observed in the presence of low field symptoms. For example, in 2004, a plot of 'Valor' under ambient conditions resulted in low disease intensity (19% incidence and 1.5% severity), while DON concentration was relatively high (16.3 ppm). To investigate further, a separate analysis on DON concentration was conducted by selectively sampling apparently healthy kernels from the harvested grain. It confirmed that samples of asymptomatic kernels could contain high levels of DON (up to 4.9 ppm was detected). However, there was no clear evidence to suggest that the prolonged wetness during milk-development was responsible for the presence of asymptomatic kernels with considerable DON concentration.

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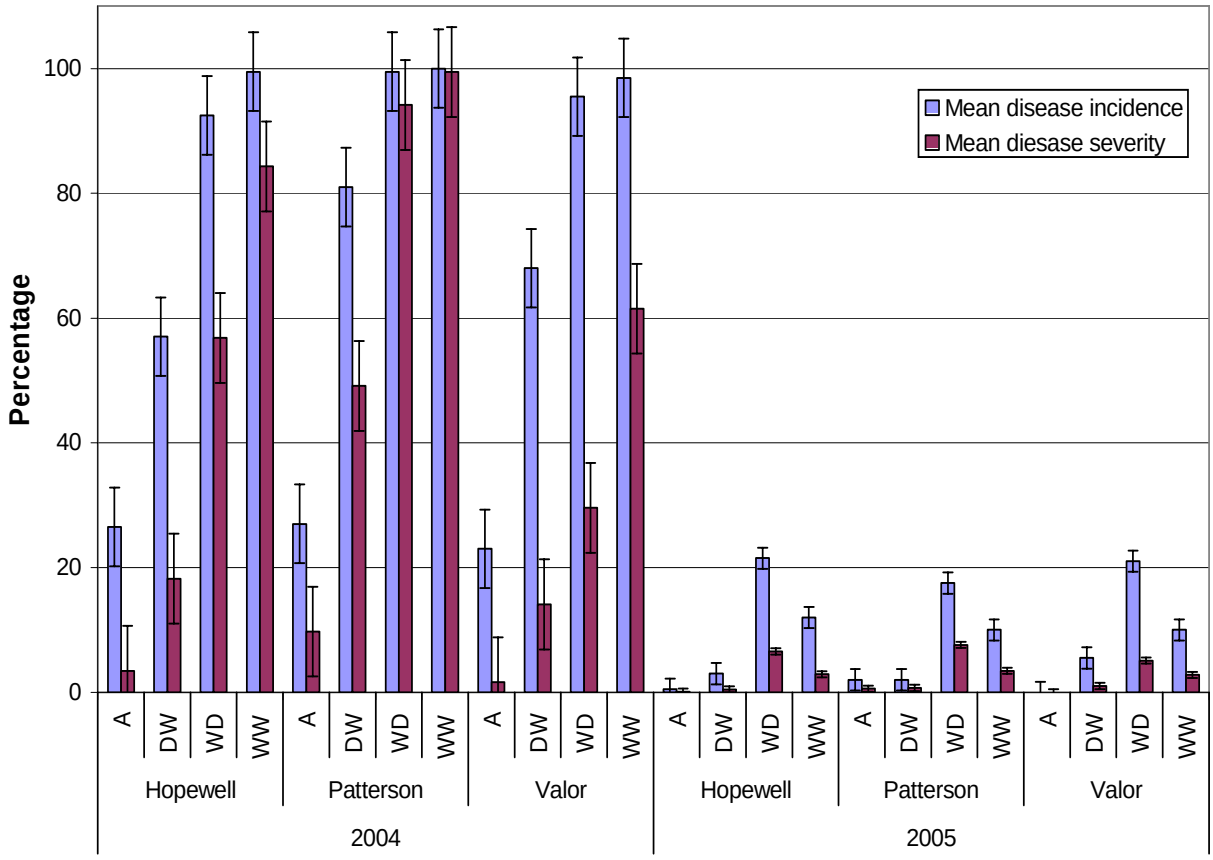

Figure 1. Effects of supplemental moisture during anthesis and grain-fill on disease incidence and severity of Fusarim head blight of wheat on three cultivars, 2004-2005, State College, PA. Treatments are: A=ambient; DW=misted only at grain-fill; WD=misted only at anthesis; and WW=misted at both anthesis and grain-fill. An error bar represents standard error of the mean across all treatments and cultivars per year.

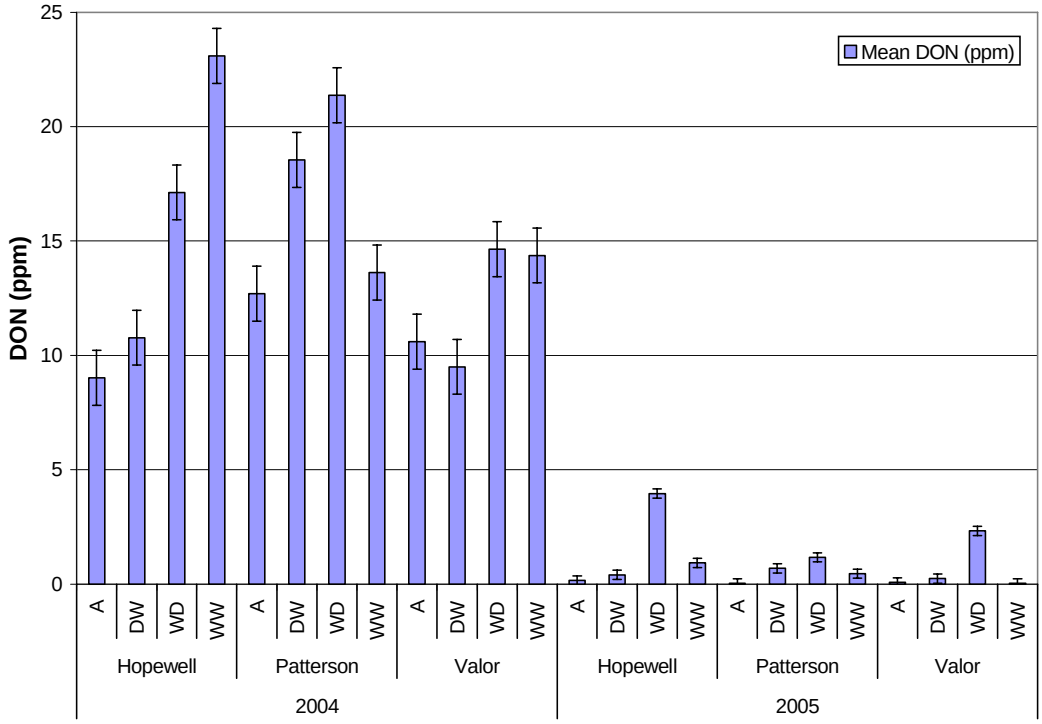

Figure 2. Effects of supplemental moisture during anthesis and grain-fill on DON (deoxynivalenol) production due to Fusarium head blight of wheat on three cultivars, 2004-2005, State College, PA. Treatments are: A=ambient; DW=misted only at grain-fill; WD=misted only at anthesis; and WW=misted at both anthesis and grain-fill. An error bar represents standard error of the mean across all treatments and cultivars per year.

AIRBORNE INOCULUM DYNAMICS FOR SEVEN LOCATION-YEARS IN RELATION TO ENVIRONMENTAL PARAMETERS

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INTRODUCTION

Worldwide, Fusarium head blight (FHB) of wheat, barley and other cereals is caused by a number of related fungi. In the United States, the primary causal agent on wheat is *Fusarium graminearum* (Fg) (teleomorph: *Gibberella zeae*, (Gz)). This fungus is homothallic and readily produces perithecia and ascospores on crop residues at the soil surface. The anamorphic form of the fungus generally produces copious conidia in sporodochial masses on suitable substrates. The fungus is not considered to be pathogenic on otherwise healthy leaf tissues, however evidence suggests there is potential for superficial, or subpathogenic colonization on vegetative tissues such as leaves and stems (Shaukat Ali, Yue Jin, pers. comm.). In the U.S., China and other areas of the world, the accepted etiology of Fusarium head blight begins with pathogen-colonized residues at the soil surface. This biomass could be corn, small grains or soybean residue, or any other tissue capable of harboring the pathogen over the winter months. In the spring, the fungus begins to produce sexual fruiting structures, called perithecia, which contain sacs (asci) of haploid spores (ascospores). Ascospores are forcible ejected from the perithecial masses and are thus able to be carried by air movement and rain splash. The spores are contained in an epiplasmic fluid while in the asci, and they retain some of this material after ejection (Trail et al., 2002), which is believed to allow the spores to adhere to contacted surfaces.

The asexual forms and stages of *Fusarium* spp. are known to be important etiologically to the FHB disease system in wheat as illustrated by those species with no sexual stage (*F. culmorum, F. avenaceum*). However, the role of conidial (asexual) inoculum is not well defined for *F. graminearum*. It is known that Fg conidia are fully capable of producing disease under controlled environments (Mesterházy, 1978; Stack and McMullen, 1985; Wang and Miller, 1988). Stack (1989) states that there is no significant difference in the efficacy of Fg conidia or Gz ascospores in producing infections and disease on wheat. There are differences in environmental limits and requirements that have been reported for each form of the fungus, and for spore survival (Doohan et al., 2003, Brennan et al, 2003). The objective of this investigation is to determine the relative abundance of conidia and ascospores of the causal agent for FHB in air samples above wheat canopies in South Dakota. A further objective is to relate quantity of airborne inoculum to environmental parameters including precipitation, temperature, and humidity.

MATERIALS AND METHODS

Several sites in northeast South Dakota were selected for sampling of airborne inoculum for Fusarium head blight in 2003 and 2004. Sites included spring wheat cultivar evaluation areas within wheat fields near Aurora, Watertown, Groton and Redfield, SD. Air was sampled using a Hirst-type spore trap, sampling approximately 5 L/min at the orifice. Airborne particulates were collected by impingement on double-sided adhesive tape affixed to a rotating drum, rotation once each 8 days. Drums were changed weekly and tapes were dissected with a razor into sections representing 24 hours of sampling. Dissection was performed while the tape remained on the drum. Each tape section was carefully lifted taking precaution not to touch the adhesive (outer) surface. Sections were placed with the non-exposed tape surface (drum-side) secured onto slide glass microscope slides. Spores were counted in the field of view of the microscope at 400X magnification. Each field measured 0.50 mm dia., or 0.20 mm^2 area. The length of each tape section was examined twice from end to end in chronological order just

above, and then just below the central (horizontal) axis of the tape to yield a continuous estimate of spores per unit time. Each section was 65+/-2 mm in length, therefore each field of view at 400X represented approximately 11 to 12 minutes. In this manner, conidia and ascospores were enumerated in a time course lasting up to three weeks at some locations. Conidia and ascospores were identified to species and differentiated based solely on spore morphology. Hyaline spores with a straight to slightly curved fusiform shape, 4-6 transverse septae, foot-shaped basal cell, and convex-conoid apical cell in a size range of approximately 50 +/- 10µm by 5 +/- 2µm were considered to be conidia belonging to *Fusarium graminearum*. Spores were counted as *Gibberella zeae* ascospores if they were hyaline, slightly crescent shaped, 1-3 septate, with convex-conoid terminal cells, and were approximately 20 to 30µm by 3-5µm in size (approx. one-half the size of *F. graminearum* conidia). For purposes of graphical and analytical comparison to weather data, the sub-hourly data was reduced to spores per hour.

RESULTS AND DISCUSSION

Ascospores and conidia were found in relatively similar abundance in both 2003 and 2004, however there were generally more ascospores for a given time period than conidia. Pearson's correlation coefficient (r) for ascospores and conidia at each location was greater than 0.90. The mean number of ascospores per hour across all locations in 2003 was 2.12 compared to 1.84 conidia per hour. In 2004, ascospores were also slightly more abundant (1.92 per hour) relative to conidia (1.55 per hour). Figures 1 through 7 represent each location-year and contain weather parameters as well as ascospore data. For simplicity, conidial concentrations are not graphed in Figures 1-7, and are not discussed further. Also note that spore concentrations reported in the figures are 'spores per hour', recorded as a 24-hr moving average (to smooth day/night fluctuations) of sub-hourly data. Moving averages, including those for temperature and RH data are reported for each hour as summary of the past 24 hours. Peaks in inoculum occurred at the rate of approximately one for every three days of monitoring, and in some cases were fairly regular. Duration of the peaks varied from less than 24 hours to as much as three days, which corresponds well with the findings of de Luna, et al. (2002). Magnitude of peaks ranged from less than one spore per hour to nearly 14 spores per hour. Peaks in spore abundance were often associated with precipitation events, particularly large events, as describe by de Luna, et al. (2002) and Markell and Francl (2003) though not always. Peaks in inoculum also occurred during long periods (up to 12 days) without recorded rainfall. Inoculum peaks appear to lag increases in mean relative humidity, and their curves appear to have some relationship, graphically. Simple correlation analysis shows that the moving average RH does correlate to moving average ascospore count in some location-years, though the r-values are not high, and for most location-years, the correlation is poor. By shifting the RH data, it was noted that the correlation became stronger. Shifting was accomplished by moving the RH data forward, to correspond with 'future' ascospore counts. For all location-years, increases in correlation coefficient were achieved in this manner. For 2003 locations, shifts of 8, 12, 16, 20, 24, 28, and 32, and 36 hours were evaluated. When the RH data is shifted forward, the correlation coefficients increase for each shift forward, to a point, after which the r-values then decline, though the maximum point varied at all three locations. For example, at Aurora, 2003 a shift forward of 8 and 12 hours maximized correlation coefficient at r = 0.79. At Groton, 2003, r-values increased from 0.44 with no shift, to a maximum of 0.88 with a 20 hour shift forward of the mean RH values. At Watertown, a shift of 32 hours forward was required to maximize r at 0.74. These results suggest the predictive value of RH as little as 8-12 hours before an inoculum release event begins. The variability in the apparent RH 'lag' suggests that temperature, or some other factor or combination of factors may help to strengthen the predictive value of a moving average of the relative humidity data.

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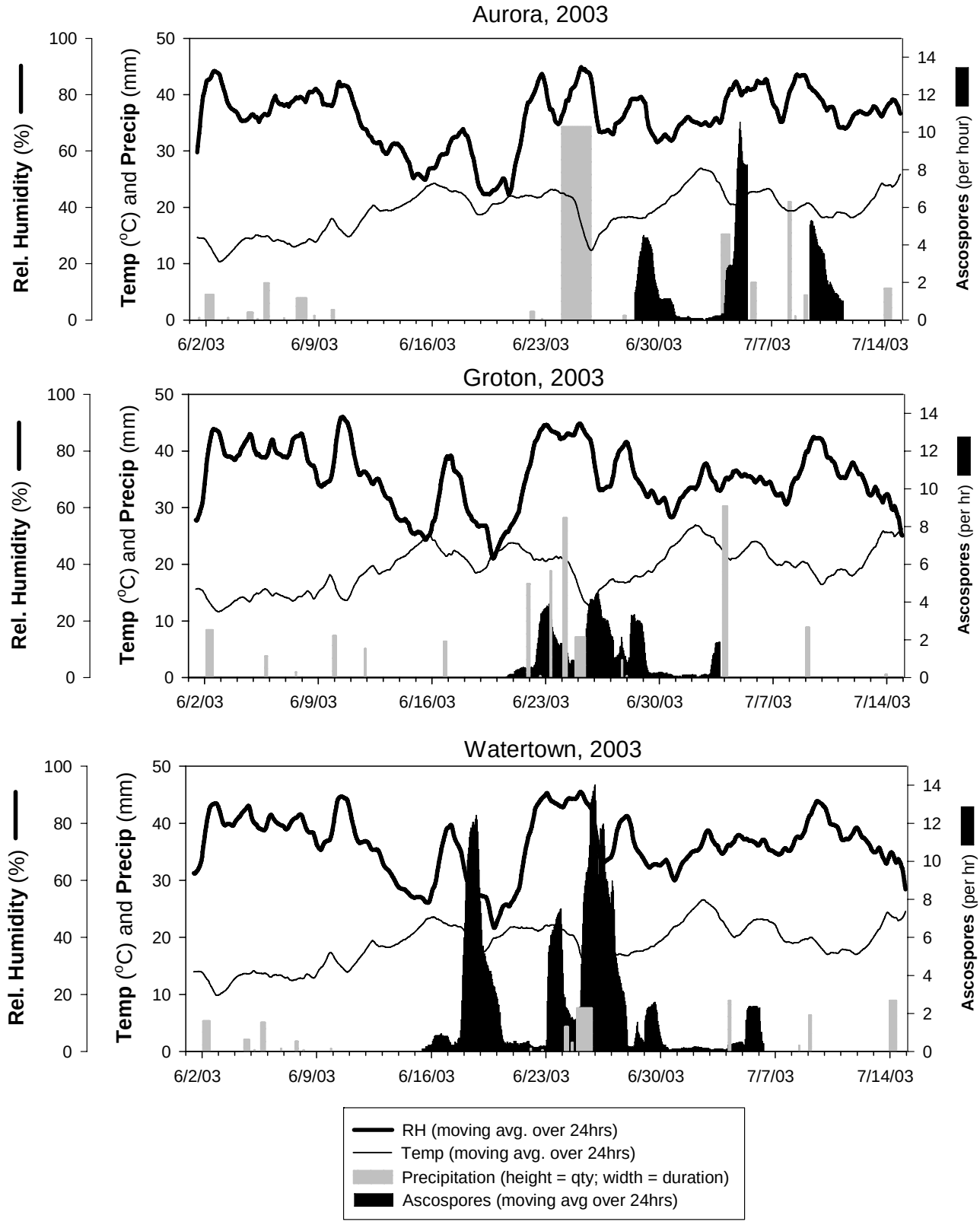
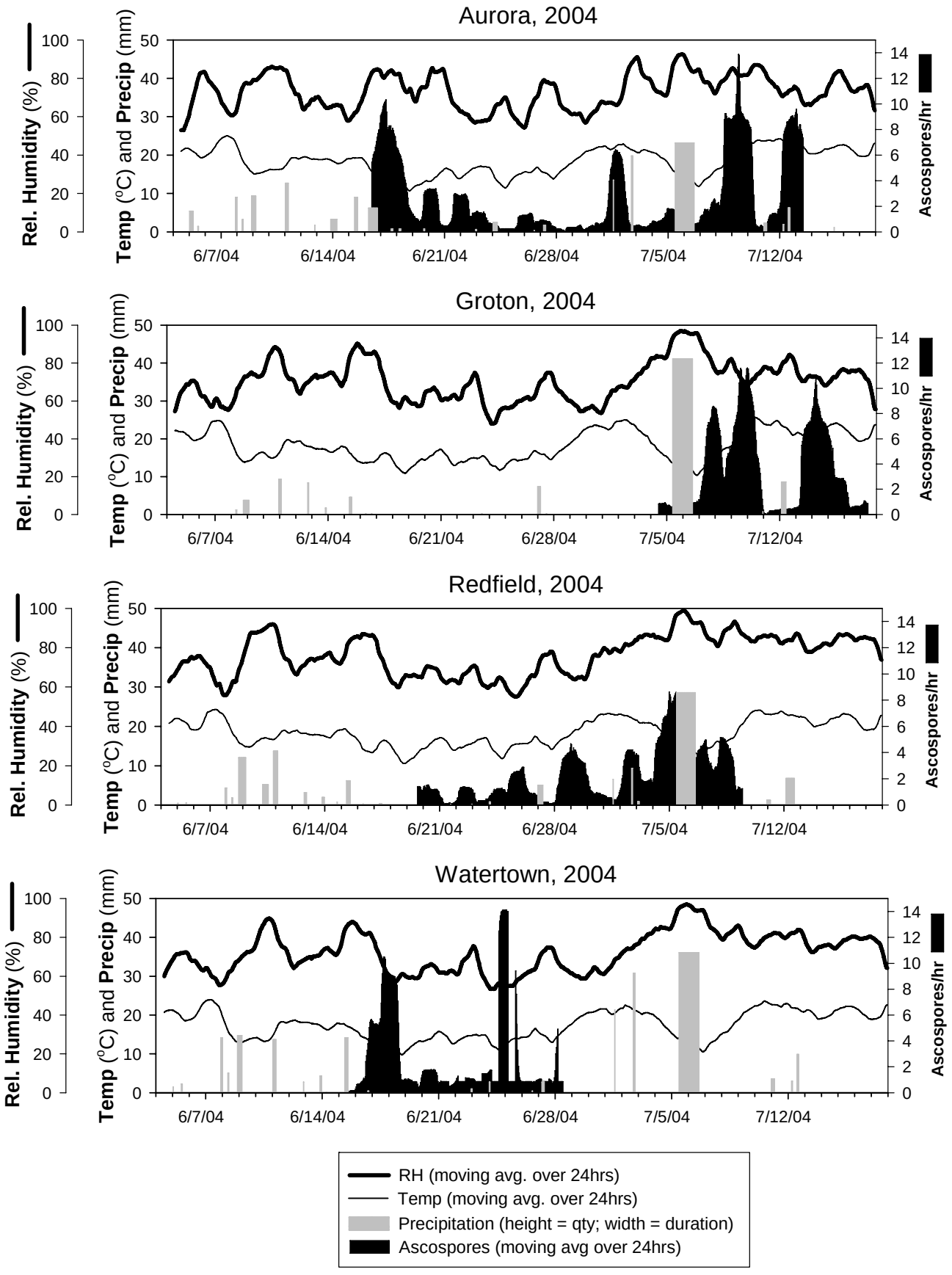
Figures 1-3.


Aurora, 2003
Groton, 2003
Watertown, 2003
Rel. Humidity (%)
Temp (°C) and Precip (mm)
Ascospores (per hour)
Ascospores (per hr)
6/2/03
6/9/03
6/16/03
6/23/03
6/30/03
7/7/03
7/14/03
RH (moving avg. over 24hrs)
Temp (moving avg. over 24hrs)
Precipitation (height = qty; width = duration)
Ascospores (moving avg over 24hrs)

Figures 4-7.


Aurora, 2004
Groton, 2004
Redfield, 2004
Watertown, 2004
Rel. Humidity (%)
Temp (°C) and Precip (mm)
Ascospores/hr
6/7/04
6/14/04
6/21/04
6/28/04
7/5/04
7/12/04
RH (moving avg. over 24hrs)
Temp (moving avg. over 24hrs)
Precipitation (height = qty; width = duration)
Ascospores (moving avg over 24hrs)

EFFECTS OF MAIZE RESIDUES AND VARIETY ON FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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INTRODUCTION AND OBJECTIVES

Fusarium head blight (FHB) of wheat and barley, caused by numerous *Fusarium* species, but primarily by *Fusarium graminearum* (teleomorph: *Gibberella zeae*) continues to occur at epidemic and at sub-epidemic levels in many regions of the U.S. and Canada including growing areas in the central and eastern U.S. By investigating the relationship of FHB incidence and severity to environmental conditions, a better characterization of the disease can be made. Environmentally-based forecasting systems have been shown to be effective in predicting epidemic levels of FHB in field situations (De Wolf et al, 2003) using temperature, precipitation and relative humidity parameters; however, the accuracy of these modeling systems is considered to be only moderate. Through the course of collecting disease and environmental data over numerous location-years, it has been observed that field disease can be highly variable under environments falling near the prediction threshold for the models mentioned above. It was hypothesized that in those instances when environment is not highly conducive to disease development, inoculum level may be more predictive of final disease than is the environment. Additionally, host resistance is an important factor that should be considered when developing and evaluating prediction systems. Although there are no wheat cultivars available having total resistance to FHB, there is wide variability among commonly planted varieties.

South Dakota State University is part of a multi-state collaborative project studying the epidemiology of Fusarium head blight (FHB) on wheat under different environments throughout the north-central and upper mid-west regions of the U.S. The ultimate goal of this collaborative effort is to refine a disease risk advisory/forecast system, and to elucidate principle components of the FHB disease cycle. In 2003, a project was established to examine the influence of varying inoculum load on field disease measurements. The study was continued for two additional years, with some modification. The primary objectives include: 1) establishment of distinct inoculum (spore) loads by varying the amount of maize stover residue on the soil surface of experimental plots; 2) to determine the effects of high and low inoculum loads and weather on final disease and mycotoxin levels in grain; and 3) to evaluate the effects of varied inoculum levels on two differentially FHB susceptible varieties under identical environments. An additional treatment and fourth objective were added in 2005 by including a fungicide in the design. The objective was to determine if fungicide treatment interacts with variety, inoculum level, and weather parameters to affect final disease estimates.

RESEARCH METHODS

Field plots were established in Brookings, SD in 2003, 2004 and 2005, based on protocols established by collaborators. In general, plots consisted of residue treatment (0, 30, and 80% soil coverage, by line-transect method) to generate corresponding low, medium and high levels of local inoculum. The medium level was discontinued for 2005. Sub-plots consisted of spring wheat varieties ('Alsen', a moderately resistant cultivar; and 'Norm', a highly susceptible cultivar). In 2005, sub-sub plots were treated with tebuconazole fungicide (trade name: Folicur, Bayer CropScience) or water (control). Plot size varied slightly from year to year due to space restrictions; however, final plot disease measurements were collected from areas no smaller that 3.1m by 4.6m, representing the smallest division of space within the design. In each year, whole-plots (residue treatments) were buffered on all sides by 8m of a tall wheat variety ('Reeder' in 2003, and 'Ingot' in 2004 and 2005) to mitigate inter-plot interference. For fungicide treated

plots in 2005, application of Folicur at 126 g a.i. per hectare (4oz product per acre) was made at Feekes 10.51. Within all sub-plots, a designated area was sampled daily after spike emergence by collecting five spikes per sub-plot for enumeration of spike-borne inoculum. At three weeks post-flowering, disease ratings were made on all plots after Stack and McMullen, (1995) and included incidence and severity estimates on 100 spikes per experimental unit. Incidence is defined as proportion of 100 rated spikes exhibiting disease symptoms. Severity is the mean severity per infected head. Disease index is the product of incidence and severity and is the overall 'amount' of disease in the field. Harvest data collected included plot yield, test weight, moisture content, assessment of *Fusarium*-damaged kernels (FDK's), and mycotoxin concentration in grain. A Burkard volumetric spore collector was placed for daily monitoring of airborne inoculum. Weather data was collected using a weather station established within the plot area, consisting of Campbell Scientific data logger and sensors. Parameters measured included temperatures and relative humidity in and above the crop canopy, wind, solar radiation, precipitation, soil temperature, soil wetness and leaf wetness estimations.

Each year, two planting dates (PD), 10-13 days apart, were utilized creating two identical studies upon which all measurements were collected. The planting dates were included to provide more opportunity for experiencing epidemic-like conditions, and were not intended to be included together in combined analysis. However as data is complete for both plantings each year, and interesting observations have been made regarding the comparison of planting dates within years, some combined analysis and comparative analysis will be discussed.

No additional inoculum in the form of spore suspension or colonized grain (for ascospore spawn) was added to the study areas. The study was dependant on inoculum formed locally (e.g.: beneath the crop canopy on plant residue), or externally (e.g.: on adjacent fields with corn or small grain residue). No environmental modification was implemented to alter the conditions for disease development.

RESULTS AND DISCUSSION

The years 2003, 2004 and 2005 were distinctly different in terms of statewide levels of FHB on spring wheat, and this difference is mirrored in the overall disease levels observed in this study. In general, 2004 had the highest levels of disease in spring wheat in the eastern and northeastern parts of SD, while 2003 was a more typical of non-epidemic years for the region, with only moderate levels of FHB in most of the state. In 2005, disease was extremely high in winter wheat throughout the state, but spring wheat crops generally had less FHB than in 2004. Therefore, within this study is represented three distinct categories: low disease (2003), moderate disease (2005), and high disease (2004). As mentioned, two planting dates (PD) were utilized, and in each year PD 2 exhibited higher disease and toxin levels than PD 1.

Establishment of local inoculum levels - The objective in placing three levels of maize residue was to establish three distinct levels of local ascospore inoculum within experimental plots. In general, head washing data from all plots over three years showed no differences among residue treatments except at distinct dates when peaks indicate a gradient of spike-borne inoculum highest for the 80% residue treatment plots, and lowest for the 0% residue plots. The data is not shown, but could indicate that when the environment is highly favorable for spore release or dispersal, local inoculum from high residue situations may play a significant role in the total inoculum load available for host infection on that date. The lack of distinct differences in spike-borne inoculum among residue treatments for most days may indicate that a high degree of interplot interference or a significant level of external inoculum was present at the study sites.

Effects of high and low residue levels on FHB - In general, residue treatments had no significant effects on visual disease estimates; however toxin concentrations were significantly affected (tables 1 and 2). Mean deoxynivalenol (DON) concentration for grain from all 0% residue plots (PD and varieties combined) was 2.9 ppm while the grain from the 80% treatment contained 3.9 ppm DON. For 'Norm' plots, which had

significantly higher toxin levels in all cases than 'Alsen', DON levels were 4.9 ppm in the 0% plots and 5.8 ppm in the 80% residue plots (years and PD's combined). For 'Alsen', DON was at 1.0 ppm and 2.0 ppm for the 0% and 80% residue treatments, respectively.

The effect of the residue on DON levels was perhaps most apparent for PD 1, though disease levels were generally lower than for PD 2. In PD 1, 'Norm', DON was 52% higher in the high residue plots compared to the 0% plots, whereas for 'Alsen', the high residue treatment had 131% more DON than the 0% treatment. The large differences in DON accumulation among residue treatments may be a result of higher levels of local inoculum incident on spikes, allowing for greater surface colonization potential, and perhaps high levels of superficial infections. The fungal colonies may not significantly exacerbate disease symptoms, however fungal biomass may be higher when local inoculum is incident and viable on the spike surface or other niches that are not good infection sites. Future investigations will focus on the relationship of disease, DON accumulation and surface colonization/ inoculum load.

Effects on differentially susceptible varieties - As indicated above, there was no strong significant effects of residue treatments on visual disease estimates (incidence, severity, or index); however, there were significant differences in disease estimates among the two varieties. As expected, 'Norm' was higher than 'Alsen' in incidence, severity and disease index in all cases. There were no significant variety by residue interactions for disease estimates. Interaction between variety and year was observed for PD 2 and for the combined PD's, but not for PD 1. The interaction in all cases represented a larger response to high disease pressure in 'Norm' than in 'Alsen'. Figure 1 and 3 represent examples of this interaction. In figures 1-3, years are arranged on the x-axis in order of lowest to highest disease pressure. It is clear that 'Norm' had greater disease relative to 'Alsen' each year, but also that greater disease pressure had a stronger influence on 'Norm' than on 'Alsen' for PD 2 and for the combined data. For PD 1 (Fig. 2), the overall disease pressure was lower each year than for PD 2. The interaction was not detected for PD 1.

CONCLUSION

In each year of this study, there was no clear effect of residue treatment on disease estimates. The purpose of the residue was to establish distinct local inoculum levels, and observe the influence on disease. It is clear that even though it appears that there was an influence on local inoculum levels on certain days, it was not noted for most days. For those instances when inoculum levels peaked, and differences among residue treatments were apparent in spike-borne inoculum levels, the peaks may or may not have coincided with peak susceptibility of the host. The residue treatments did have an effect on toxin accumulation and this is likely due to increased fungal colonization that was not detectable visually, during disease rating. The disassociation between visual estimates of disease and DON contamination has been noted numerous times by other researchers (Paul, et al., 2005) and this set of experiments may show that, indeed, increased levels of inoculum may result in higher levels of toxin, though not necessarily in higher disease levels, for a given variety.

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Table 1. Mean DON Concentrations for 'Norm' grain

	Planting Date 1		Planting Date 2		PD1+2	
(ppm)	0%	80%	0%	80%	0%	80%
2003	0.80	0.40[1]	0.78	1.28	0.79	0.84
2004	4.43	7.93	17.18	18.23	10.81	13.08
2005	1.75	2.05	2.15	2.43	1.95	2.24
3 year avg	2.46	3.74	7.24	7.86	4.85	5.80

1. Limit of detection = 0.5 ppm, values below detection assigned value of 0.25 ppm. therefore some averages may result from a large number of such samples.

Table 2. Mean DON Concentrations for 'Alsen' grain

	Planting Date 1		Planting Date 2		PD1+2	
(ppm)	0%	80%	0%	80%	0%	80%
2003	0.25[1]	0.25[1]	0.25[1]	0.25[1]	0.25[1]	0.25[1]
2004	0.53	1.53	3.75	7.38	2.14	4.46
2005	0.25[1]	0.52	0.70	1.00	0.48	0.76
3 year avg	0.35	0.81	1.69	3.11	1.00	1.96

1. Limit of detection = 0.5 ppm, values below detection assigned value of 0.25 ppm. therefore some averages may result from a large number of such samples.

Fig. 1: Disease Index (Inc*Sev), PD1+2

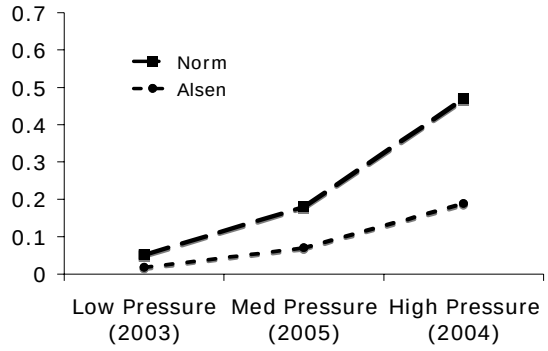


Fig. 2: Disease Index (Inc*Sev), PD 1 only.

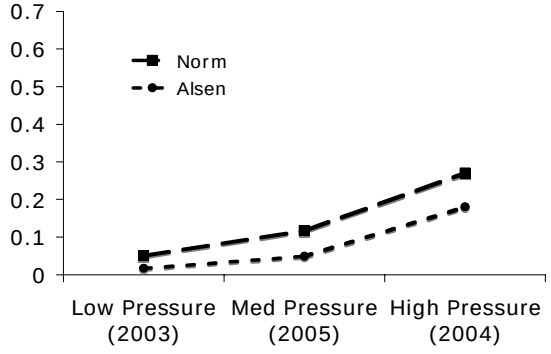
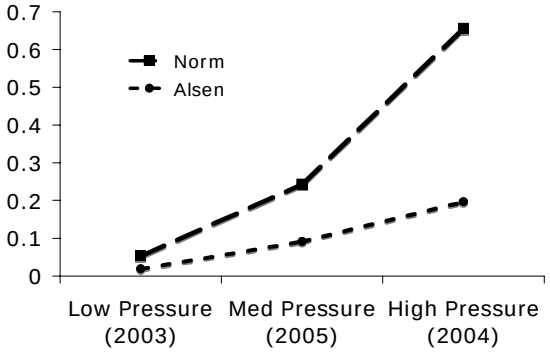


Fig. 3: Disease Index (Inc*Sev), PD 2 only.



RELATIONSHIP BETWEEN FHB INDEX AND DON: A QUANTITATIVE SYNTHESIS OF EIGHT YEARS OF RESEARCH

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ABSTRACT

The reemergence of Fusarium head blight (FHB) in the 1990s has forced researchers from multiple institutions, representing various states and wheat-growing regions, to collaborate in an effort to better understand this disease and to reduce its detrimental impact on the wheat and barley industries. Among the many collaborative research efforts currently in progress are Uniform Fungicide Trials designed to investigate fungicide effectiveness in managing FHB and deoxynivalenol (DON), and Uniform FHB Screening Nurseries for the development of resistant cultivars. In both types of investigation, trials have been conducted according to standard protocols over multiple years and locations. Collaborative research allows for the collection of a large amount of data from a range of environmental conditions and wheat cropping systems, enabling the evaluation of various responses (effects) under different disease pressures. The inherent variability among years and locations, however, has led to contrasting conclusions being drawn about several of the responses being investigated. Of notable mention are the relationship between FHB and DON and the percent control of FHB and DON achieved through Folicur application.

Meta-anslyses were conducted to evaluate the relationship between FHB and DON and the overall effectiveness of Folicur at reducing FHB and DON. Meta-analysis is the quantitative synthesis of the results from multiple individual studies. It is regarded as an objective approach for integrating and interpreting results and drawing conclusions from multiple studies, and allows the investigator to evaluate study-specific characteristics likely to influence relationships and treatment effects. In meta-analysis, some measure of magnitude of treatment effect or association between variables (called effect size) is gathered from the results of individual studies, converted to a common metric, and analyzed to determine the magnitude, significance, heterogeneity, and precision of the mean effect size across studies. For the purpose of evaluating the relationship between FHB and DON, correlation and regression coefficients (intercepts and slopes) were used as measures of the strength of the relationship between the two variables. Response ratio and percent control were used as measures of the effectiveness of Folicur against FHB and DON.

The results from eight years of fungicide trials and resistance screening nurseries were gathered for this analysis. The effects of wheat type, study type, study location, disease level, and DON level on the relationship between FHB and DON were determined, and the influence of wheat type on the effectiveness of Folicur was evaluated. There were significant positive relationships between DON and all commonly used measures of Fusarium head blight intensity. The overall mean correlation (r) between index (IND) and DON was 0.62. Approximately 70% of the 158 studies analyzed had r values greater than 0.50. Correlations were significantly affected by wheat type (spring versus winter wheat), study type (fungicide versus. genotype trials) and study location (U.S. spring- and winter-wheat-growing regions, and other wheat-growing regions). The strongest correlations were observed in studies with spring wheat cultivars, in fungicide trials, and in studies conducted in U.S. spring-wheat-growing regions. There were minor effects of magnitude of disease intensity (and, indirectly, environment) on the correlations. The overall mean regression slope and intercept for the relationship between IND and DON, 0.22 and 2.94, respectively, were significantly different from zero ($P < 0.001$). Thus,

for every unit increase in IND there was on average a 0.22 ppm increase in DON; furthermore, when there was no visual symptoms of Fusarium head blight (IND = 0), the overall mean DON level was 2.94 ppm.

In preliminary investigations, the overall percent control of FHB index (across 118 studies) resulting from the application of Folicur was approximately 41%; however, the overall percent control of DON (across 91 studies) was only 22%. Wheat type significantly affected the percent control of IND and DON. These analyses are still ongoing and will be repeated following the inclusion of data from 2005. Results to date confirm the high variability in disease control by Folicur and indicate that the fungicide is considerably more effective in reducing disease index than in reducing DON concentration.

COLONIZATION OF WHEAT CULTIVARS BY *FUSARIUM GRAMINEARUM* AT HARVEST AND IN OVERWINTERED RESIDUES

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OBJECTIVE

To establish the effect of the overwintering of residues and resistance in wheat to FHB on the colonization, survival and inoculum production of *Fusarium graminearum*.

ABSTRACT

Fifteen wheat cultivars were grown at two locations in Minnesota (Oklee and Humboldt) in 2004. The experimental design was a randomized complete block design with two replications. Immediately prior to harvest, 30 bundles (3-5 plants/bundle) were arbitrarily sampled from each plot. Thirty plants per plot were assayed to determine the incidence of colonization of nodes by Fusaria in mature plants to harvest. The remaining plants (25-35 per plot) from each plot were buried superficially (2-5 cm soil cover) at Umore Park,Rosemount, MN in October 2004. The buried plants were recovered in April 2005 and assayed to determine colonization of nodes by *F. graminearum* and other Fusaria in wheat residues left in the field over the winter. Isolations from nodes on *Fusarium*-selective Komada's medium indicated that there was a significant (P = 0.01) increase in the colonization of nodes by *F. graminearum* in the six months from harvest 2004 (5.8%) to spring 2005 (41.6%). Overwintered nodes of the wheat cultivars Norpro (53.6%), Mercury (50.1%), Parshall (48.4%), MN97803 (47.5%), Dapps (47.1%), Oxen (44.4%), Briggs (42.6%) and Reeder (41.1%) had higher levels of *F. graminearum* colonization than nodes of cultivars Oklee (39.3%), Granite (37.1%), Hanna (36.95%), Alsen (34.6%), Walworth (34.4%), Knudson (34%), and Verde (33%). The data suggests that *F. graminearum* continues colonizing wheat residues over the winter, and the rate of this colonization is cultivar dependent.

INTRODUCTION

Fusarium head blight (FHB), caused predominantly by *Fusarium graminearum*, is a devastating disease of wheat and barley. Understanding the factors affecting the epidemiology of FHB in the U.S. is a high priority for researchers. Among the factors influencing epidemics is the primary inoculum of *F. graminearum*. The impact of wheat cultivars with some resistance to FHB on the colonization of host residues and the subsequent production of inoculum is generally unknown. The main objectives of this study were 1) to examine the colonization by *F. graminearum* of nodes from wheat plants at harvest, 2) to compare colonization by *F. graminearum* at harvest with that of nodes of plants which overwintered in the field, and 3) to examine the effect of host resistance to FHB on the colonization of wheat residues.

MATERIALS AND METHODS

Fifteen wheat cultivars included in the 2004 Red River On-Farm Yield Trials were sampled at two trial locations in Minnesota (Oklee and Humboldt). Each cultivar was grown in 7.6 m x 2.0 m plots arranged in a randomized complete block design with two replications. Cultivars were subject to natural infection by Fusarium head blight fungi. Prior to harvest 30 bundles (3-5 plants/bundle) of mature plants were arbitrarily sampled from each plot. Thirty plants per plot were used to determine the incidence of Fusariua colonizing nodes prior to harvest. On Oc-

tober 20, 2005 the remaining plants (25-35) were placed in a furrow in a field (UMore Park, Rosemount, MN) and covered loosely with 2-5 cm of soil known to have 735-819 CFU/g of *F. graminearum* in the surface soil (0-2 cm deep). The buried plants were recovered the following spring (April 2005) and used to determine the incidence of Fusaria colonization of nodes following six months in the field.

To isolate Fusaria, the nodes were excised from plants collected at harvest and from residues recovered the following spring. The excised nodes were split in two and the node pieces surface sterilized with 70% ethanol for 30 s and 0.5% NaOCl for 60 s then rinsed three times in sterile distilled water. Surface sterilized nodes were then plated onto Komada's medium (selective for *Fusarium* spp.). Nodes plated onto Komada's medium were incubated at 20-24ºC under fluorescent lights (12:12, light:dark) for ca. 12 days. *Fusarium* isolates were identified to species according to Burgess et al. (1994). The incidence of colonization of nodes by *F. graminearum* was determined as the percentage of plated nodes from which *Fusarium* spp. were recovered.

Data obtained were analyzed using SAS PROC ANOVA.

RESULTS

Regardless of trial location and sampling date, *F. graminearum* was the pathogenic *Fusarium* species most frequently isolated at harvest and in the following spring (Fig. 1 and Fig. 2). Other pathogenic Fusaria were isolated at much lower frequencies (Fig. 1 and Fig. 2).

There was a significant increase of colonization by *F. graminearum* of nodes from harvest (October 2004) to the following spring (April 2005). At harvest, the overall incidence of *F. graminearum*-colonized nodes at Oklee and Humboldt was 5.4% and 2.2%, respectively, whereas, in the following spring, the incidences were 41.7% (Oklee) and 41.6% (Humboldt).

Not all the wheat cultivars were colonized at the same level (P = 0.03). The wheat cultivars Norpro (53.6%), Mercury (50.1%), Parshall (48.4%), MN97803 (47.5%), Dapps (47.1%), Oxen (44.4%), Briggs (42.6%) and Reeder (41.1%) had higher levels of *F. graminearum*-colonized nodes than Oklee (39.3%), Granite (37.1%), Hanna (36.95%), Alsen (34.6%), Walworth (34.4%), Knudson (34%), and Verde (33%) (Fig 3).

DISCUSSION

Previously, we have shown the increase of colonization of nodes by *F. graminearum* in overwintered standing wheat plants in comparison with the levels of colonization immediately prior to harvest (Salas and Dill-Macky, 2004). The data presented here supports this finding and indicates that the burial of residues may promote the colonization of residues by *F. graminearum* (harvest = 5.8% vs. spring = 41.6%) over the winter months. The high level of colonization of nodes presented in this study may have resulted from Fusarium spp. within the plant or from inoculum present in the soil and other residues in the proximity of the buried residues.

Our findings may help explain the high incidence of FHB frequently observed in wheat produced using conservation tillage systems (Dill-Macky and Jones, 2000). Residues which are partially buried residues during chisel plowing in the fall may be subject to further colonization by *F. graminearum* over the winter. If these residues are then brought to the surface through cultivation operations in the spring (including planting), perithecia and ascospores may develop providing inoculum to infect subsequent wheat crops. It is known that *F. graminearum* can colonize and survive as a saprophyte on buried crop residues (Sutton, 1982); perithecia can develop in Fusarium-infected kernels at the soil surface or buried at 5 or 10 cm (Inch and Gilbert, 2003); and resurfacing of previously buried residues can lead to the production of perithecia and ascospores (Pereyra et al., 2004).

It is interesting to note that the rate of colonization of residues of wheat cultivars was directly correlated to the differential colonization of the plants of those wheat cultivars by *F. graminearum* (Salas et al., 2004). Moderately FHB resistant cultivars such as Alsen,

Hanna, or Verde had lower levels of colonization by *F. graminearum* than the susceptible or moderately susceptible cultivars Norpro, Mercury, Reeder, or Oxen. Thus this study supports the finding that cropping resistant cultivars may help lower the inoculum of Fusarium in subsequent growing seasons as reported in a previous study by Salas and Dill Macky (2005).

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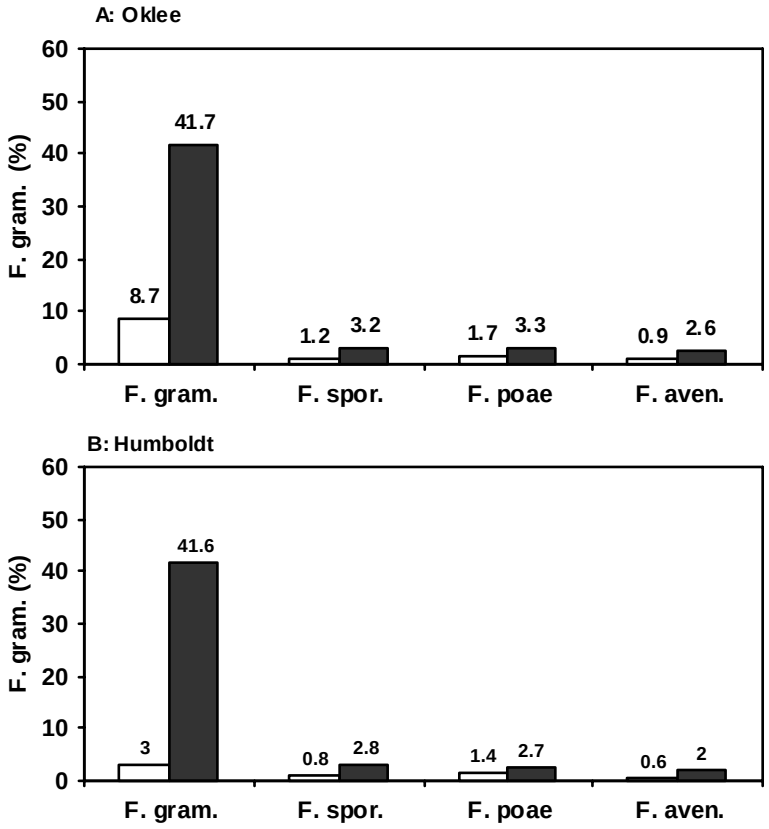

Fig. 1. Overall incidence of colonization of wheat nodes by pathogenic Fusaria (*F. graminearum, F. sporotrichioides, F poae,* and *F. avenaceum*) in plants collected prior to harvest (□) or overwintered buried residues (■) (nodes) at Oklee (A) and Humboldt (B), Minnesota.

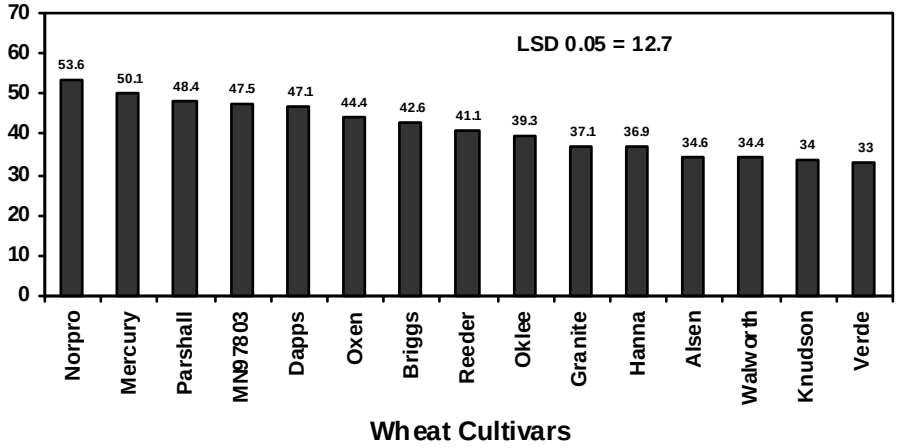

Fig. 2. Overall incidence of colonization of nodes from overwintered residues by *Gibberella zeae* (*F. graminearum*) in fifteen wheat cultivars in the 2004 Red River Valley On-Farm Yield Trials.

EFFECT OF RESIDUE MANAGEMENT AND HOST RESISTANCE ON THE EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT

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OBJECTIVE

To examine the effect of the post-planting burning of wheat residues and host resistance on the *F. graminearum* population in soil, airborne *F. graminearum* inoculum, and the subsequent colonization of a Fusarium head blight susceptible wheat crop by *F. graminearum*.

ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* limits wheat production in the U. S.. Despite the importance of the inoculum of *F. graminearum* in the epidemiology of FHB, little is known about factors affecting levels of inoculum. We examined the effect of burning residue(unburned control, light and severe burns) and resistance in wheat to FHB (FHB susceptible - Wheaton, Norm; FHB moderately susceptible - 2375, Ingot; FHB moderately resistant - BacUp, Alsen) in plots established at Rosemount, MN in May 2003. All plots were left in situ (unharvested) over the winter. In April 2004, plots were chisel plowed, planted to Wheaton, and burned as in 2003. *F. graminearum* was isolated from wheat residues and surface soil at planting, air within the canopy at anthesis and early dough, and from Wheaton plants at hard dough in 2004. The severe burn treatment significantly reduced; the survival of *F. graminearum* in straw, the populations of the pathogen in soil and in the air, and the colonization of Wheaton, in comparison with the lighter burn or control treatments. Plots previously planted to FHB susceptible wheat cultivars (Wheaton and Norm) had higher populations of *F. graminearum* in soil and air samples, and the subsequent crop of Wheaton was more heavily colonized than in plots of Wheaton following Ingot, BacUp, 2375 and Alsen. Our data confirms that grain producers would benefit from reducing wheat residues and by cropping FHB resistant cultivars.

INTRODUCTION

Fusarium head blight is a major disease of wheat in the U. S.. Although cereal residues are considered to be the major source of inoculum inciting FHB epidemics (Salas and Dill-Macky, 2004), little is known about the role of residues in determining inoculum levels. This study examined the impact of residue destruction and host resistance on; the *F. graminearum* population in soil, airborne inoculum, and the subsequent colonization of a FHB susceptible wheat crop.

MATERIALS AND METHODS

A field experiment with two factors, burn (control, light and severe burns conducted after planting) and FHB host resistance (FHB susceptible - Wheaton, Norm; FHB moderately susceptible - 2375, Ingot; FHB moderately resistant - BacUp, Alsen) was established at UMore Park in Rosemount MN in 2003 over residue of a 2002 Norm wheat crop. Cultivars (plots, 3.7 m x 7.6 m) were grown to maturity and left in situ over the winter (unharvested). In April 2004, plots were chisel plowed, planted to Wheaton and the residue burning treatments repeated so that each plot had the same burn treatment as in 2003.

From each plot in 2004, all wheat straw visible on the soil surface of a 0.5 m^2 quadrat, five surface soil samples (collected at points 1 m apart along a 5-m transect in each plot, 0-2 cm depth) and 30 Wheaton plants at hard dough growth stage were collected.

Nodes were excised from the residue of the wheat grown in 2003 and crowns, nodes and kernels from the 2004 Wheaton plants. The excised node and crown tissues and kernels were surface sterilized with 70% ethanol for 30 s and 0.5% NaOCl for 60 s then rinsed three times in sterile distilled water. Surface sterilized tissues were then plated onto Komada's medium (selective for *Fusarium* spp.). Soil samples were air dried for 4 days at 20-24ºC, sifted, and 6 mg of fine soil particles (<250 μ) from each plot was dispersed onto Komada's agar medium on each of five Petri plates. Airborne inoculum of *F. graminearum* within the canopy was trapped by exposing three Komada plates/plot at soil level between 9 AM and 10 AM for ten minutes.

All plates were incubated at 20-24ºC under cool white and UVA (1:1) fluorescent lights (12 hr photoperiod) for 14 days. Fusaria, including F. *graminearum*, were identified according to Burgess et al. (1994). The incidence of colonization of nodes by *F. graminearum* was determined as the percentage of plated nodes from which *Fusarium* spp. were recovered.

All data obtained was analyzed using SAS PROC ANOVA.

RESULTS

In comparison with the non-burned treatment, residue burning significantly reduced; the amount of straw at the soil surface, the survival rate of *F. graminearum* in nodes and the population of *F. graminearum* in soil (Table 1). Burning also reduced the airborne inoculum within the canopy at anthesis and early dough, and the subsequent colonization of the wheat crop (Table 1).

Populations of *F. graminearum* in soil after a crop of the FHB-susceptible cultivars (Norm and Wheaton) were higher than those following moderately susceptible or resistant cultivars (Fig. 1). Similarly, airborne inoculum was higher in plots previously planted to Norm and Wheaton (Fig. 2) and high levels of colonization by *F. graminearum* was seen in the Wheaton planted into these plots (Fig. 3).

While *F. graminearum* colonized nodes throughout the whole canopy, kernels were the most heavily colonized tissue examined (Fig.4).

DISCUSSION

Our data support the findings of others and indicate that wheat residues can harbor *F. graminearum*, provide a local source of inoculum (Pereyra et al., 2004) and need to be managed. Heavily colonized residues were demonstrated to increase inoculum levels in soil and air, thus providing greater inoculum for FHB epidemics.

Wheat cultivars were shown to be differentially colonized by *F. graminearum*, as in our previous studies (Salas and Dill-Macky, 2004). This differential colonization of cultivars affects the production of *F. graminearum* inoculum, thus cultivar selection could impact the risk of future FHB epidemics. Residues of FHB susceptible cultivars are likely to release more inoculum than FHB moderately resistant cultivars.

Cultural practices that eliminate wheat residues and/or the cropping of FHB resistant cultivars may help producers to reduce their risk of FHB.

ACKNOWLEDGEMENTS

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Salas, B. and Dill-Macky, R. 2004. Incidence of *Fusarium graminearum* in pre-harvest and overwintered residues of wheat cultivars differing in Fusarium head blight-resistance. In: Canty, S. M., Boring, T., Wardwell, J. and Ward, R. W. (Eds.), Proceedings of the 2nd International Symposium of Fusarium Head Blight; Incorporating the 8th European Fusarium Seminar; 2004, 11-15 December; Orlando, Fl, USA. Michigan State University. East Lansing, MI. pp 502-503.

Table 1. Effect of residue destruction on; residue dry matter (number of nodes/m^2), survival of *F. graminearum* (FG) in nodes, the population of FG in soil at the time of planting, airbone inoculum of FG at anthesis and early dough, and colonization by FG of a subsequent wheat crop of the FHB-susceptible cultivar Wheaton.

Burning[1]	Nodes (no./$m^{2)}$	FG survival (%)	FG in soil (cfu/g)	Airborne FG (cfu/Petri plate)		Wheaton FG Colonization (%)
				Anthesis	Early Dough	
Control	62 a[2]	33.0 a	693 a	7.6 a	15.1 a	18.4 a
Light	46 b	13.1 b	598 b	6.6 a	12.2 b	17.7 a
Severe	36 c	9.0 b	522 b	4.8 b	9.8 c	11.3 b

[1]Control, non-burned residues; Light, one pass with an alfalfa burner (1.3 m/s); Severe, one pass with an alfalfa burner (0.5 m/s)
[2]Means followed by different letters within a column are significantly different at P=0.05 level.

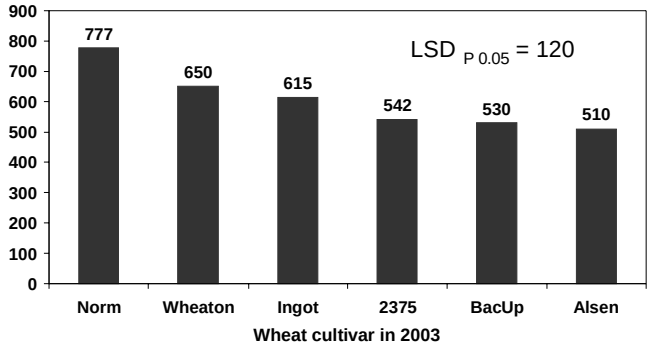

Fig. 1. Effect of the cultivar of 2003 wheat crop on populations of *F. graminearum* (FG) in soil in 2004.

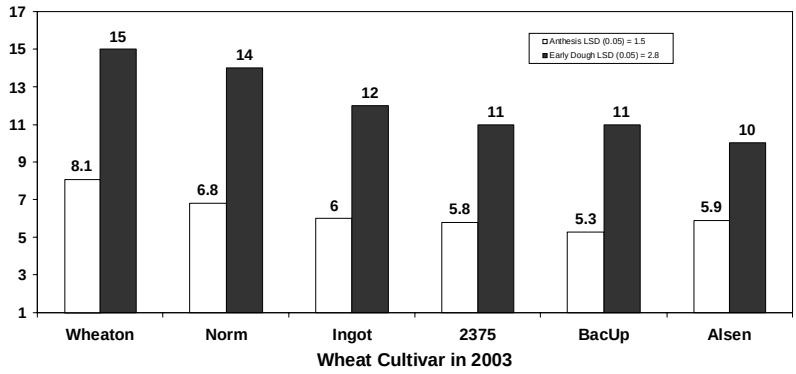

Fig. 2. Effect of the wheat cultivar in 2003 on airborne inoculum (cfu/Petri plate) of *F. graminearum* (FG) within the canopy of the 2004 wheat (cv. Wheaton) at anthesis and early dough growth stages.

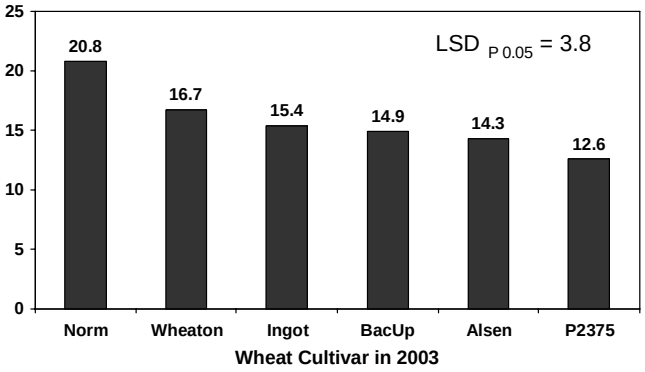

Fig. 3. Effect of wheat cultivar in 2003 on the colonization by *F. graminearum* (FG) of 2004 wheat (cv. Wheaton) at the hard dough growth stage.

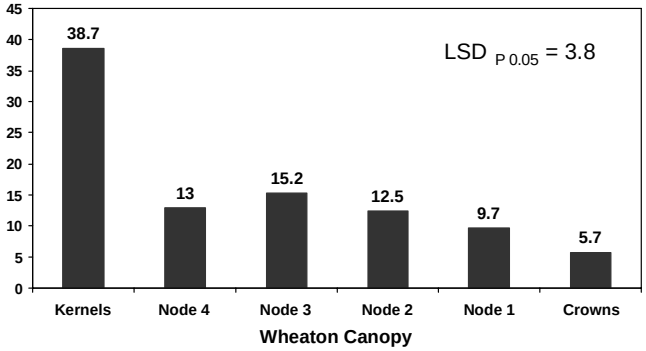

Fig. 4. Incidence of *F. graminearum* (FG) in kernels, node and crown tissues of Wheaton wheat (2004) at the hard dough growth stage.

VALIDATION OF THE DONCAST PREDICTION TOOL IN WHEAT ACROSS FRANCE AND URUGUAY

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ABSTRACT

Forecasting *Fusarium* toxins is useful as a tool to help prevent entry of toxins into the food chain. Wheat fields under an array of agronomic practices were sampled for deoxynivalenol (DON) content at harvest across Ontario from 1996 to 2004. A robust site-specific, DON forecast (DONcast) was developed and commercialized for wheat and has been used to make fungicide spray decisions in Ontario, Canada, for more than 5 years. This model is delivered online, and is sponsored by the crop protection industry and the Ontario Wheat Producers Marketing Board. (http://www.ownweb.ca/ lib/fusarium.cfm). There is growing interest amongst producers to use this tool pre-harvest to make marketing decisions and for grain handlers to use the tool for grain sourcing. For example, in the country of Uruguay in South America, DONcast is being used to alert growers, regulators, and grain handlers of pending problems with DON in locally grown wheat (http://www.inia.org.uy/online/site/157852I1.php). In France, a pilot study is underway to investigate the forecasting tool under European weather and cropping systems. From field data collected in France during 2004, 72% of samples were predicted correctly to contain either above or below 1.0 ppm DON, and 83% of samples were predicted correctly at a 2.0 ppm threshold. Most of the inaccurate predictions were false positives. Similarly, in Uruguay in 2004, 68.3 and 74.8% of samples were predicted correctly to be above or below a threshold of 1.0 and 2.0 ppm, respectively. It is well known that DON predictions are very sensitive to coincidental weather around heading, varietal susceptibility to *Fusarium* and DON accumulation, and to the management of previous crop residue. DONcast has successfully taken these factors into account, and we have demonstrated its robustness across varied environments.

GENETIC STRUCTURE OF ATMOSPHERIC POPULATIONS OF *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae, causal agent of Fusarium head blight (FHB) of wheat and barley and Gibberella ear rot (GER) of corn, may be transported over long- distances in the atmosphere. Epidemics of FHB and GER may be initiated by regional atmospheric sources of inoculum of *G. zeae*, but little is known about the origin of inoculum for these epidemics. We hypothesized that atmospheric populations of *G. zeae* are genetically diverse—potentially originating from multiple locations, and mixed over large geographic distances. We tested this hypothesis by examining the genetic structure of the New York atmospheric populations (NYAPs) of *G. zeae*, and comparing the structure of these NYAPs to populations of *G. zeae* collected from seven different states across the continental United States. Viable, airborne spores of *G. zeae* were collected in rotational (lacking any apparent within-field inoculum sources of *G. zeae*) wheat and corn fields in Aurora, NY in May through August over three years (2002-2004). In all, 780 isolates of *G. zeae* were used for the analysis; 257 isolates were used from four NYAPs, and 523 isolates were used from eight populations collected across the United States. We observed the presence or absence of alleles at 23 amplified fragment length polymorphism (AFLP) loci, based on three separate primer-pair combinations. Normalized genotypic diversity was high (ranging from 0.91 to 1.0) in NYAPs of *G. zeae*, and nearly all of the isolates in each of the populations represented unique AFLP haplotypes. Pairwise calculations of Nei's unbiased genetic identity were uniformly high (> 0.99) for all of the possible NYAP comparisons, and tests for differences among allele frequencies at each of the 23 loci demonstrated that the NYAPs differed at only a single locus. Although the NYAPs were genotypically diverse, they were genetically similar and potentially part of a large, interbreeding population of *G. zeae* in North America. Estimates of the fixation index (G_{ST}) and the effective migration rate (*Nm*) for the NYAPs indicated significant genetic exchange among populations. Low levels of linkage disequilibrium in the NYAPs suggested that sexual recombination in these populations may not be exclusive. When NYAPs were compared to populations of *G. zeae* collected across the United States, the observed genetic identities between the populations were relatively high (ranging from 0.92 to 0.99). However, there was a significant negative correlation ($R = -0.59$, $P < 0.001$) between genetic identity and geographic distance, suggesting that genetic isolation may occur on a continental scale. While the contribution of long-distance transport of *G. zeae* to regional epidemics of FHB and GER remains unclear, diverse atmospheric populations of *G. zeae* suggest that inoculum may originate from multiple locations over large geographic distances. The long- distance transport of *G. zeae* suggests that the management of inoculum sources on a local scale, unless performed over extensive production areas, will not be effective for the management of FHB and GER.

TEMPORAL SCALES OF GENETIC DIVERSITY WITHIN NEW YORK ATMOSPHERIC POPULATIONS OF *GIBBERELLA ZEAE*

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ABSTRACT

Ascospores of *Gibberella zeae* are transported through the atmosphere to wheat spikes and corn ears, where they cause Fusarium head blight (FHB) and Gibberella ear rot (GER), respectively, on susceptible cultivars. We hypothesized that atmospheric populations of *G. zeae* remain genetically diverse over time. We tested this hypothesis by examining various temporal scales of genetic diversity within New York atmospheric populations (NYAPs) of *G. zeae*. We analyzed data from 23 amplified fragment length polymorphism (AFLP) loci for 30 temporal sub-populations comprising a total of 218 isolates of *G. zeae*. Genetic identities were uniformly high (very close to 1) when comparing temporal sub-populations of *G. zeae* collected over consecutive calendar dates, during day and night sample periods, during two-hour sampling intervals throughout the night, and collected during consecutive day and night sample periods at two different field locations in a similar year. We did not observe a significant correlation between genetic identity and time for any of the temporal sub-population comparisons. Tests for differences in allele frequencies across all 23 AFLP loci demonstrated that temporal sub-populations of *G. zeae* collected over consecutive day and night sample periods at two different field locations in a similar year differed at only a single locus. Although field isolates of *G. zeae* are homothallic and may reproduce sexually without a partner, outcrossing under natural conditions may contribute to high levels of diversity in local atmospheric populations of the pathogen. The perpetuation of high levels of genotypic diversity within atmospheric populations of *G. zeae* may result from the continued mixing of atmospheric inoculum sources over time, potentially being transported and mixed over large geographic distances. Our findings suggest that spore sampling during any temporal period would provide an accurate measure of the diversity present in atmospheric populations of *G. zeae*.

ENVIRONMENTAL FACTORS INFLUENCING SCAB OF BARLEY IN THE NORTHERN GREAT PLAINS

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ABSTRACT

We are investigating the relationship between environmental factors, crop stage, and barley genotype with Fusarium head blight (FHB) and DON accumulation in the grain. This project is associated with the established spring and winter wheat FHB-modeling effort and aims to produce the information required to either validate one of the current wheat models for barley, or generate novel models.

Varieties of regionally adapted barley of both 2- and 6-row types were planted at 18 locations in Minnesota, North Dakota, and South Dakota. At least two varieties were common to each location. Plots were a minimum of 1.5m x 4.6m in size and replicated four times in an RCBD. Additional varieties were planted based upon availability and local producer preference. Crop stage was monitored regularly throughout the season and the date at which each plot was at Feekes 10.3 stage was noted. No additional inoculum was introduced into the plots. The incidence and severity of FHB was recorded on a minimum of 25 heads per plot at the soft-dough stage (approximately 21 days after 10.3). Environmental variables consisting of temperature, relative humidity, and precipitation were recorded with an on-site, or nearby, weather station.

When field severity (disease index) was averaged across all blocks and varieties, locations had varying levels of disease with values ranging from 0.20 to 27.68%. Locations in western Minnesota all had relatively low disease (< 3%), whereas those in the Dakotas had a much broader range (<1 to 25%). DON data is pending for many locations; however, the concentrations were relatively low (<2.7 ppm) for the ones currently available. Preliminary investigations into the relationship between disease and weather indicates that the relationship is similar to that described for wheat. Locations where plants were heading when temperature was between 20 and 30°C and mean relative humidity was high (> 70%) and/or frequent rainfall events had occurred resulted in correspondingly high disease severity. Sub-optimal combinations of temperature and RH/precipitation had correspondingly reduced final levels of disease. Winter-habit, feed barley was planted at collaborators' locations in Indiana, Pennsylvania, and Ohio and had trace levels of disease.

THE FUSARIUM HEAD BLIGHT EPIDEMICS OF THE WINTER AND SPRING WHEAT CROPS IN SOUTH DAKOTA FOR 2005

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ABSTRACT

South Dakota is a transition state for wheat production and resides at the Northern and Southern boundaries of the (hard, red) winter and spring wheat "belts", respectively. In South Dakota, FHB has historically only been a concern for spring wheat producers. Winter wheat production was focused in regions with limited precipitation and the crop usually escapes infection because flowering occurs when temperatures are cooler than optimum for FHB. Winter wheat has recently been expanding into the eastern, and southeastern regions of the state. These regions receive regular precipitation, increasing the yield potential for wheat crops substantially. However, they are also the center of corn production and corn residue serves as an excellent source of *Fusarium graminearum* inoculum. The dominant winter wheat varieties grown in SD are also susceptible to scab. The planting of a susceptible winter wheat crop into a region with high precipitation and substantial inoculum potential greatly increased the probability of an epidemic. Rain events and optimal temperatures for infection during flowering of the wheat crop in southeastern SD resulted in a widespread, severe epidemic in 2005.

Spring and winter wheat fields were sampled throughout SD for FHB field severity approximately 3 weeks after flowering and used to generate yield loss estimates. Counties in regions with FHB epidemics were sampled more intensively and data were pooled by crop reporting districts for all instances. FHB field severity in SD ranged from trace to 7% and 60% for spring and winter wheat, respectively. The weighted impact of each district was calculated using the % acreage each represented in SD * average yield/a * the estimated % FHB. Mean estimated % FHB field severity in winter wheat was greatest in the east central (30%) and southeast (60%) districts, with the latter having the highest weighted impact. For spring wheat, the northeast and east-central districts both had 7% estimated FHB severity, with the former having the greatest weighted impact. Using the average price/bushel for 2004, monetary loss estimates were also generated. Losses in SD due directly to FHB infection (direct kernel blighting) were estimated to be $11.4 million and $24.7 million for the 2005 spring and winter wheat crops, respectively.

ACCUMULATION MANNER OF DEOXYNIVALENOL AND NIVALENOL IN WHEAT INFECTED WITH *FUSARIUM GRAMINEARUM* AT DIFFERENT DEVELOPMENTAL STAGES

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ABSTRACT

The manner in which the accumulation of deoxynivalenol (DON) and nivalenol (NIV) progresses in wheat grain infected with *Fusarium graminearum* and the influence of the infection timing on the toxins' contamination were investigated. Five wheat cultivars with different resistance levels were tested in a greenhouse environment, and one of the cultivars was also tested in a field. In both the experiments, the wheat cultivars were spray inoculated with a mixture of two isolates of different chemotypes of *F. graminearum* at three stages: at anthesis, 10 days after anthesis (DAA), and 20 DAA; these were sampled at 10 DAA, 20 DAA, and at maturity and were subsequently analyzed for their toxin content. The results indicated that high levels of DON and NIV can be produced after 20 DAA even by early infection. In addition, it was also indicated that infection at a late stage, at least as late as 20 DAA after which clear Fusarium head blight symptoms were not observed on the spikes, can cause grain contamination with these toxins. Thus, our results indicated the importance of the late stage in grain development in addition to the early stage in DON and NIV contamination, suggesting that the development of control strategies that cover the late stage as well as the early stage would be desirable to reduce the risk of toxin contamination in wheat.

PATHOGEN GENETICS AND GENOMICS

Chairperson: Frances Trail

FUNCTIONAL ANALYSIS OF POLYKETIDE SYNTHASE GENES IN *GIBBERELLA ZEAE*

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ABSTRACT

Polyketides are a complex class of secondary metabolites that include some of the most potent mycotoxins (i.e. aflatoxin, zearalenone, fusarin). In fungi, polyketides are synthesized by large, multi-domain enzymes called polyketide synthases. We have identified from the genomic sequence 15 polyketide synthase genes and functionally disrupted them. Five of these genes are responsible for producing the mycotoxins zearalenone, aurofusarin, fusarin C and the black perithecial pigment. We have shown that each of the 15 genes show relatively unique expression patterns during grain colonization, plant colonization, sexual development, and mycelial growth. None of these is essential to pathogenicity, however, our results indicate that they play important roles in the life cycle of this fungus.

DISPLACEMENT OF THE NATIVE POPULATION OF *FUSARIUM GRAMINEARUM* IN NORTH DAKOTA AND PARTS OF MINNESOTA BY A GENETICALLY DIVERGENT AND MORE TOXIGENIC POPULATION

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ABSTRACT

Population genetic analyses of *Fusarium graminearum* that examined hundreds of strains collected from 13 mainly Midwestern states from 1999-2002 revealed the presence of a genetically distinct population of *F. graminearum* that was restricted in distribution to North Dakota (ND) and Minnesota (MN) and that was characterized by a 3ADON chemotype. Systematic sampling in 2003 and 2004 of more than 7,000 wheat heads from 83 fields in 51 counties of MN, ND and South Dakota (SD) resulted in 4,957 strains. While previous surveys from these states recovered strains with a 3ADON chemotype only occasionally, these more recent and extensive surveys show that its frequency has dramatically increased over just a few years. In the 2003 collection 3ADON strains were at frequencies of 19% and 23% in ND and MN, respectively, while in 2004, 3ADON strain frequencies were again higher, with averages of ca. 35% for both ND and regions of MN north of the counties of Ottertail and Wilkin. A field in Pembina County, ND showed the highest frequency of 3ADON strains at 59.6%. While combined data from 2003 and 2004 indicate that the 3ADON type is now widespread and frequent in most ND and MN areas surveyed, the 3ADON type is less common in MN for regions south of the Fargo/Moorhead area. Among nine southern MN counties surveyed in 2004, the average frequency of 3ADON strains was only 1%, though the 3ADON type still could be detected in seven out of the nine counties. Also, the 3 ADON chemotype has so far not been detected in SD. We also discovered that the emerging population actually consists of both 3ADON and 15ADON chemotype strains. By use of three unlinked VNTR markers we identified 15ADON strains that otherwise could not be distinguished from the 3ADON strains, indicating that they belong to the same population. When the data are combined, the emergent population was at a frequency of about 45% both in ND and MN (north of the counties Wilkin and Ottertail) in 2004, up from about 30% in 2003. Preliminary data indicate that the emergent population has a higher toxigenic potential compared to the native population that consists of 15ADON strains only. Compared to strains of the native population on the susceptible cultivar Norm, 3ADON and 15ADON strains of the emergent population produced on average 68% and 32% higher DON concentrations, respectively in inoculated spikelets. We are currently in the process of further determining the selective advantages of this emergent population in addition to identifying recombinants and determining the amount of recombination between the two populations. Identification and study of recombinants will not only be valuable for potentially mapping regions of the genome that contribute to the higher toxigenic potential, but also for determining whether the high toxigenic potential displayed by the emergent population will be reduced in the future by recombination with the native, less toxigenic population.

FUSARIUM HEAD BLIGHT OF WHEAT IN LOUISIANA IS CAUSED LARGELY BY NIVALENOL PRODUCERS OF *FUSARIUM GRAMINEARUM* AND *FUSARIUM ASIATICUM*

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ABSTRACT

Annual collections of Fusarium head blight (FHB)-symptomatic wheat by the USDA-ARS Cereal Disease Laboratory (CDL) not only cover midwestern states with a history of FHB epidemics, but also southern states that are not generally considered epidemic regions. Since the year 2000, several hundred plant samples have been examined from Texas, Oklahoma, Alabama, Florida, Mississippi, and Louisiana. While *Fusarium* species other than *F. graminearum* are occasionally present in wheat seed, *F. graminearum* itself appears to be scarce in southern states. So far we have not encountered the species in samples from Oklahoma, Texas, and Florida, and our collections only hold one *F. graminearum* isolate each from Mississippi and Alabama. In contrast, genetically diverse populations of the *Fusarium graminearum* (Fg) species complex are present in Louisiana. Our collection of more than 150 strains from four parishes in Louisiana (from 2002, 2003, and 2005) consists predominantly of nivalenol (83%) and 3ADON (13%) chemotype strains; the 15ADON chemotype, which is otherwise predominant in the Midwest was only observed for seven strains. All strains also have been genotyped using five molecular markers based on variable number of tandem repeats (VNTRs) and species identification using the multilocus genotyping array has been performed for some VNTR genotypes (see T.J. Ward *et al*., this Proceedings). Our preliminary analysis of the diversity in Louisiana indicates the following: the largest proportion of the population consists of *F. graminearum* isolates that may possibly belong to the same population as isolates commonly present in the Midwest, though nearly all strains of *F. graminearum* in Louisiana are of a nivalenol chemotype. A smaller proportion of isolates belong to a divergent lineage of *F. graminearum* that displays all three chemotypes. Nivalenol producers of *F. asiaticum* have also been identified that may represent about 25% of the population in Louisiana. The predominance of nivalenol producers from different Fg complex species in Louisiana strongly implies a selective advantage for this chemotype in this particular environment that will be addressed in future studies. Also, as nivalenol has a higher overall toxicity than deoxynivalenol, the risk of a potential spread of nivalenol producers to other U.S. wheat growing regions needs to be evaluated. While deoxynivalenol producers have been reported to have a selective advantage on wheat over nivalenol producers in Nepal this needs to be confirmed and evaluated taking into account the genetic background on which these chemotypes reside.

RELATIVE PATHOGENICITY OF 3-ADON AND 15-ADON ISOLATES OF *FUSARIUM GRAMINEARUM* FROM THE PRAIRIE PROVINCES OF CANADA

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ABSTRACT

Eighteen isolates of *Fusarium graminearum* Schwabe, 3 producing 15-ADON and 3 producing 3-ADON from each of the Canadian provinces of Manitoba, Saskatchewan and Alberta, were tested for relative pathogenicity and consistency of production of toxin, on two Canadian spring wheat cultivars, 'Roblin' (S) and '5602 HR' (MR). The experimental design was a 3-replicate randomized complete block. Each replicate consisted of a pot containing 2 or 3 plants of one cultivar, and were grown in a cooled greenhouse. At anthesis, 2 to 5 heads per pot were inoculated with one isolate and heads covered with glassine bags for 48 h to promote a favourable environment for disease development. Disease was scored at 14 d and 21 d after inoculation and recorded as percentage infected spikelets. These preliminary results showed no significant differences in pathogenicity among isolates from the three provinces and producing either 3-ADON or 15-ADON. Toxin analysis by GC/MS of seeds from the inoculated heads found higher levels of DON in the susceptible (45 ppm) vs resistant (14 ppm) wheat. All isolates formed their respective 3-ADON or 15-ADON analogs. The 3-ADON isolates formed, on average, 16.1 ppm DON on 5602HR and 56.9 ppm on Roblin, higher than the 15-ADON isolates which averaged 11.9 ppm and 33.6 ppm respectively.

GENES IMPORTANT IN ASCOSPORE DEVELOPMENT IN *GIBBERELLA ZEAE*

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ABSTRACT

With the availability of the genome sequence and the Affymetryx Genome Chip for gene identification and gene expression, our ability to identify genes important to ascospore development and perithecium function has greatly increased. We have targeted several gene families using these methods and are now characterizing their role in inoculum development. Myosins are proteins found in a wide variety of organisms and are important in cellular transport and cellular movement. As asci must stretch at the appropriate time to discharge their contents, we hypothesize that they are important in ascus function. Fueled by ATP hydrolysis, myosins move along actin filaments and cause cell movement. In this study 3 myosin genes were deleted from the genome of *G. zeae* and their phenotypic characterization will be presented. Several other groups of genes have been targeted for gene deletion and the results of these experiments will also be presented. Through this process, we have identified several genes whose products are vital to the development of asci and ascospores.

TRIACYLGLYCERIDE ACCUMULATION IN ANTICIPATION OF SEXUAL DEVELOPMENT IN *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae accumulates fats in dikaryotic hyphae prior to host plant senescence and in anticipation of over-wintering and sexual reproduction. Using NMR and gas chromatography, we have analyzed the lipid profile from haploid mycelium to perithecium initials both in culture and *in planta.* Results show that the major sequestered lipid storage products are triacylglycerides. These lipid stores increase dramatically following perithecium induction in culture. In plants, they increase as the fungus approaches sexual development. The triacylgycerides consist mainly of C18 fatty acids with varying degrees of saturation. These results show that lipids play an integral role in survival and reproduction by this fungus. The ability of the fungus to colonize senescing vegetative tissue and accumulate fats has strong implications for the following seasons epidemic.

DELETION OF THE TRICHOTHECENE GENE CLUSTER OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

The trichothecene gene cluster (~ 27 kb) of *Fusarium graminearum* is comprised of genes involved in the synthesis of trichothecene mycotoxins. Strains of *F. graminearum* differ in the chemical profile of trichothecene derivatives produced. These different profiles, known as chemotypes, correspond to strains that produce predominantly 1) deoxynivalenol (DON) and 15-acetyl DON, 2) DON and 3-acetyl DON or 3) nivalenol. Our objective is to develop isogenic lines of *F. graminearum* that differ only in the toxin biosynthesis cluster. These lines may be used to ascertain whether chemotype is determined solely by genes within the cluster, and will be essential for understanding how trichothecenes influence fungal pathogenicity, selection and fitness. As the first step towards creating isogenic lines, the trichothecene gene cluster has been deleted from *F. graminearum* strain PH-1. Split-marker recombination was used for targeted deletion of the trichothecene gene cluster via homologous recombination, resulting in replacement of the cluster with a hygromycin resistance gene. Hygromycin resistant mutants were screened for deletion of the cluster using PCR for genes in the cluster as well as Southern hybridization using as a probe a BAC containing the trichothecene gene cluster.

STRATEGIES TO CONTROL SCAB USING RNAI TECHNOLOGY: PROGRESS AND OBSTACLES

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ABSTRACT

RNA interference (RNAi) technology takes advantage of a conserved eukaryotic mechanism that degrades mRNAs. A key component of this mechanism is the production of double stranded RNA (dsRNA) which can be digested into small interfering RNAs (siRNAs) 21-26 nt in length by a RNAse III helicase-containing protein called Dicer. siRNAs enter into a Dicer containing complex known as RISC (RNA induced silencing complex) and anneal to same sequence mRNAs. These mRNAs are cleaved by the RISC complex leading to the degradation of mRNA. Here we demonstrate that trichothecene production can be down regulated by RNAi technology in the scab pathogen *Fusarium graminearum*. Current studies focus on (a) investigating the ability of fungi to uptake and amplify siRNAs in the cell through activity of RNA-dependent RNA polymerases and (b) creating transgenic siRNA producing wheat lines that will target *Fusarium* virulence genes.

EXPRESSION ANALYSIS OF DEFENSE-RELATED GENES IN WHEAT IN RESPONSE TO INFECTION BY *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB), caused by the fungus *Fusarium* species, is a worldwide disease of wheat (*Triticum aestivum* L.). The Chinese cultivar, Ning7840, is one of few wheat cultivars with resistance to FHB. GeneCalling, an open-architecture, mRNA-profiling technology, was used to identify differentially expressed genes induced or suppressed in spikes after fungal infection in FHB resistant cultivar Ning7840-*Fusarium graminearum* interaction. Over 150 individual cDNA fragments representing different transcripts expressed in wheat spikes were examined and sequenced, and putative functions assigned to some of the unigenes based on BLASTN and BLASTTX. Of the unigenes identified, 28 were assigned function in primary metabolism and photosynthesis, 7 were involved in defense response, 14 were in gene expression and regulation, 25 encoded proteins associated with plant cell wall degradation, 42 were without a known function with sequences in the database, and interestingly, 3 genes showed similarities to cloned disease resistance proteins. Of particular interest in this study were genes associated with resistance and defense genes to pathogen infection. Real-time quantitative reverse-transcriptase PCR indicated that, of 51 genes tested, 19 genes showed 2-fold or greater induction in the FHB resistant wheat lines KS24-2 containing FHB resistance derived from *Lophopyrum elongatum* and Ning7840, in contrast to susceptible wheat line Len, while another 32 genes were not significantly induced in either of the FHB resistant wheat lines compared with susceptible Len. Characterization of these genes, whose activity is correlated with FHB resistance and may be involved in wheat-fungal interactions, is ongoing in this study.

FIELD POPULATIONS OF *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae is homothallic and the sexual stage occurs commonly where the fungus is found as part of wheat/maize cropping systems. In the northern United States the amount of genetic diversity is large, most individuals are unique (not clones) and linkage disequilibrium is detectable only at levels approaching the error rate associated with the statistical analysis. These patterns hold over wide distances, *i.e.* Virgina to Montana, and across years. In limited samples, isolates associated with maize fields are not significantly different from those associated with wheat fields. Outside the United States, patterns are different and sometimes more complex. Although isolates in the United States differ greatly, all of the isolates we analyzed can be associated with lineage 7 *sensu* O'Donnell *et al.* (2000). Near CIMMYT in Mexico, field isolates belong to lineage 3. In Uruguay and southern Brazil lineage 7 again dominates the field collections, but other lineages are present as are putative interlineage hybrids. In Australia and Viet Nam, the genetic boundaries for the lineages are not distinct with AFLP markers, but we have not analyzed diagnostic sequences to determine if interlineage hybrids are present. In Korea, the dominant lineage present depends on the host, with lineages 7 and 3 dominating in maize and lineage 6 dominating in rice/barley rotations. There is evidence for putative interlineage hybrids occurring in Korea as well. Thus, genetic variation in *G. zeae* occurs at all levels measured. The genetic isolation of the lineages remains an open question, but laboratory crosses and the existence of putative hybrids under field conditions suggest that these differences are not sufficient to stop significant gene flow between the lineages if more than one lineage is present at the same location.

DEVELOPMENT OF A NOVEL BIOASSAY SYSTEM FOR FHB MOLECULAR INTERACTIONS

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ABSTRACT

Fusarium head blight (FHB) of wheat and barley, caused by *Fusarium graminearum*, is a destructive disease that occurs in warm and humid regions. FHB causes serious yield and quality losses, but of greater concern is the contamination of cereal food and feed with mycotoxins. Further investigations of the molecular interactions between the plant and pathogen are necessary to more effectively combat this disease. We developed a novel bioassay system using primary leaves to evaluate *Fusarium graminearum* pathogenicity and gene expression *in planta*. When a drop of the conidial suspension was placed on a wound (approximately 1 mm in diameter) of a primary leaf, the pathogen succeeded in infection and produced an oval lesion. The diameter of oval lesions produced on resistant cultivars (according to field data) was significantly less than the diameter of lesions produced on susceptible cultivars. When wounds were treated with purified toxin (DON) alone, water-soaked symptoms were observed. Lesion size was remarkably increased when wounds were treated with a mixture of toxin and conidial suspension. These results indicate that DON plays an important role in lesion development by the fungus. DON & NIV mycotoxins have been shown to be virulence factors in FHB and some toxin synthesis genes have already been cloned. However, their role in host-pathogen interactions is not clearly understood. Therefore, fungal gene expressions were investigated using total RNA prepared from infection sites produced by conidial inoculation of wounds on primary leaves. Transcript accumulation of two constitutive genes (Actin and ß-tubulin) and two trichothecene genes (*Tri4* and *Tri5*) were detectable by RT-PCR no later than 24h after inoculation. Notably, Actin and b-tubulin gene expressions were observed even at 3h after inoculation. This suggests that the new bioassay system, using conidia, mycotoxin, or conidia plus mycotoxin, is useful for analyzing early mycotoxin regulation by *Fusarium* at the molecular level.

GENE EXPRESSION ANALYSIS OF CONIDIUM MATURATION AND GERMINATION ON *FUSARIUM GRAMINEARUM*

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ABSTRACT

To understand the infection cycle of the head blight pathogen *F. graminearum*, gene expression profiles were monitored in newly formed conidia, conidia that had been desiccated for 10 days and germinating conidia using the 18K feature *F. graminearum* Affymetrix GeneChip. A total of 6,384 positive signals were detected in newly formed spores with detection p value <0.001. Enhanced expression of many genes involved in transcription or transcriptional regulation and metabolism such as glycolysis, the glyoxylate cycle, and â-oxidation imply that newly formed conidia are not dormant cells but rather are metabolically active. Surprisingly, a total of 2, 916 positive signals were detected even in mature conidia. Among 543 genes that were up-regulated more than 2-fold upon spore maturation were many genes involved in autophagy, proteolysis, protein secretion, and cell wall synthesis. After suspending conidia in liquid complete medium for 2h, a total of 5,587 signals were detected (p value <0.001) and 2,593 signals were up-regulated more than 2-fold in these swollen spores. Genes involved in transcription, RNA splicing, protein synthesis, and amino acid and nucleotide metabolism were highly induced during the initiation of spore germination. Up-regulation of proteasome components and secretory proteins was observed as germlings established polarized growth after 8h of incubation. Many stage-specific genes and events during spore maturation and germination were identified and will be discussed.

A MULTILOCUS GENOTYPING ASSAY FOR IDENTIFICATION OF FUSARIUM HEAD BLIGHT SPECIES AND TRICHOTHECENE TOXIN CHEMOTYPES

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ABSTRACT

Fusarium head blight (FHB) places a serious constraint on the production of wheat and barley worldwide. In addition to yield reductions, infested grains may be contaminated with trichothecene mycotoxins that pose a serious threat to human and animal health. Recent evolutionary analyses have revealed unexpected diversity among FHB pathogens. Fourteen different species within the B-trichothecene lineage (B-clade) of *Fusarium*, including nine within the *Fusarium graminearum* species complex (*Fg* complex), have been described. In addition, B-trichothecene toxin chemotype polymorphism (NIV, 3ADON, and 15ADON) was found to be transspecific and maintained by balancing selection. Using a unique multilocus DNA sequence database (13.6 kb of DNA sequence from 47 strains) we have developed a high-throughput single tube assay for the simultaneous identification of all described B-trichothecene species and chemotypes in order to improve pathogen surveillance and facilitate a greater understanding of the ecology and epidemiology of FHB pathogens. The multilocus genotyping (MLGT) array, consisting of 37 probes targeting single nucleotide differences unique to individual species or chemotypes, was validated using a panel of 218 isolates with known chemotype and species identity. Over 99.6% of the genotypes produced with the MLGT array matched expectations, and due to probe redundancy, chemotype and species identity was correctly determined for all 218 isolates. Use of this assay in our ongoing molecular surveillance has identified unexpected FHB species and chemotype diversity in North America (see related posters by Ward *et al*. and Gale *et al*.).

FHB SPECIES AND TRICHOTHECENE TOXIN DIVERSITY IN NORTH AMERICA

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ABSTRACT

Our previous phylogenetic and molecular evolutionary analyses demonstrated that the primary etiological agent of FHB, the morphospecies *F. graminearum*, actually comprises at least nine distinct species (*Fg* species complex). In addition, we have demonstrated that the three trichothecene toxin chemotypes (NIV, 3ADON and 15ADON) segregating within these species have been maintained by balancing selection, indicating that these differences are adaptive. Until recently, it appeared that *F. graminearum* (*sensu stricto*) isolates with a 15ADON chemotype were the only significant cause of FHB in North America. However,molecular surveillance with a multilocus genotyping assay for FHB species and chemotype determination has revealed an East-West chemotype cline in Canada and evidence that *F. graminearum* isolates with a 3ADON chemotype may be displacing those with a 15ADON chemotype. In addition, 3ADON isolates have been found to produce significantly ($P < 0.001$) higher levels of trichothecene than those with a 15ADON chemotype. Significant 3ADON populations have also been detected in the Northern Plains (Gale *et al*. poster) and within the Northeast U.S. We have also identified a novel member (*Fusarium gerlachii*) of the *Fg* species complex from the Northern Plains of the U.S. Isolates of this previously unrecognized species produce nivalenol (NIV chemotype), which has higher vertebrate toxicity than15ADON. In addition, NIV-producing *F. asiaticum* as well as 3ADON and NIV-producing *F. graminearum* have been identified in the Southern U.S. (see Gale *et. al*. poster for additional details), and a highly divergent evolutionary lineage of *F. graminearum* from the U.S. Gulf Coast has been found to segregate for all three B-trichothecene chemotypes. Taken together, these results indicate significant changes in B-FHB pathogen composition are taking place in North America, which could have significant implications for food safety, disease control efforts and regulatory policy regarding trichothecene-contaminated grain.

FUNCTIONAL GENOMIC STUDIES OF PATHOGENICITY IN *FUSARIUM GRAMINEARUM*

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ASBSTRACT

Head blight or scab caused by *Fusarium graminearum* is a disease of wheat and barley that occurs world-wide and that has great impact on U.S. agriculture and society. Infested cereals are often contaminated with trichothecene and estrogenic mycotoxins. To better understand fungal pathogenesis and development in this important pathogen, we have generated over 30,000 ESTs from three cDNA libraries and a draft sequence of the *F. graminearum* genome. A whole-genome *Fusarium* Affymetrix GeneChip (representing ~14000 putative genes) has been developed. Hybridizations with the *Fusarium* GeneChip were used to identify genes differentially expressed in cultures grown under different nutritional conditions or developmental stages. Transcript profiles of several mutants defective in plant infection have been generated and compared with that of the wild-type strain. Microarray analysis also was used to identify fungal genes differentially expressed during plant infection. In addition, a collection of over 10,000 random insertional mutants have been generated and screened for mutants defective in conidiation and pathogenicity. We also have constructed mutants deleted for over 50 candidate genes and thereby have identified novel virulence factors in *F. graminearum*. Details of the microarray data and phenotypes of selected mutants will be presented.

FOOD SAFETY, TOXICOLOGY AND UTILIZATION OF MYCOTOXIN-CONTAMINATED GRAIN

Chairperson: Stephen Neate

MULTIPLEX REAL-TIME PCR METHOD TO SIMULTANEOUSLY DETECT AND QUANTIFY DEOXYNIVALENOL AND OCHRATOXIN A PRODUCING FUNGI

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ABSTRACT

Cereal crop plants are colonized by many fungal species such as *Aspergillus ochraceus* and *Penicillium verrucosum,* which produce ochratoxins, and *Fusarium graminearum,* which produces trichothecene mycotoxins. A multiplex real-time PCR method using TaqMan probes was developed to simultaneously detect and quantify trichothecene producing *Fusarium* species and ochratoxin A producing *Penicillium* and *Aspergillus* species in cereal grains. Primers and probes were designed targeting the *Tri5* gene in trichothecene producing *Fusarium, rRNA* gene in *Penicillium verrucosum* and polyketide synthase gene in *Aspergillus ochraceus.* The method was highly specific to the species containing these genes and sensitive, detecting 3 pg of genomic DNA. These products were detectable over five orders of magnitude (3 pg to 30 ng of genomic DNA). Thirty barley samples were evaluated for the presence of deoxynivalenol (DON) and ochratoxin A (OTA) producing fungi using the above method. Among these samples, 9 tested positive for *Fusarium* spp, 5 tested positive for *Penicillium* spp and 2 tested positive for *Aspergillus* spp. Results were confirmed by traditional microbiological methods. These results indicate that DON and OTA producing fungi can be detected and quantified in a single reaction tube using this multiplex real-time PCR method.

TOWARD BICHROMATIC OPTICAL SORTING OF SCAB-DAMAGED WHEAT

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ABSTRACT

Our previous optical work exhaustively examined the best single wavelength and best two-wavelength combination that maximized class separation between normal and scab-damaged wheat kernels, using linear discriminant analysis. This was accomplished by single kernel visible (410-865 nm) and near-infrared (1031-1674 nm) scanning of more than 4500 kernels from 100 commercial varieties, equally divided between normal and scab-damaged categories. Generally, the best two-wavelength models were approximately 95% accurate in classification. From there, we have studied the application of a good-performing wavelength pair (675 nm and 1480 nm, as selected from a handful of manufacturers stock combinations) in a high-speed commercial sorter. Unknown until this study was the effectiveness of such classification models when applied under real-time, high-speed sorting conditions. More than 40 samples of soft red winter and soft white wheat, each approximately 5 kg, obtained from commercial mills in the eastern United States, primarily from the 2003 harvest. Of these, 35 samples were from regular process streams (selected because of a priori knowledge of elevated *Fusarium* damage), with the remaining 7 samples taken from discard piles of cleaners. The sorter, which used detectors and interference filters, one in the visible region and the other in the near-infrared region (see above), consisted of a series of parallel inclined channels. The level of DON ranged from 0.6 to 20 mg/kg. Samples were processed by the sorter operating at a throughput of 0.33 kg/(channel-min) (four of ten available channels used) and a kernel rejection rate of 10%. Visual measurements of the proportion of *Fusarium*-damaged kernels were collected on incoming and sorted specimens. Sort effectiveness was assessed by two means: percentage reduction of *Fusarium*-damaged kernels and percentage reduction in DON concentration. Results indicated that the fraction of DON contaminant level in the sorted wheat to that in the unsorted wheat ranged from 18 to 112 percent, with an average of 51 percent. Nine of the 35 regular samples and all 7 of the discard pile samples underwent a second sort, with 5 from this second set undergoing a third sort. Multiple sorting was effective in yielding product whose DON concentration was between 16 and 69 percent of its original, unsorted value. In summary, sorting resulted in a reduction of DON concentration by approximately one half on average, with further reduction arising from the resorting of accepted material. This study is fully described in our recent publication [Delwiche, et al., Plant Disease, vol. 89, p. 1214-1219 (2005)].

CORRELATION OF SEED SIZE AND DON ACCUMULATION IN SPRING WHEAT

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ABSTRACT

Fusarium head blight (FHB) reduces grain yield, test weight, grade, and may also contaminate kernels with mycotoxins. The most common mycotoxin associated with FHB infected grain, deoxynivalenol (DON or vomitoxin) reduces the marketability of grain since milling, baking, and feed quality may be adversely impacted. In this study we explored the relationship between DON accumulation within, and seed size of, thirty six spring wheat varieties and advanced breeding lines, where seed size represents an indirect estimate of the glume: endosperm ratio. Test entries were selected from South Dakota State University (SDSU) and North Dakota State University (NDSU) spring wheat breeding programs and represented a sample of germplasm which resulted from FHB resistance breeding efforts conducted from 1998 to 2003. Field tests were carried out as a Randomized Complete Block Design with four replications at Brookings, SD and Prosper, ND during the 2004 and 2005 growing seasons. Seed weight was obtained by weighing a 1000 seed sample of each entry, while DON concentration were collected on ground wheat samples by NDSU Veterinary Diagnostic Services. Analysis of seed size and DON concentration data over years from Brookings revealed the significant effect of years for both seed size and DON concentrations. Entry mean values for seed size and DON concentrations were calculated over years at Brookings for correlation analysis. Pearson's product moment correlation coefficients between seed size and DON concentration were computed individually for each year, the results showed negative association between seed size and DON concentration ($r=-0.35906$, $p=0.0315$; $r=-0.40772$, $p=0.0136$) in 2004 and 2005 respectively. Results from Brookings over years revealed a negative association between seed size and DON concentration but not statistically significant ($r=-0.22957$; $p=0.1780$). Both seed size and DON concentration observations were highly correlated over years ($r=0.79216$, $p=<.0001$; $r=0.56983$, $p=0.0003$) respectively. Breeders may eventually favor the selection of large-seeded lines as a means of lowering DON concentration in wheat varieties, although additional studies will be required to fully understand these relationships.

THE TRICHOTHECENE TRIANGLE: TOXINS, GENES AND POPULATIONS

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ABSTRACT

Fusarium trichothecenes have been classified based of structural properties. Type A and Type B trichothecenes differ in their A ring oxygenation pattern. Type A trichothecenes have a C-8 hydroxyl (neosolaniol), C-8 ester (T-2 toxin) or no C-8 substitution (diacetoxyscirpenol) and Type B trichothecenes having a C-8 keto group (deoxynivalenol, nivalenol). *F. graminearum* Type B trichothecenes can be divided into two chemotaxonomic groups. The DON chemotype produces deoxynivalenol and its acetylated derivatives (3-ADON, 15-ADON) and the NIV chemotype produces nivalenol and its acetylated derivative (fusarenone X). Nivalenol production is the ancestral trait based on sequence analysis. DON producers are more aggressive and virulent than those producing nivalenol (Desjardins et al. 2005). DON producers occur worldwide and predominate in North American, South America and Europe.

Trichothecenes are produced by a complex series of oxygenations, isomerizations, cyclization, and esterifications. Biochemical and genetic investigations of *Fusarium sporotrichioides*, *F. graminearum*, *F. culmorum* and other species have elucidated both the steps required (Zamir et al. 1999; Hesketh et al. 1991; McCormick et al. 1990) as well as the genes controlling trichothecene biosynthesis. Trichothecene genes have been mapped to four loci: the main 26 kb trichothecene cluster of twelve genes (*Tri8, Tri7, Tri3, Tri4, Tri6, Tri5, Tri10, Tri9, Tri11, Tri12, Tri13, Tri14*), a smaller cluster containing *Tri1* and *Tri16*, and two other loci with *Tri101* and *Tri15*, respectively (Jurgenson et al. 2002; Meek et al. 2003; Brown et al. 2001; Kimura et al. 1998).

The DON and NIV chemotypes have a clear genetic basis. DON-producing strains lack functional *Tri7* and *Tri13* genes (Kim et al. 2003). *Tri13* encodes the C-4 oxygenase and *Tri7* encodes a C-4 transacetylase. Similarly, differences for Type A and Type B trichothecene producing strains can be found in the small *Tri1-Tri16* gene cluster. T-2 toxin producing strains of *F. sporotrichioides* have *Tri1* that controls C-8 hydroxylation adjacent to *Tri16* that controls C8 esterification (Meek et al., 2003; Peplow et al., 2003). In DON-producing strains, *Tri16* is non-functional and a *Tri1* homolog controls hydroxylation at both C-7 and C-8 (McCormick et al. 2004). Finding the genetic basis for additional chemotypes (3-ADON/DON or 15ADON/DON) (Ward et al., 2002) may require the analysis of the genes involved in C-3 and C-15 acetylation and deacetylation. *Tri101* and *Tri3* encode the C-3 acetyltransferase and C-15 acetyltransferase, respectively (Kimura et al., 1998; McCormick et al., 1996). *Tri101* acts in concert with *Tri8*, the C-3 esterase gene (McCormick et al. 2002) to add and remove the C-3 acetyl group during biosynthesis. *F. graminearum Tri8* mutant strains lack the C-3 esterase and accumulate 3,15-diADON. Since biosynthesis of both 3-ADON and 15-ADON is likely via a 3,15-diADON intermediate, the esterase genes may be more important in distinguishing the 3ADON and 15ADON chemotypes. A C-15 esterase gene has not been identified. Infection with either 3-ADON or 15-ADON producing strains can result in the accumulation of DON in infected plant tissue. Defining chemotypes based on which toxins are found in infected plant tissue is problematic since plant esterases can modify the toxins normally produced in culture.

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PERSPECTIVES ON THE RISK ANALYSIS OF THE TRICHOTHECENE MYCOTOXINS

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ABSTRACT

Risk analyses for trichothecene mycotoxins and other food contaminants, which are to a significant extent unavoidable, present considerable challenges. The international risk assessments completed for deoxynivalenol, T-2 and HT-2 toxins, and nivalenol have noted a number of issues regarding the lack of adequate intake data for exposure assessment and significant gaps in toxicological studies for hazard characterizations. This is in spite of the fact that animal health problems have been associated with these mycotoxins for at least twenty-five years and occasional outbreaks of human illness have been reported. Risk assessment is constrained by uncertainties associated with the lack of adequate data, and risk management must consider the fact that mycotoxin contamination can have serious impacts on trade and food sufficiency. Risk assessment must be an iterative process, since the problem formulation and the risk assessment may need to be revised to reflect new data and theories. In addition to providing advice to risk managers, risk assessment should provide a blueprint for future research by illustrating what observations will influence a prediction. The objective, unbiased, and adequate evaluation of the association of exposures to specific agents or mixtures of agents with particular health outcomes can be extremely difficult. This is particularly true of complex, interactive phenomena, such as risk-risk and risk-benefit analyses, where there is often a lack of a common currency for comparisons. Two analytic approaches that can provide a more structured framework for such considerations are weight-of-evidence (WOE) and value-of-information (VOI). WOE approaches to human health risk analyses provide a framework for the interpretation of information about the harmful effect, including quality of testing methods, size and power of study designs, consistency of results across studies, biological plausibility of the exposure-response, and statistical associations. While most risk assessments involve WOE considerations, no general criteria or guidance have been established. VOI approaches can provide the basis for deciding whether it is better to make a decision now based on an inherently uncertain risk assessment or to collect additional information first and then decide. VOI analyses, based on decision analysis principals, can focus on the potential cost, including health impacts, from errors due to the uncertainties in decision making (based either on false-negatives or false positives) and identify the most valuable information for reducing the uncertainties. Addressing these uncertainties would provide risk managers with better guidance for control measures. The World Health Organization/International Programme on Chemical Safety has been developing criteria for WOE approaches and has initiated work on general considerations and guidance for VOI approaches.

FUSARIUM INFECTION, DON CONTENT AND MICROBIAL LOADS IN DURUM WHEAT FROM THE NORTHERN PLAINS: 2001-2004

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ABSTRACT

Durum wheat samples for years 2001 to 2004 were collected from five districts in North Dakota and one district in Montana and were analyzed for their microbiological quality, including deoxynivalenol (DON) content, aerobic plate count (APC), mold and yeast count (MYC), mesophillic aerobic spore formers (MASF), *Fusarium* infection (FI), and total mold infection (TMI). Composite samples from different regions were also analyzed for APC, MYC, FI, TMI, MASF. Processed samples such as bran, dust, flour, pasta, semolina, and shorts were also analyzed for APC, MYC and DON content. DON content for durum samples, district composite samples, and processed samples averaged 1.76 ± 2.05 (range: 0.47-4.79), 1.83 ± 1.81 (range: 0.5-3.89), and 1.76 ± 1.86 (range: 0.52-4.5) µg/g respectively. APC for durum samples, district composite samples, and processed samples averaged 8.18 ± 8.44 (range: 5.68-8.75), 7.91 ± 7.98 (range: 5.55-8.27), and 6.97 ± 7.13 (range: 5.12-7.46) $\log_{10}$ cfu/g respectively. MYC for durum samples, district composite samples, and processed samples averaged 4.49 ± 4.26 (range: 4.07-4.71), 4.25 ± 4.22 (range: 3.91-4.63), and 3.98 ± 3.88 (range: 2.41-4.27) $\log_{10}$ cfu/g respectively. MASF for durum samples and district composite samples averaged 2.5 ± 1.53 (range: 2.47-2.54) and 2.48 ± 0.76 (range: 2.47-2.48) $\log_{10}$ cfu/g respectively. FI for durum samples and district composite samples averaged 35.76 ± 19.42 (range: 11.13-57.36) and 40.76 ± 28.98 (range: 11.67-75.67) % respectively. TMI for durum samples and district composite samples averaged 83.32 ± 8.11 (range: 75.39-94.01) and 85.72 ± 8.44 (range: 77.17-97.17) % respectively. When the whole grains were processed, DON content was reduced significantly from an average of 1.76 ± 2.05 (whole grain) to 0.92 ± 0.67 (pasta) µg/g. APC were reduced from an average of 8.18 ± 8.44 (whole grain) to 5.48 ± 5.78 (pasta) $\log_{10}$ cfu/g. MYC were reduced from an average of 4.49 ± 4.26 (whole grain) to 2.51 ± 2.44 (pasta) $\log_{10}$ cfu/g. Overall, microbial loads and DON were reduced as a result of milling and processing. Data for all four years will be presented.

DEOXYNIVALENOL AND 15-ACETYLDEOXYNIVALENOL PRODUCTION BY *FUSARIUM GRAMINEARUM* STRAINS GROWN IN SEMI-DEFINED MEDIUM WITH DIFFERENT CARBOHYDRATE SOURCES

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ABSTRACT

Two *Fusarium graminearum* strains (FRC # R-9828 and FRC # R9832) were cultured in yeast extract peptone broth limited to single carbohydrate sources (at 1%), including xylan, cellulose, starch or glucose, to measure production of deoxynivalenol (DON), 15-acetyldeoxynivalenol (15Ac-DON) and mycelium. R-9828 was an isolate from South Africa belonging to Lineage 3 of *Fusarium graminearum*, while R9832 was a strain from the United States and belongs to Lineage 7. The two strains were chosen for the study because they demonstrated virulence to wheat in greenhouse trials and were good DON producers in solid substrates such as rice, corn, wheat and barley. A random complete block design with factorial arrangement was used. Each treatment was done in duplicate and repeated once on different days (n = 4). Analysis of variance with Duncan's multiple comparison was employed to test for treatment differences at a significance level of 5% ($p<0.05$). On average, xylan yielded the highest mycelial mass (22.22 ± 10.63 mg), followed by glucose (13.56 ± 8.73 mg), starch (11.36 ± 7.39 mg), and finally cellulose (4.21 ± 6.95 mg). There were significant differences between the four carbohydrate sources for DON production with glucose (12.94 ± 19.65 ppm/mg mycelia) and xylan (6.30 ± 9.17 ppm/mg mycelia) yielding significantly higher DON than starch (4.72 ± 8.84 ppm/mg mycelia) or cellulose (0.39 ± 0.70 ppm/mg mycelia). There were no significant differences between the four carbohydrates for 15Ac-DON production.

These preliminary results support the theory that *Fusarium graminearum* invades through xylan in the cell walls of wheat and barley and suggests that xylan may induce *Fusarium* growth and DON production to help with the invasion process.

CHEMICAL, BIOLOGICAL AND CULTURAL CONTROL

Chairperson: Martin Draper

CHARACTERISTICS, INCLUDING TOLERANCE TO ELEVATED HEAT AND ELEVATED SALT CONCENTRATION, OF A *BACILLUS* STRAIN USED AS A BIOCONTROL AGENT TO CONTROL FUSARIUM HEAD BLIGHT

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ABSTRACT

Selected strains of *Bacillus* can be used as biocontrol agents (BCAs), to antagonize *Fusarium graminearum* which causes Fusarium Head Blight (FHB) of wheat and barley. The ability of the BCA strain 1BA to survive during storage at different temperatures was examined. Cells of strain 1BA grown for five days at 27°C in tryptic soy broth were stored at either 4°C, -20°C, or room temperature (27°C) for one month, then enumerated for viable cells. Best survival of cells was at 27°C (a 2 log decrease), with a 4-log decrease in cell numbers at -20°C and at 4°C. This was unexpected, since bacterial numbers usually remain more stable at refrigerator temperature rather than at room temperature. This has implications for using these BCAs for control of FHB, since due to unpredictable weather conditions and/or maturation of wheat or barley in the field, storage of BCAs before their use in the field is often required.

The production of endospores by strain 1BA at different temperatures was examined, using nutrient agar amended with manganese sulfate to help encourage spore production. Large numbers of spores were produced at selected incubation temperatures, ranging from 27°C to 50°C. Endospores survived pasteurization at 80°C for 10 minutes, indicating that endospores of this BCA could remain viable at elevated storage temperatures. Using plate assays, amylase but not chitinase activity was verified at all examined temperatures for 1BA.

To assay numbers of 1BA after spraying its cells onto wheat or barley heads in the field, a selective and/or differential growth medium is needed to either discourage growth of native wheat or barley microflora while allowing growth of this BCA. Temperature and salt stresses on 1BA were examined to develop such a medium. Strain 1BA grew on tryptic soy agar (TSA) and nutrient agar (NA) at various temperatures, ranging from 27°C to 50°C. 1BA also grew on TSA and NA amended with various NaCl concentrations, ranging from 2.5% NaCl to 10% NaCl . Strain 1BA also grew at elevated temperature and salt concentrations in the defined broth medium used for producing inoculum for field application. There were distinct colonial morphology changes in 1BA depending on temperature or NaCl concentration. The elevated temperature and NaCl concentrations that 1BA could withstand were used in preliminary plate counting of the microflora of wheat heads. Little or no growth of the native microflora was noted with these conditions, suggesting that we will be able to apply and count 1BA inoculum that has been sprayed onto wheat heads using a plate count methodology. The ability of these BCAs to grow at elevated temperature and salt concentration indicates these stresses can be used to select for these BCAs in plate count assays, and possibly to enrich for them on plant surfaces by spraying salt solutions on aerial plant parts.

2005 UNIFORM FUNGICIDE PERFORMANCE TRIALS FOR THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and a serious epidemic impacted the state's wheat and barley crop in 2005. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Two hard red spring wheat cultivars, Briggs and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore/ Watertown) and Robust barley was planted at Brookings. A winter wheat study site at South Shore/Watertown, was lost due to poor stand and heavy cheatgrass pressure. Only the spring wheat data is presented in this report. Trial treatments were from the Uniform Fungicide Trial treatments list for the suppression of FHB and included an untreated check, Folicur (tebuconazole) applied at 4.0 fl oz/A, Prosaro (a premix of prothioconazole and tebuconazole) applied at 6.5 fl oz/A, BAS 555 (metconazole) applied at 10 or 13.5 fl oz/A, and Punch (flusilazole) applied at 6 or 8 fl oz/A. All treatments included Induce, a non-ionic surfactant, applied at 0.125% v/v. Spring wheat trials were planted in a factorial randomized complete block design with six replications. Trial treatments were applied at anthesis (Feekes growth stage 10.51). Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation applied for 3 min out of every 20 minutes from 5:00 pm until 9:00 am each day for two weeks following anthesis at the Brookings location only. Other sites had natural inoculum from corn stalk residue and natural moisture conditions. Twenty-one days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity. Samples were collected for Fusarium damaged kernels (FDK), deoxynivalenol (DON), grain yield, and test weight. Under dryland conditions at South Shore/Watertown FHB was moderate, with 17.5% FHB field severity on the untreated. FHB incidence and severity were both reduced by all treatments except either rate of Punch. Prosaro and BAS 555 show a numeric improvement over tebuconazole alone. Folicur, Prosaro and BAS 555 provided the best yield and test weight response of the treatments. A similar response was noted at Groton, SD with BAS 555 generally providing the best response under those environmental conditions and slightly less FHB, 12.7% field severity in the untreated. At the irrigated Brookings location FHB was severe, 46% field severity on the untreated. Under these extreme conditions, no treatments reduced FHB incidence and only Prosaro and the BAS 555 treatments reduced FHB field severity and increased yield. Test weight was increased and FHB severity decreased by all treatments at Brookings except for the low rate of Punch.

2005 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat and barley producers in South Dakota for ten years and was very severe in parts of SD in 2005. The objective of this study was to continue to evaluate the efficacy of various fungicides and fungicide combinations for the suppression of Fusarium head blight and other wheat diseases. Ingot hard red spring wheat and Robust barley were planted at Brookings, South Dakota. Trial treatments included an untreated check; Folicur (tebuconazole) applied at 4.0 fl oz/A; C3R5 (*Lysobacter enzymogenes*) from University of Nebraska, Lincoln, NE, C3 + Folicur coapplied, 1BA (*Bacillus subtilus*) from South Dakota State University, Brookings, SD, 1BA + Folicur coapplied, TrigoCor 1448 (*Bacillus* sp.) from Cornell University, Ithaca, NY, and TrigoCor 1448 + Folicur coapplied. Treatments were grown on site according to specifications from their originating labs and applied at anthesis. Plots were inoculated by spreading *Fusarium graminearum* (isolate Fg4) inoculated corn (*Zea mays*) grain throughout the field at least ten days prior to flowering (wheat) or head emergence (barley) throughout the field and providing overhead mist irrigation applied for 3 min out of every 20 minutes from 5:00 pm until 9:00 am each day for two weeks following treatment. Twenty-one days following treatment, plots were evaluated for FHB incidence, FHB head severity, and FHB field severity. Plots were harvested for yield and test weight and samples were collected for Fusarium damaged kernels (FDK) and deoxynivalenol (DON). Substantial lodging occurred in the barley plots so yields of barley were highly variable.

Under the misted environment in 2005, FHB was severe at this location. FHB incidence was as high as 96% on spring wheat and near 100% on barley. FHB plot severity ranged from about 41-53% in spring wheat. Barley data is forthcoming. No significant improvements over the untreated were observed among the biological treatments for disease suppression or yield enhancement. Also, the Folicur treatment did not provide the level of disease suppression observed in other trials and in other years. The combined treatments of BCAs with Folicur also provided no greater response that any component individually. Test weight was impacted by two BCAs; C3 + Folicur and 1BA + Folicur improved test weight significantly over the untreated check, but not more than Folicur alone. However, the addition of the BCA allowed means separation from the untreated. Folicur alone, while numerically better than the untreated, did not significantly improve test weigh unless the BCAs were present.

FLUID BED DRYING OF *CRYPTOCOCCUS NODAENSIS* OH 182.9; A BIOCONTROL AGENT OF FUSARIUM HEAD BLIGHT

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OBJECTIVE

To evaluate the feasibility of using fluid bed drying to prepare stable wettable granules of *Cryptococcus nodaensis* OH 182.9 for control of Fusarium head blight.

INTRODUCTION

Cryptococcus nodaensis (nomen nudem) OH 182.9 (NRRL Y-30216) has been shown to be effective in controlling Fusarium head blight (FHB) in greenhouse and field studies (Khan et al. 2004; Schisler et al. 2002). In an effort to transform *C. nodaensis* into a commercially viable FHB biological control option, studies in our laboratory have been performed to optimize its production and bioefficacy (Zhang et al. 2005a; Zhang et al. 2005b). Additional research is still needed to develop cost effective methods of drying and stabilizing *C. nodaensis* to produce a product with a suitable shelf-life. Optimization of the formulation and application parameters are critical steps in the product development process.

Wettable powders and granules have long been the ideal method of formulating microbial biological control agents. Successful wettable powders and granules offer ease of use, convenience for transportation, improved shelf-life and consumer acceptance. These dried products can be formed by a variety of methods such as, air drying, spray drying or fluid bed drying. Spray drying and fluid bed drying have long been used to produce active dry yeast for the food industry due to their reliability, low costs and high throughput (Grabowski et al. 1997; Luna-Solano et al. 2003; Luna-Solano et al. 2005). Fluid bed drying is generally considered the less stressful of the two for drying microbial cells.

MATERIAL AND METHODS

Biomass Production - *Cryptococcus nodaensis* (nomen nudem) OH 182.9 (NRRL Y-30216) was produced in a B Braun D-100 fermentor charged with 80 L of SDCL medium (Slininger et al., 1994). To initiate a production run, cells from a log-growth stage SDCL culture served as a 5% seed inoculum for the D-100 fermentor initially set at 25°C. Twenty –four hours after inoculation, the temperature was reduced to 15°C to cold shock the cells for 24 hrs prior to harvest. After completion of biomass production at approximately 48 h, colonized reactor broth was concentrated into a paste using a Sharples 12-V tubular bowl centrifuge. The cell paste was frozen at -80°C until use.

Granulation -Uniform spheres of *C. nodaensis* were produced by dropping droplets of 10% (w/w) aqueous solution into a rotating bed of perlite (Harborlite 1500 S, Harborlite Corp. Santa Barbara, CA) using a 20 ga needle and peristaltic pump. The rotating bed was a modified seed coater spinning at approximately 45 rpm. Excess free perlite was removed with sieving with 1 mm screen.

Fluid bed drying - was performed with a Niro-Aeromatic fluid bed dryer type STR-1 (Niro-Aeromatic Inc, Columbia, MD). Five hundred g batches of the wet *C. nodaensis* spheres were dried at 30°C and an air volume of 90 m^3/h. Five replicate dryings were performed. The relative humidity of the ambient air was ~60% with no humidity controls in place. Samples were taken every 3 minutes of drying

and assayed for moisture content and viability. Moisture content ((wet-dry)/wet) of the samples was determined with a moisture analyzer (Mark I, Denver Instruments, Tempe,AZ).

Viability testing - Air-dried samples were resuspended in 50 ml of weak (0.03%) phosphate buffer, mixed in a Stomacher 80 (Seward Inc., England) with the normal setting for 60 s. Serial dilutions were made for each sample and plated on TSBA/5 media. Plates were incubated at 25ºC for 2 days until colony counting.

RESULTS AND DISCUSSION

Fluid bed drying requires the product to be in granular form for the most cost-effective method of drying. To address this problem, a simple process for forming small (~3 mm) spheres of cell slurry was developed to provide a uniform product for fluid bed drying trials. The spheres had good flow behavior in the dryer with no problems of clumping and easily form a fluid bed. The process was developed after initial efforts to form extruded pellets proved unsuccessful. The method is applicable to other organisms, scalable and allows for easy incorporation of a number of adjuvants.

The results of fluid bed drying of *C. nodaensis* are presented in figure 1. Moisture content and viability of the cells were monitored over the course of drying. The results show *C. nodaensis* viability was well maintained until the moisture content decreased below 20%. Below 20% moisture content, a gradual loss of viability is observed until a final moisture content of 2-3%. The viability loss across this range of moisture is on the magnitude of one log. Additional experiments are needed to identify the optimum moisture content for storage. For comparison, the optimum moisture content for storage of baker's yeast is 7.0-8.5 % (Beker and Rapoport 1987).

The current work demonstrates fluid bed drying is a viable option for the drying of *C. nodaensis*. Additional optimization of the drying parameters, such as, temperature, humidity of incoming air, residence time, final moisture content and incorporation of stabilizing adjuvants should enhance product shelf life and bioefficacy.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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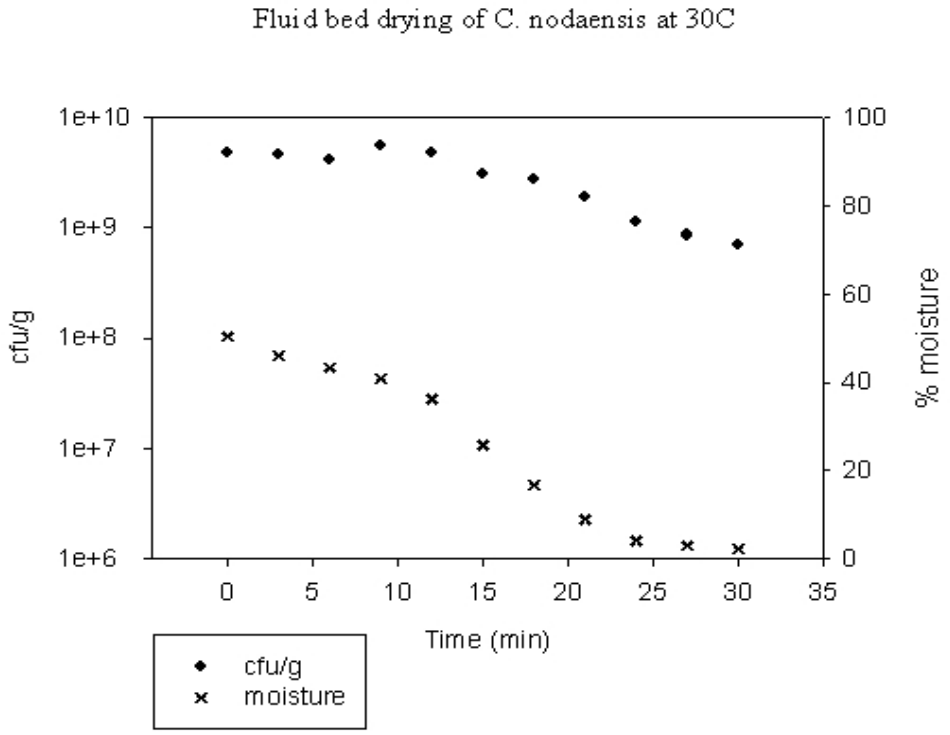

Figure 1. *C. nodaensis* viability and moisture content during fluid bed drying.

OSMOTIC SHOCK TOLERANCE AND MEMBRANE PROPERTIES OF *CRYPTOCOCCUS NODAENSIS* OH 182.9; A BIOCONTROL AGENT OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Drying and stabilizing microbial biological control agents present a challenging problem. Drying and subsequent rehydration puts the microbe in high osmotic pressure gradients which can damage the cells. Understanding how cells respond to these pressures should lead to better methods for drying and rehydrating these cells. Our laboratory has previously shown *Cryptococcus nodaensis* OH 182.9 should be a suitable commercial biological control candidate for Fusarium head blight. Developing *C. nodaensis* into a commercially viable biocontrol agent requires knowledge of its environmental limitations. Our laboratory has previously shown *C. nodaensis* becomes more desiccation tolerant after cold shocking at 15°C for twenty-four hours. The current study evaluates the osmotic shock tolerance of *C. nodaensis* with and without cold shocking. In addition, the membrane transition temperature of the cells is determined through fluorescence anisotropy experiments. The results show cold shocking *C. nodaensis* results in improved osmotic shock tolerance and changes in the cell membrane.

EFFECT OF NOZZLES ON FUNGICIDE EFFICACY FOR CONTROL OF FUSARIUM HEAD BLIGHT ON BARLEY

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OBJECTIVES

The study objective was to determine if nozzles with differing drop formation technologies, differing drop sizes, and differing orientations affected the efficacy of fungicide for control of Fusarium head blight (FHB) on barley.

INTRODUCTION

Fungicide applications to small grains for control of FHB have often given results that are inconsistent and need to be improved. Preliminary results from ongoing studies at the Langdon Research Extension Center have shown spray deposition on the grain head can be improved with certain spray volumes and angle orientations. Barley has been the most difficult crop to show consistent control of FHB with fungicide application due to the extensive and tight structure of awns when heading is complete which the recommended time for fungicide application is. The awns provide a filter to minimize the amount of spores that contact and infect the developing grain kernels, but also provide a structure that makes it more difficult to deposit fungicide on the lemma and palea to protect against infection from FHB.

MATERIALS AND METHODS

A study was initiated using ground application equipment to compare several nozzles with different type and size drop formation. The nozzles evaluated were Spraying Systems Co.® (Spraying Systems, 2005) Flat Fan, XR TeeJet XR8002 and XR8003 oriented forward (F), Turbo Teejet TT11001 and TT11002 oriented forward + backward (F+B) and F, respectively, Air Induction AI110015 and AI11002 oriented F, and AirJet 49880A (liquid orifice # 31) F+B at air pressure of 3.5,5.0,and 8.5 psi. An untreated plot was included as a control. The classification for the nozzles were XR8002 (fine), XR8003 (medium), Turbo Teejet 11001 and 11002 (medium), Air Induction 110015 (coarse) and 11002 (very coarse), and AirJet 3.5 (very coarse), 5.0 (coarse) and 8.5 (fine) (Spraying Systems, 2005). The study was arranged as a randomized complete block with four replicates. The site was established to 'Tradition' barley on a Barnes/Svea soil on the Langdon Research Extension Center in May of 2005. Previously the site was fallowed. A Fusarium barley inoculum was hand spread 21 and 14 days prior to heading at 122 grams per plot. The most commonly used method to evaluate spray technology is the use of water and oil sensitive paper (WSP Spraying Systems Co.®, Wheaton, Illinois 60189). Water sensitive cards were placed on stands at grain head height in the center of two plots. The card data had not been compiled yet and will be presented in another manuscript at a later date. The tractor mounted sprayer traveled on the left side of the plots with a boom extending to the right of the tractor sprayed area measured 6 x 20 ft. The spray solution contained Bayer CropScience's Prosaro SC fungicide at 6.5 fl. oz. / Acre, Induce adjuvant at 0.125% v/v, and a dye (F D&C Blue #1) at 22 grams/acre. Additionally, barley heads were sampled after spray application from three replicates. Each sample consisted of 5 heads. The heads were shaken for 2 minutes to remove the dye in 80 ml of 95% ethanol and the absorbance determined by a Jenway spectrophotometer. A regression curve was established from a dilution of the original spray sample and the absorbance of the spectrophotometer is presented as dilution data for a comparison of head coverage among the nozzle treatments. The tractor traveled at 6 mph for the study and the spray volume was

10 GPA. North Dakota State University Extension recommended production practices for Northeast North Dakota were followed. A visual estimation was made from 20 samples per plot collected 20 days after fungicide application to estimate the incidence (number of spikes infected) and field severity (number of FHB infected kernels per head divided by total kernels per individual spike) of FHB in each plot. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield, protein, and test weight determination and plump on barley. A sub sample was ground and analyzed for the toxin deoxynivalenol (DON) by North Dakota State University. Data was analyzed with the general linear model (GLM) in SAS. Least significant differences (LSD) were used to compare means at the 5% probability level.

RESULTS AND DISCUSSION

The North Dakota Agricultural Weather Network Weather Station recorded over 7 inches of precipitation in June. The total was more than double what is considered the 30 year normal. No additional misting was added to the trial. Disease levels were moderate to high considering the low levels of inoculum present in the area. Disease incidence was greater than 90% on all treatments but not different among treatments (Table 1). The greatest amount of dye on the head was with nozzles that produced fine or medium drops, dilution 4.3-Flat Fan XR8002, 4.9-Turbo Teejet 11001, and 4.3 AirJet 8.5 psi and smallest amount of coverage with coarse type drops. Yield, plump, and proteins were not different among treatments. Deoxynivalenol concentration was not different among fungicide treatments but coarse type drops trended toward less reduction in DON compared to the untreated. There were strong correlations between FHB incidence and severity, test weight and incidence and severity, plump and test weight, and dilution on protein. Weaker correlations were determined between DON and plump and dilution and incidence and test weight (Table 2). F+B facing nozzles offer no measurable advantage when travel speeds were 6 mph. Data trends indicate that coarse and very coarse drops may reduce fungicide deposition on the grain head and result in increased DON. A study conducted on hard red spring wheat in 2004 (unpublished) showed increased fungicide levels on the heads with small medium drop size (XR8002) nozzles, compared to fine drop size (XR8001) nozzles and large medium drop size (XR8003) nozzles.

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Table 1. FHB Incidence and Field Severity, Yield, Test Weight, Plump, Deoxynivalenol, Protein, and Dilution by Nozzle Type, Size, and Orientation on Tradition Barley, Langdon 2005.

Nozzle Class[Y] and Type	Size, Orient., or Air (Water) Pressure	FHB Incidence	Field Severity	Yield	Test Weight	Plump	DON	Protein	Dilution
	PSI	%	%	Bu/A	Lb/Bu	%	PPM	%	10^{-4}
Fine									
Flat Fan	XR8002 (35) F	96	13.3	98.9	47.8	93	1.1	11.7	4.3
Air Jet	8.5 psi (21) F+B	98	9.2	99.5	47.6	95	1.2	11.8	4.3
Medium									
Flat Fan	XR8003 (13) F	90	8.0	103.7	48.6	96	1.0	11.7	3.2
Turbo Teejet	11001 (35) F+B	100	17.9	102.2	47.3	95	1.0	12.0	4.9
Turbo Teejet	11002 (35) F	96	11.7	96.5	47.8	95	1.0	12.0	3.9
Coarse									
Air Jet	5.0 psi (21) F+B	91	9.4	101.6	48.0	94	1.6	11.6	3.9
Air Induction	110015 (60) F	96	10.9	101.2	47.8	95	1.0	11.7	3.5
Very Coarse									
Air Induction	11002 (35) F	94	10.2	104.6	48.1	95	1.2	11.8	3.8
Air Jet	3.5 psi (21) F+B	96	13.0	99.6	48.3	95	1.7	11.8	4.0
Untreated		98	14.9	101.2	47.8	94	2.6	11.3	2.9
LSD		NS	6.1[z]	NS	0.8*	NS	0.8	NS	1.0
% C.V.		7	36	7	1	1	40	6	11

[Y]Spraying Systems Mobile Systems Products Catalog 49A. 2005. TeeJet Mid-Tech North 403 North Main Hartford, South Dakota 57033.

[z] P=0.1

Travel Speed 6 MPH and application rate 10 GPA on all plots.

Table 2. Pearson Correlation of Dependent Variables in Nozzle Study, Langdon 2005.

Dependent Variable	FHB Incidence	Severity	Yield	Test Weight	Plump	DON	Protein	Dilution
Incidence	1.0	0.4746 0.0020	-0.2335 0.1470	-.03217 0.043	0.0417 0.7985	-0.0251 0.8779	-0.0582 0.7213	0.3458 0.0613
Severity		1.0	-0.1473 0.3645	-0.3237 0.0416	-0.1720 0.2887	-0.0683 0.6754	0.1786 0.2702	0.2873 0.1237
Yield			1.0	0.2805 0.0800	0.2527 0.1156	0.0971 0.5510	0.0305 0.8517	0.0895 0.6381
Test Weight				1.0	0.3305 0.0372	0.0522 0.7489	0.0978 0.5483	-0.3483 0.0593
Plump					1.0	-0.3079 0.0533	0.0998 0.5401	0.1765 0.3508
DON						1.0	-0.1574 0.3322	0.3017 0.1051
Protein							1.0	-0.4004 0.0283
Dilution								1.0

Correlation Coefficient
Prob > |r| under H0: Rho=0

THE EFFECT OF PREVIOUS CROP RESIDUE, CHAFF MANAGEMENT, AND TILLAGE SYSTEM ON FUSARIUM HEAD BLIGHT OF BARLEY

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ABSTRACT

The effects of durum or barley crop residue, chaff collection and removal from the field, and tillage system on Fusarium head blight (FHB) were examined in 2004 and 2005 at the Langdon Research Extension Center, Langdon North Dakota. Robust barley was planted to plots previously cropped durum or barley with the previous crops' chaff either dropped in the plot (typical of most producer management strategies) or collected and removed. A conventional tillage system, spring tooth cultivation both fall and spring before planting, was compared to rototill, both fall and spring to simulate moldboard plowing, and notill. The management systems evaluated typically leaves three distinct residue levels in the field. Barley as a previous crop left more residue in the field after planting. Collecting and removing chaff at harvest reduced residue levels the following spring but only slightly. Residue levels at planting were greater with notill than conventional or rototill. Yields were greater following durum and slightly greater when chaff was collected and removed. Yields were similar among tillage systems in 2004 when inoculum amounts were small but less with notill in 2005 when environment conditions provided greater amounts inoculum. Previous crop barley had greater plump than durum indicating advantage to rotation. Notill had less plump than the other systems. No differences were determined in FHB incidence, field severity, or deoxynivalenol levels regardless of treatment except for a small incidence difference one year. While it is likely that previous crop may contribute to FHB, this study indicates that localized residues may make minimal contribution to FHB when disease levels are moderate or high in the surrounding area.

AERIAL APPLICATION OF FUNGICIDE ON BARLEY

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ABSTRACT

Fusarium head blight (FHB) has been a major problem for cereal grain producers during the past decade. To combat this disease, growers have applied fungicide by both aerial and ground application. About 50% of the small grains acreage sprayed with fungicide in the Dakota-Minnesota region of the Great Plains are applied with spray planes. An aerial application study was conducted near Esmond to evaluate fungicide application for control of FHB on 'Drummond' barley. The study was designed as a randomized complete block with three replicates. The treatments included the fungicide Folicur (tebuconazole) applied with spray of 3 or 7 GPA applied with a fine and a medium size drop and a volume of 5 GPA applied with a medium size drop. The applications were made to heading barley (greater than 50% of main stem heads fully extended from the boot). The fungicide was applied with Induce adjuvant at 0.125% v/v and F D&C Blue #1 dye at 22 grams per acre. The dye is a food grade type. A common method to evaluate spray technology is the use of water and oil sensitive paper. Water sensitive cards, were placed at grain head height on stands. One card was placed horizontal. Other cards were placed vertical, back to back and oriented forward and backward and right and left on stands within the sprayed plots. Stain size was determined with WRK Droplet Scan system. Three 50 ft spray passes were made side by side (150 ft.) on each plot. All data was collected from the center of the plot. Additionally, 5 heads were collected at 5 points across the swath width and placed in Erlenmeyer flasks for determination of head coverage by washing the food dye with a 90% alcohol solution and recording absorbance with a spectrophotometer. Field counts were determined by a visual assessment of FHB and foliar disease at mid dough growth stage by assessing twenty heads in two locations per plot and determining the incidence of the disease and the severity of the individual head. The incidence x the severity of the 20 heads gave a field severity per plot. Foliar disease differences were determined by estimating the infected area on 5 leaves at two locations. Data were analyzed with the general linear model (GLM) in SAS. Least significant differences (LSD) were used to compare means at the 5% probability level. Differences in all measurement parameters were not significant due to almost 100% incidence in both the treated and untreated plots and high field severity. No differences in yield, test weight, plump, protein and DON were found. Drop size differences were also found to be insignificant due to large variations in coverage and drops deposited on the water sensitive cards were likely due to orientation of the plots and the variable wind speed recorded during spray application. Coverage on the head, although not significantly different trended to greater amounts with smaller spray volumes. This is due to the exceptional efficiency of small drops depositing on small collectors (awns).

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2005 FHB UNIFORM FUNGICIDE TRIAL ON HARD RED SPRING WHEAT IN MINNESOTA

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OBJECTIVE

Evaluate and compare Fusarium head blight (FHB) control efficacies of non-registered fungicide products when applied on hard red spring wheat in Minnesota.

INTRODUCTION

Fusarium head blight was originally described more than a century ago. Since that time the disease has caused severe and repeated epidemics on small grain crops resulting in billions of dollars in crop losses (McMullen et al., 1997; Wood, 2002). More specifically, Nganje et al. (2004) estimated the recent 1993-2001 FHB epidemics caused economic losses of greater than $5.2 billion in Minnesota and North Dakota alone. The disease remains a constant threat to the economic stability of small grain growers in production areas with rain, humidity, or heavy dews during critical fungal infection periods (McMullen, 1997).

Successful plant infection by *Fusaria graminearum* is largely dependent on environmental conditions prior to, and during the time when the crops are in susceptible growth stages. Cultural disease management strategies (i.e.: crop rotation, tillage, and field sanitation) have resulted in partial disease suppression. Likewise, additional suppression has been achieved from the application of select fungicide products at Feekes 10.51 (early flowering growth stage). Ongoing research into disease control efficacies of experimental fungicides is needed to preserve yield and quality of small grains in regions most at risk for catastrophic crop losses.

MATERIALS AND METHODS

Hard red spring wheat cultivar 'Oxen' was planted 4 May 2005 into wheat residue at 1.25 million live seed $acre^{-1}$ in a randomized complete block design with four replicates. Each plot was inoculated with 112 kg ha^{-1} of *F. graminearum* infested corn grain six weeks after planting. Early morning misting (10 minute intervals every 80 minutes between 4:00 am to 8 am) was initiated seven weeks after planting. When the weather moderated and soils were not saturated, the misting duration was increased. Misting was continued until approximately the hard dough growth stage (Feekes 11.2), but was discontinued temporarily during the growing season if weather events caused soil saturation. On 6 June, an application of tank mixed herbicides (MCPA, Harmony GT, and Puma) was made to control weeds.

Eight weeks after planting (1 July), fungicide treatments were applied to wheat at the early-flower growth stage. Treatment applications were made with a CO_2 backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. On 15 July, disease severities were recorded from flag leaves, and 50 spikes $plot^{-1}$ were collected and frozen until rated for FHB symptoms. The test was harvested 14 weeks after planting, on 9 August.

Fusarium head blight severities were estimated according to the visual scale published by Stack and McMullen (1995), while percent visually scabby kernels (VSK) was estimated using a set of grain standards based on Jones and Mirocha (1999). Percent leaf disease was estimated using James (1971). Grain deoxynivalenol (DON) levels were determined by the University of

Minnesota Toxicology Lab in St. Paul utilizing the gas chromatography/mass spectrometry (GC/MS) method. ANOVAs were performed with SAS using PROC GLM. Fisher's protected least significant difference (LSD) mean comparisons were used to identify statistically different treatments.

RESULTS AND DISCUSSION

Two fungicide treatments resulted in the least amount of FHB incidence symptoms (Prosaro 6.5 fl oz/a and BAS555 13.5 fl oz/a) and were not significant from the duplicate Folicur 4 fl oz/a treatments (P=0.01) (Table 1). Two treatments (Punch 8 fl oz/a and Prosaro 6.5 fl oz/a) resulted in less FHB severity, but were not significant from either Folicur 4 fl oz/a treatment, BAS555 10 fl oz/a, or BAS555 13.5 fl oz/a (P=0.1). FHB index values ranged from a low of 3.4% to a high of 6.%. The Prosaro 6.5 fl oz/a treatment resulted in the smallest FHB index value, but it was not significantly different from BAS555 13.5 fl oz/a, Folicur 4 fl oz/a, and Punch 8 fl oz/a (P=0.001). Visually scabby kernels varied from 5.5% to 10.3%. Numerically, the BAS555 (10 fl oz/a) treatment resulted in the most scabby kernels, but it was not significantly different than most other treatments (P=0.03). Application of Prosaro 6.5 fl oz/a and BAS555 13.5 fl oz/a resulted in significantly fewer scabby kernels compared with BAS555 10 fl oz/a. DON means were relatively similar across treatments, ranging from 2.2 ppm (Prosaro 6.5 fl oz/a) to 4.8 ppm (nontreated). The treatment resulting in the least amount of DON (Prosaro 6.5 fl oz/a) was not significantly different from BAS555 13.5 fl oz/a or Folicur 4 fl oz/a ($P<0.0001$). Fungicide treatments had increased kernel protein levels compared with the nontreated control. Folicur 4 fl oz/a, BAS555 13.5 fl oz/a, and Prosaro 6.5 fl oz/a treatment means were similar (15.3% to 15.4%) and were not statistically different from most of the other treatments (P=0.0003). Overall, Prosaro 6.5 fl oz/a, BAS555 13.5 fl oz/a, Punch 8 fl oz/a, and Folicur 4 fl oz/a performed well for test weight (P=0.0003) and yield ($P<0.0001$).

In general, the non-registered fungicides showed increased FHB control over the current industry standard treatment (Folicur 4 fl oz/a), but the difference was not significant. However, compared with the nontreated control, non-registered fungicides resulted in significantly better disease control (e.g.: FHB incidence, FHB index, DON levels, percent protein, leaf disease severity, and yield).

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Table 1. Fusarium head blight (FHB) and leaf spot disease responses from 'Oxen' hard red spring wheat in Crookston, Minnesota during 2005.

Treatment/Active Ingredient	Fusarium Head Blight								
	*DI[2] (%)	**DS (%)	*DX (%)	*VSK (%)	*DON (ppm)	*Protein (%)	*LD[3] (%)	*Test Wt. (lb/bu)	*Yield (bu/A)
1. Nontreated control.....	50.0a	12.3ab	6.1a	9.0abcd	4.8a	14.8c	2.5a	57.6c	57.1d
2. Folicur[1] 432SC 4.0 fl oz/a... • tebuconazole	36.0cd	10.3bc	3.7cd	6.5cd	3.0def	15.4a	0.3d	59.4a	64.6ab
3. Folicur[1] 432SC 4.0 fl oz/a... • tebuconazole	39.5bcd	11.5abc	4.5bc	7.0bcd	3.7bcd	15.3ab	1.2c	59.2ab	63.9b
4. Prosaro[1] 6.5 fl oz/a..... • tebuconazole & prothioconazole	33.0d	10.2c	3.4d	5.5d	2.2f	15.4a	1.9b	60.0a	67.3a
5. BAS555[1] 01F 13.5 fl oz/a.... • metconazole	33.0d	10.3bc	3.5cd	6.0d	2.5ef	15.4a	0.2d	59.7a	65.7ab
6. BAS555[1] 01F 10.0 fl oz/a.... • metconazole	45.5ab	11.7abc	5.3ab	10.3abc	4.6ab	15.2b	0.2d	59.4a	60.6c
7. Punch 6.0 fl oz/a..... • flusilazole	43.5abc	12.7a	5.4ab	7.5abcd	4.3abc	15.2ab	0.3d	59.1ab	64.0b
8. Punch 8.0 fl oz/a..... • flusilazole	45.5ab	9.8c	4.4bcd	6.5cd	3.4cde	15.3ab	0.2d	59.2ab	64.7ab
CV	*16*	*13*	*18*	*33*	*16*	*1*	*46*	*1*	*3*

[1]Fungicide treatment included 0.125% Induce.
[2]Treatment abbreviations are DS, FHB severity; DI, FHB incidence; DX, FHB index; VSK, visually scabby kernels; DON, deoxynivalenol; LDS, leaf disease severity; Test wt., bushel test weight.
[3]Fungal foliar diseases consisted of Septoria/Stagonospora blotch complex (*Septoria tritici* and *Stagonospora nodorum*) and tan spot (*Pyrenophora tritici-repentis*). Data were logarithmically transformed.
NOTE: LSD significant at 0.10 probability level (**) and at 0.05 probability level (*).

2005 FHB UNIFORM FUNGICIDE TRIAL ON HARD RED WINTER WHEAT IN MINNESOTA

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ABSTRACT

Hard red winter wheat cultivar 'Jerry' was planted on 29 Sept. 2004, into wheat residue at the Northwest Research and Outreach Center near Crookston as part of the uniform fungicide trials for Fusarium head blight control. The objectives of the trial were to evaluate and compare Fusarium head blight (FHB) control efficacies of non-registered fungicide products when applied to winter wheat in Minnesota. The test was arranged in a randomized complete block design with four replicates. Each plot was inoculated 10 June 2005, with 112 kg ha^{-1} of *F. graminearum* infested corn grain. Fungicide treatments were applied on 20 June when plants were at the early flowering growth stage (Feekes 10.51). Fungicide treatments were applied using a CO_2 backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. Treatments consisted of: (1) nontreated control; (2) duplicate Folicur (tebuconazole) 4 fl oz $acre^{-1}$ treatments; (3) Prosaro (tebuconazole + prothioconazole) 6.5 fl oz $acre^{-1}$; (4) BAS555 (metconazole) 13.5 fl oz $acre^{-1}$; (5) BAS555 (metconazole) 10.0 fl oz $acre^{-1}$; (6) Punch (flusilazole) 6 fl oz $acre^{-1}$; and (7) Punch (flusilazole) 8 fl oz $acre^{-1}$. On 7 July, flag leaf disease severities were recorded. The same day 50 spikes $plot^{-1}$ were collected and frozen until rated for FHB symptoms. The test was harvested on 29 July. ANOVAs were performed with SAS using PROC GLM. Fisher's protected least significant difference (LSD) mean comparisons were used to identify statistically different treatments. Results of FHB incidence means ranged from 16.5% (Prosaro 6.5 fl oz) to 7.0% (BAS555 13.5 fl oz), FHB severity means ranged from 10.8% (Punch 8 fl oz) to 7.7% (BAS555 10 fl oz), and FHB indexes ranged from 1.7% (Punch 6 fl oz) to 0.7% (BAS555 13.5 fl oz). Treatment means were not significant for most test parameters (e.g.: FHB severity, FHB index, protein, test weight, thousand kernel weight and yield). FHB incidence, grain DON content, and leaf disease severity means were significant at P=0.05, P=0.1, and P=0.05 respectively. Test results will be published in the *2006 Fungicide and Nematicide Tests*.

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2005 FHB UNIFORM FUNGICIDE TRIAL ON SPRING BARLEY IN MINNESOTA

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ABSTRACT

A six-row spring barley cultivar 'Robust' was planted on 3 May 2005 into wheat residue at the Northwest Research and Outreach Center near Crookston as part of the uniform fungicide trials for Fusarium head blight control. The objectives of the trial were to evaluate and compare Fusarium head blight (FHB) control efficacies of non-registered fungicide products when applied to spring barley in Minnesota. The test was arranged in a randomized complete block design with four replicates. Each plot was inoculated six weeks after planting with 112 kg ha^{-1} of *F. graminearum* infested corn grain and misted when weather was dry. Misting was discontinued during the hard dough development stage. Seven weeks after planting, fungicide treatments were applied to barley at Feekes 10.5 (early heading growth stage). Fungicide treatments were applied using a CO_2 backpack-type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward positioned 'XR' Teejet flat fan 8001 VS nozzles. Treatments consisted of: (1) duplicate nontreated control treatments; (2) duplicate Folicur (tebuconazole) 4 fl oz $acre^{-1}$ treatments; (3) duplicate Tilt (propiconazole) 4 fl oz $acre^{-1}$treatments; (4) Prosaro (tebuconazole + prothioconazole) 6.5 fl oz $acre^{-1}$; (5) BAS555 (metconazole) 13.5 fl oz $acre^{-1}$; (6) BAS555 (metconazole) 10.0 fl oz $acre^{-1}$; (7) Punch (flusilazole) 6 fl oz $acre^{-1}$; and (8) Punch (flusilazole) 8 fl oz $acre^{-1}$. On 8 July, flag leaf disease severities were recorded. The same day 50 spikes $plot^{-1}$ were collected and frozen until rated for FHB symptoms. The test was harvested 13 weeks after planting, on 1 August. ANOVAs were performed with SAS using PROC GLM. Fisher's protected least significant difference (LSD) mean comparisons were used to identify statistically different treatments. Results of FHB incidence means ranged from 56% (nontreated) to 32% (Punch 6 fl oz), FHB severity means ranged from 2.6% (Tilt 4 fl oz) to 2.0% (BAS555 10 fl oz), and FHB indexes ranged from 1.4% (nontreated) to 0.6 (Punch 6 fl oz). Treatment results were not significant for most test parameters (e.g.: DON content, FHB incidence, FHB severity, FHB index, leaf disease severity, protein, test weight, and yield). Barley plump and thousand kernel weight mean results were significant at P=0.1. Test results will be published in the *2006 Fungicide and Nematicide Tests*.

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The authors would like to thank the U.S. Wheat and Barley Scab Initiative and the Northwest Research and Outreach Center for supporting this research; BASF Corp., Bayer CropScience, and DuPont Crop Protection for supplying fungicides; and the University of Minnesota Mycotoxin lab for providing DON results. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-9-053. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

COOPERATIVE STUDY FOR IMPROVED MANAGEMENT OF FUSARIUM HEAD BLIGHT USING AERIAL APPLICATION OF FUNGICIDE

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OBJECTIVE

To evaluate the efficacy of aerial fungicide application with different spray volumes and droplet sizes for Fusarium head blight (FHB) management in hard red spring wheat.

INTRODUCTION

Fusarium head blight is difficult to manage in small grains if environmental conditions promote infection and disease development when crops are at susceptible growth stages. Extension plant pathologists, and others, suggest an integrated approach for managing the disease. Recommendations focus on crop rotation, residue management, cultivar resistance, and a timely application of fungicide (Watkins and Doupnik, 1996; McMullen and Stack, 1999; Hollingsworth, 2004; Stromberg and Thomason, 2005). During 1996, Wilcoxson published a comprehensive review of reported research that contributes to our body of knowledge regarding fungicide control efficacies. Progression of FHB disease symptoms after application of fungicides has been studied extensively. For more information on this topic, see the 'Chemical, Cultural and Biological Control' section of this publication for recent cooperative results from the Fusarium head blight uniform fungicide trial.

New fungicide chemistries offering increasingly effective control of FHB are limited, making it imperative that existing fungicides are applied in such a manner to achieve maximum disease control. Spreading a fungicide active ingredient uniformly onto glume tissues in sufficient volumes to manage the disease is difficult. Non-target plant tissues (e.g.: awns, leaves) compete for droplets, reducing the amount of fungicide product available to protect susceptible plant tissues. Our objectives were to establish whether aerial fungicide application technologies could be modified in such a way as to increase fungicide deposition on plant tissues by adjusting droplet sizes and dilution volumes for increased disease control using fungicide.

MATERIALS AND METHODS

Treatment parameters. This cooperative research effort included three Red River Valley experimental locations situated within commercial fields of hard red spring wheat (locations north to south: St. Thomas, ND; Crookston, MN; Hunter, ND). A total of three wheat cultivars, susceptible to FHB, were tested ('Reeder' cv at St. Thomas, 'Polaris' cv at Crookston, and 'Briggs' cv at Hunter). All three fields were planted during late April and production inputs were managed by cooperating growers to achieve optimum yields. Three replications of treatments (five fungicide and one nontreated control) were arranged in a randomized complete block design at each test location. Treatments tested at each location included combinations of two fungicide dilutions (3.0 and 7 gpa) in two sizes of spray droplets (200 and 350 μm), as well as the industry standard treatment (5 gpa, 275 μm). Each plot area measured approximately 150 x 700-1,000 ft. to accommodate three 50 ft. wide fungicide application swaths from an aircraft. Folicur 3.6 F fungicide (e.g.: tebuconazole active ingredient) mixed with Induce adjuvant (0.125% v/v), and a blue food grade

type dye (22 g/a FD&C #1) were applied by a Cessna Ag Truck aircraft operated by Dakota Aviation of Grafton, ND. Different treatment spray volumes were attained by selecting CP-03 nozzles with two orifice sizes (0.125 and 0.171). One application of the fungicide mixture was applied at the labeled rate of 4 oz. $acre^{-1}$ during June or July (Hunter, 30 June; St. Thomas, 5 July; Crookston, 6 July) when the crop was at early flowering (Feekes growth stage 10.51).

Data collection. Droplet patterns and deposition data were determined from water sensitive cards and grain spikes. Coverage data will be reported elsewhere. Disease data as well as grain yield and quality parameters are reported here. FHB incidence, severity, and index ratings (incidence x severity/100) were recorded from 50-100 heads $plot^{-1}$ at soft dough stage of kernel development. Leaf disease severity ratings from 10-55 flag leaves $plot^{-1}$ also were collected at two test sites (Crookston and St. Thomas) during dough development (Feekes growth stage 11.2). At grain maturity, cooperating growers harvested one swath from each plot using commercial grain combines. Harvested grain was transferred into a weigh wagon. Yield (Bu/a) was calculated from swath area and grain weight. Grain sub-samples were collected to determine kernel moisture, protein, test weight, and deoxynivalenol (DON) concentration. A combined statistical analysis of data was conducted across test sites.

RESULTS AND DISCUSSION

Moderate FHB disease pressure occurred at two of three experiment locations (Hunter and St. Thomas, ND), while pressure was much less at Crookston (Table 1). All fungicide treatments resulted in less FHB incidence compared with the nontreated control (P=0.002;). Severity of FHB was reduced at all test locations from two treatments (5 gpa, 275 μm; 7 gpa, 200 μm), at two of three test locations from two treatments (3 gpa, 350 μm; 7 gpa, 350 μm), and at one location from one treatment (3 gpa, 200 μm) compared with the nontreated control (P=0.001; Fig. 1). A significant location x treatment interaction occurred with FHB index data (P=0.0002), Compared with the nontreated control, all treatment combinations reduced FHB index at Hunter and St. Thomas, but not at Crookston. Flag leaf disease severities were reduced from all fungicide treatments at St. Thomas (P=0.05). Those treatments with fine droplet sizes (3 or 7 gpa with 200 μm) resulted in increased grain yield at all test locations over the nontreated control while treatments with larger droplet sizes (5 gpa, 275 μm; 3 or 7 gpa with 350 μm) increased yield at the St. Thomas and Hunter, ND test sites (P=0.0001). Kernel test weight was increased at all test locations from one treatment (3 gpa, 200 μm); at two locations from two treatments (5 gpa, 275 μm; 7 gpa, 350 μm), and at one location by two treatments (3 gpa, 350 μm; 7 gpa, 200 μm) compared to the nontreated control (P=0.004). Only one treatment (3 gpa, 200 μm) at one location (Crookston) increased kernel test weight significantly compared with the industry standard treatment (5 gpa, 275 μm). Kernel protein and DON concentration results were not significant.

Overall, this research establishes that aerial application of fungicide on spring wheat, regardless of droplet size or dilution volume, was of benefit in locations with moderate FHB disease levels. The industry standard treatment (275 μm, 5 gpa) and the '200 μm, 7 gpa' treatment appear to offer a slightly greater and significant level of FHB control (Fig. 2), when averaged over all three locations.

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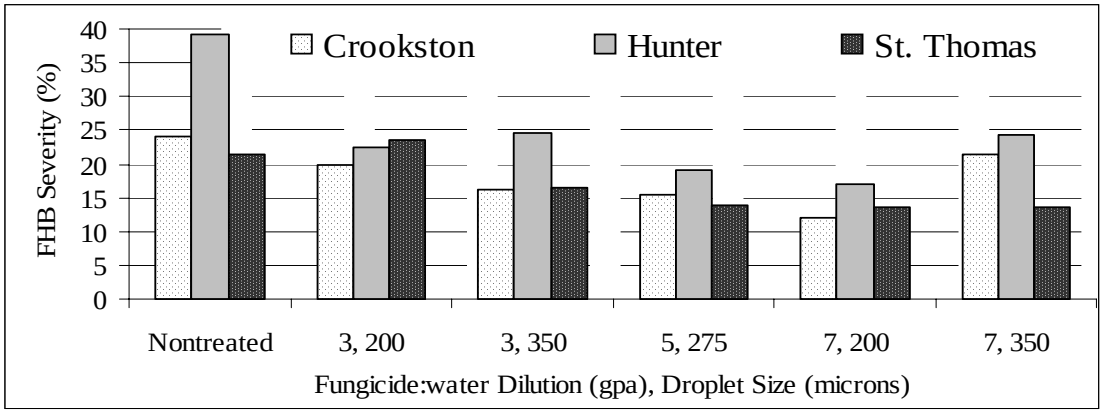

Fig 1. FHB severity of three spring wheat cultivars and locations using aerial application of fungicide with different treatment combinations of fungicide dilutions and droplet sizes.

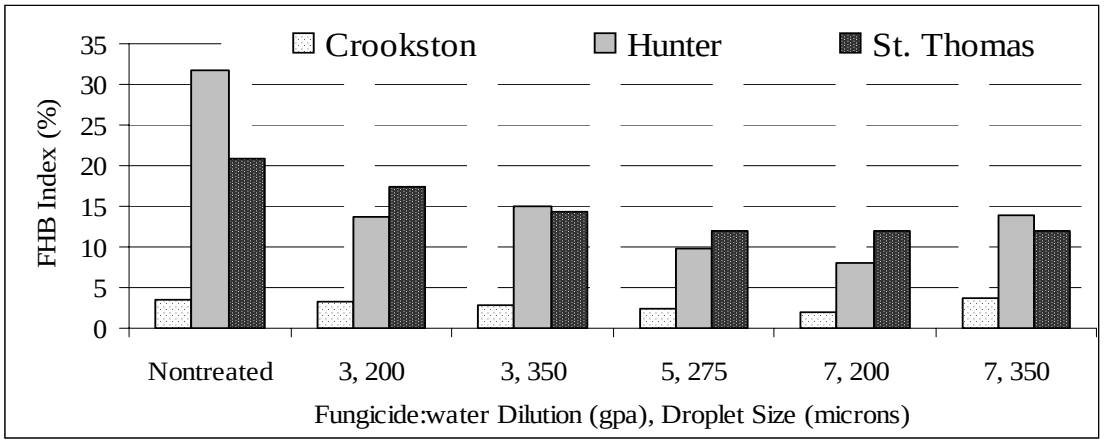

Fig. 2. FHB index values for three spring wheat cultivars and locations using aerial application of fungicide with different treatment combinations of fungicide dilutions and droplet sizes.

Table 1. FHB disease, crop yield, and grain quality parameters tested at three Red River Valley locations and from three spring wheat varieties during 2005.

Spray Volume (gpa)	Drop Size (µm)	Fusarium head blight Incidence (%)	Severity (%)	Index (%)	Yield (Bu/a)	Test Weight (lb/Bu)	DON (ppm)	Protein (%)
Crookston 'Polaris'								
3	200 µm	16.7	19.8	3.3	63.4	61.5	1.5	13.0
3	350 µm	16.7	16.2	2.8	60.6	60.9	1.3	12.5
7	200 µm	16.7	12.0	2.0	63.6	60.6	1.3	12.9
7	350 µm	16.0	21.4	3.7	61.3	60.8	1.3	12.5
5	275 µm	16.0	15.5	2.5	61.5	60.6	1.0	12.8
Untreated		16.0	24.0	3.4	59.3	60.4	1.2	12.9
Hunter 'Briggs'								
3	200 µm	59.2	22.6	13.8	60.6	61.0	3.1	15.0
3	350 µm	62.5	24.6	15.0	61.0	60.6	2.9	15.0
7	200 µm	47.5	17.0	8.1	56.2	60.5	3.2	15.4
7	350 µm	57.5	24.3	13.9	56.3	60.8	3.4	14.7
5	275 µm	52.5	19.2	9.8	59.4	60.8	2.8	15.5
Untreated		80.8	39.1	31.8	52.8	60.0	5.2	15.6
St. Thomas 'Reeder'								
3	200 µm	79.2	23.4	17.4	45.0	56.4	6.4	15.5
3	350 µm	77.5	16.4	14.3	45.2	56.5	7.2	15.5
7	200 µm	79.2	13.5	12.0	45.1	55.6	7.3	15.6
7	350 µm	76.7	13.7	12.0	45.1	57.0	4.9	15.5
5	275 µm	73.3	13.8	12.0	44.8	56.6	5.8	15.3
Untreated		89.2	21.4	20.9	38.9	54.8	11.8	15.6
Location		0.0001	0.0017	0.0001	0.0001	0.0001	0.0001	0.0001
Treatment		0.0024	0.0006	0.0001	0.0001	0.0040	0.1433	0.1599
Loc*Trt		0.0627	0.4165	0.0002	0.1422	0.5337	0.5952	0.5149
LSD		7.1	5.8	3.0	2.2	0.6	NS	NS
C.V.		14	31	30	4	1	60	3

EFFECTIVE APPLICATION OF FUNGICIDES ON WHEAT HEADS: WHAT'S THE BEST?

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ABSTRACT

Effective application of fungicides for protection against Fusarium head blight is a challenging goal for current application systems. For example, uniform coverage of a fungicide on wheat heads is critical. Spray coverage using a conventional sprayer with single-spaced nozzles on a boom has not been satisfactory. Timing of fungicide application is also critical. Unfortunately, the window of fungicide application in wheat is extremely narrow – just a few days – and the time often coincides with herbicide application in other crops. Therefore, both growers and custom applicators need methods to increase efficiency in chemical coverage within and among wheat heads, and to increase sprayer efficiency across land areas by increasing forward travel speeds – economically – using inexpensive nozzle configurations.

We've been investigating various sprayers and nozzle configurations since the late 1990s. In 2001, UV dye in various sprayers showed extremely variable coverage on wheat heads from various spray configurations. During each year between 2002, and 2005, water sensitive papers (Spraying Systems Co., Wheaton, IL) were used to evaluate spray coverage. These papers were transformed into cylinders to mimic wheat heads before spraying. After each spray treatment, the papers were unfolded, scanned, and analyzed for coverage using SigmaScan Pro Version 5.0 software. Labels on the papers were used to mark the position of the "wheat head" relative to the forward travel of the sprayer. Copper sulfate was used in the spray solution in all years, except 2001, to assess the amount of chemical applied on each "side" of the paper cylinders. The ground sprayer nozzle configurations included the use of Turbo TeeJet® nozzles in a forward-back configuration, TwinJet® nozzles, air induction nozzles, Turbo FloodJet® (single nozzles alternating forward and backward along the boom), FullJet nozzles, and the use of Twin Caps; except in 2005, all nozzle configurations on ground sprayers were compared at forward speeds of 10 and 19 kph (6 and 12 mph) and sprayed at the same water volumes. Ground sprayer configurations were compared with the airplane and helicopter in 2002. All of the nozzle configurations before 2005 were tested in optimal wind conditions (winds <5 kph) and boom heights above the wheat heads. In 2005, the best sprayer nozzle configurations from previous years were tested in sub-optimal spray conditions – wind conditions of approximately 15 kph, and with boom heights higher than the ideal, to mimic field conditions using wide spray booms. In 2005, we also assessed both coverage and fungicide efficacy of various nozzle configurations on field-scale strip plots.

Briefly, the backward-forward nozzle configuration and the FloodJet configuration produced the highest coverage and apparent distribution of chemical on the simulated wheat heads when compared to all other spray applicators. In these two sprayer configurations, a forward speed of 19 kph was equal in total coverage and uniformity of coverage compared to 10 kph at the same water volumes. All other spray nozzle configurations, however, had lower total coverage and higher variability when spraying at 19 kph compared to 10 kph. TwinJet nozzles at 10 kph produced half the coverage of the backward-forward nozzles, but coverage was relatively uniform compared to the Twin Cap and flat fan configurations. Although the spray coverage from the airplane and helicopter was relatively low (<3%), the amount of chemical that reached the "heads" was com

parable to most of the other ground applicator systems, but still less than the backward-forward and FloodJet configurations. Boom height and wind effects had a great impact on total coverage and uniformity of coverage. These data will be presented, along with a ranking of sprayer systems for effective application of fungicides for controlling Fusarium head blight.

EFFECTS OF INDUCED SYSTEMIC RESISTANCE-ACTIVATING AGENTS ON FUSARIUM HEAD BLIGHT

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OBJECTIVES

I. To determine whether some of the known biocontrol agents for Fusarium head blight (FHB) can activate induced systemic resis tance (ISR) in wheat against FHB.

II. To determine the potential of using ISR-in ducing strains of plant growth promoting rhizobacteria (PGPR) to control FHB.

III. To evaluate PGPR strains and autoclaved fungal biomass (AFB) preparations for effec tiveness in controlling FHB in the field.

INTRODUCTION

Chemicals and microorganisms have been reported to activate ISR in wheat and barley against a number of fungal diseases (Gorlach *et al*., 1996; Steiner and Schonbeck, 1995). Theoretically, ISR could provide several advantages to controlling FHB: 1) protection using ISR-activating agents could be achieved without having to target flowering heads in spray treatments; and 2) application of the agents could be made prior to anthesis because ISR, once activated, can provide longer-term protection than conventional fungicides; and 3) ISR might provide control of other foliar pathogens as well, thus making application of ISR activators more economically practical (Van Loon et al., 1998). The potential of any ISR activator to induce resistance against FHB, however, cannot be determined without direct testing. For example, benzothiadiazole, a commercially-available chemical activator, can induce ISR against powdery mildew in wheat (Stadnick and Buchenauer, 2000), but it did not provide protection against FHB (Yu and Meuhlbauer, 2001). There are very few other reports of materials being evaluated for induction of ISR against FHB. In one of them, autoclaved fungal biomass (AFB) prepared from several fungal isolates, reduced the severity of FHB in greenhouse experiments through induced resistance (Khan and Tisserat, 2004). In another, the bacterial biocontrol agent *Lysobacter enzymogenes* strain C3 did not induce systemic resistance in wheat against FHB when applied to flag leaves or roots, but presumably affected FHB partly through localized induced resistance (Yuen and Jochum, 2003). No other reported biocontrol agent for FHB has been investigated for induction of ISR in wheat. Numerous strains of PGPR are known to induce ISR (Kloepper et. al. 2004; Ramamoorthy et al., 2001; Van Loon et al., 1998). While some were reported to induce resistance against fungal pathogens when applied to foliage of gramineaceous plants (Ekici-Kilic and Yuen, 2004; Ramamoorthy et al., 2001), none have been tested for control of FHB.

MATERIALS AND METHODS

Biological control agents against FHB, strains of PGPR, and AFB preparation used in this study are listed in Table 1. Strain C3 of *L. enzymogenes* was included in all experiments for comparison as a presumed inducer of localized resistance. The PGPR strains were selected for this study because they induced resistance against a fungal pathogen when applied to tall fescue foliage in a previous study (Ekici-Kilic and Yuen, 2004). Strain C3 was cultured in chitin broth for 7 days, while all other bacterial strains were cultured on nutrient broth with yeast extract for 3 days. Whole broth cultures were used in treating plants in greenhouse and field experiments. AFB preparations were made from an isolate of *Aspergillus niger* (AFB1) and *Penicillium* 2 (AFB2) by freeze-drying autoclaved mycelia and grinding to powder form. The

preparations were added to water at a rate of 400 mg/L for application in field experiments. All microbial and AFB treatment liquids were amended with the surfactant Induce (0.125%) prior to application to plants.

A greenhouse experiment was conducted to determine whether FHB biocontrol agents can induce ISR against FHB. Scab-susceptible spring wheat cultivar 'Bobwhite' was grown in 15 cm pots (6 plants per pot). Plants were treated with the biocontrol strains or water, as the control, by either spraying flag leaves 3 days prior to pathogen inoculation or spraying flowering heads 1 day prior to inoculation (6 pots per treatment). In the flag-leaf treatment, the heads were shielded from the spray by enclosing them in plastic bags during treatment. All plants were inoculated with the pathogen by spraying a conidial suspension of *Fusarium graminearum* ($5X10^5$ spores/ml) onto the heads at anthesis. Inoculated plants were placed in a mist chamber for 48 hours to stimulate infection and then transferred back to the greenhouse for scab development over a 14 day period. Scab severity (percent of spikelets on each inoculated head exhibiting scab symptoms) was assessed. Results from multiple heads per pot were averaged prior to performing analysis of variance.

A separate greenhouse experiment was conducted to evaluate PGPR strains for control of FHB. They were compared with *L. enzymogenes* C3 and a water control. Six pots with heads entering anthesis were sprayed with each treatment and inoculated 3 days later with the pathogen. All other methods were as described above.

Two field experiments were conducted, one in Lincoln, NE on winter wheat '2137', the other in Brookings, SD on spring wheat 'Russ'. The treatments in both experiments were *Pseudomonas fluorescens* WCS417, AFB1, AFB2, and *L. enzymogenes* C3. These were compared with tebuconazole (Folicur 3.6F; 295 ml/ha) and a water control. There were six replicate plots (1.2 m X 2.5 m) per treatment. All treatments were applied at 6.6 L/ha at early flowering (Feekes 10.5.1) using a CO_2-pressurized sprayer. Both trials were inoculated with *F. graminearum* in the form of pathogen-infested corn kernels and utilized mist irrigation systems to stimulate infection. FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. Results from the two trials were pooled for analysis of variance.

RESULTS AND DISCUSSION

None of the FHB biocontrol agents (1BC, Trigocor 1448, and C3) provided protection against the disease when they were applied to flag leaves of wheat plants (Table 2). Therefore, we conclude that none of them can induce systemic resistance against FHB. The possibility that the biocontrol agents activated resistance locally when applied to wheat heads cannot be ruled out.

Among the three PGPR strains evaluated in the greenhouse, only *Pseudomoans fluorescens* WCS417 reduced FHB severity, but it was not as effective as *L. enzymogenes* C3 (Table 3). This supports findings from a previous study comparing the strains using a different pathogen-host system (Ekici-Kilic and Yuen, 2004); greater efficacy of C3 in that study was attributed to its ability to inhibit the pathogen through antibiosis and localized induced resistance.

In the two field experiments comparing the PGPR strain WCS417, AFB1, and AFB2 with C3 and tebuconazole, there were no significant treatment effects; none of treatments significantly inhibited FHB development compared to the control (Table 4). Thus, the identification of microbial agents that can induce a sufficiently high level of ISR to provide effect control of FHB in the field remains elusive. The number of agents evaluated for this trait in this study, however, is small. Further screening of strains among the large number reported to induce ISR is warranted. Performance of microbial agents in this field study may have been affected by such factors as application coverage and retention on foliage in response to environmental influences (e.g. rain). These also are problems that need to be addressed in subsequent research.

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Table 1. Biocontrol agent strains and activators of ISR used in this study

Strain/material	Organism	Source
FHB biocontrol agents		
1BC	*Bacillus sp.*	B. Bleakley, South Dakota State Univ.
TrigoCor1448	*Bacillus subtilus*	G. Bergstrom, Cornell University
C3	*Lysobacter enzymogenes*	G. Yuen, Univ. of Nebraska-Lincoln
PGPR strains		
INR7	*Bacillus pumilus*	J. Kloepper, Auburn University
89-B61	*Psuedomonas fluorescens*	J. Kloepper, Auburn University
WCS417	*Psuedomonas fluorescens*	L. Van Loon, Utrect University
Autoclaved fungal biomass		
AFB1	*Aspergillus niger*	B. Tisserat, NCAUR, USDA-ARS
AFB2	*Penicillium* 2	B. Tisserat, NCAUR, USDA-ARS

Table 2. Results from greenhouse experiment evaluating FHB biocontrol agents for induction of systemic resistance in wheat to FHB.

	Severity (%)	
Treatment	Flag leaf treatment	Head treatment
Control (water)	49	93
Bacillus sp. 1BC	55	65
Bacillus subtilis TrigoCor1448	56	21
Lysobacter enzymogenes C3	63	33
P	NS	0.002
$LSD_{0.05}$	---	20

Table 3. Results from greenhouse experiment testing ISR activating strains of PGPR for control of FHB.

Treatment	Severity (%)
Control (water)	67
Pseudomonas fluorescens WCS417	45
Pseudomonas fluorescens 89B-61	56
Bacillus pumilus INR7	50
Lysobacter enzymogenes C3	26
P	0.007
$LSD_{0.05}$	19

Table 4. Results from 2005 field experiments evaluating bacterial strains and autoclaved fungal biomass (AFB) preparations for control of FHB. Values are means of two experiments.

Treatment	Severity (%)	Incidence (%)	Index (%)
Control (water)	16	79	27
Lysobacter enzymogenes C3	15	82	31
Pseudomonas fluorescens WCS417	13	87	27
AFB1	12	76	27
AFB2	16	81	27
Tebuconazole	11	76	24
P	0.130	0.350	0.095

EFFECT OF ADJUVANTS ON EFFICACY OF FOLICUR FUNGICIDE FOR FHB CONTROL

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ABSTRACT

Currently, the triazole fungicide Folicur (tebuconazole) has special exemptions in some states within the United States (US) for use on wheat and barley to suppress Fusarium head blight (FHB). A standard adjuvant recommended for use with tebuconazole is Induce, a petroleum-based non-ionic surfactant. Various private companies in the US sell other non-ionic surfactants or have other adjuvants for sale that are silicone-based or are encapsulating products, and these companies also are experimenting with many new formulations of adjuvants. With so many products on the market, more information is needed about their efficacy with triazole fungicides such as Folicur. Our preliminary tests indicated few differences among adjuvants when combined with Folicur. We continued studies in the greenhouse with adjuvants in combination with Folicur on hard red spring wheat, durum wheat and barley and measured effects on reductions in Fusarium head blight severity index (% incidence x % head severity). All fungicide applications were applied once, at early flowering (Feekes 10.51) in spring wheat and durum wheat, and at early full head emergence (Feekes 10.5) in barley. Fungicide plus adjuvant applications were made using a track sprayer mounted with XR8001 flat fan nozzles oriented forward and backward at a 60° angle from vertical, delivering 18.3 gpa at 40 psi. Plants were inoculated with a mixture of three *F. graminearum* isolates, delivering 10,000 spores/ml, 20 ml/pot per spray event, with a DeVilbiss atomizer, 4 hrs after the fungicide was applied. Immediately following inoculation, plants were misted for 48 hours using a closed mist system at or near 100% RH at 23°C (+ or – 5°C).

Results showed that Interlock, an encapsulating adjuvant, in combination with an experimental adjuvant, both manufactured by Agriliance LLC, resulted in significant reduction of the FHB severity index as compared to Folicur without adjuvant, and had the lowest FHB rating of all adjuvants tested across three grain classes. In a separate study on durum and barley only, several experimental adjuvants from Wilbur Ellis Co. resulted in lower FHB severity ratings than Folicur plus Induce, but not significantly so, while other experimental adjuvants provided by Wilbur Ellis resulted in significantly higher FHB severity indices, indicating that appropriate adjuvant use can benefit or hinder efficacy of fungicides for FHB control.

ACKNOWLEDGEMENT

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REAL WORLD RESULTS IN FHB MANAGEMENT

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ABSTRACT

In 1981, the Canadian Phytopathological Society held a symposium on the "Epidemiology and control of mycotoxigenic fusaria on cereal grains" as part of their 52nd annual meeting. In the Introduction to that Symposium, W. L. Seaman, from the Ottawa Agriculture Canada Research Station, described important steps for prevention of head molds: "development of resistant cultivars, rotation with nongramineous crops, effective cultivation of small grain and corn stubble, early planting, the use of high quality seed, prompt harvesting at maturity, and aerating and drying damp grain immediately after harvest". J. C. Sutton, also a participant in that symposium, stated in his article on the epidemiology of wheat head blight, that "Disease forecasts and schemes to warn growers of the risks of mycotoxins… should be made… Given timely warnings, growers may find it possible to take actions to reduce the intensity and impact of disease". This symposium in 1981 provided valuable information to scientists working with *Fusarium* diseases of cereals at that time, but it wasn't until severe widespread epidemics in the 1990s in the US and Canada that an impetus for large regional research efforts to revisit these management guidelines occurred. And by 1998, a US national effort was organized, the US Wheat and Barley Scab Initiative, which funded multi-state and multi-grain class projects directed at finding resistant germplasm, developing resistant varieties, studying epidemiology and developing disease forecasting models, evaluating effects of modern cultural practices on disease severity, and finding new chemistries and biological agents for disease control. As a result of these efforts, today more tolerant wheat cultivars are now available to growers, disease forecasting tools are widely available, more information is available about crop rotation and tillage effects on the disease, and newer fungicide chemistries have shown improved reduction of FHB severity and DON levels. How have these research results been presented to growers, how have they been translated into use by growers, and how effective have they been under real world situations, including recent FHB epidemics in the US from 2003-2005? This presentation will focus on the above mentioned management strategies and how they performed in the real world in the US from 2003-2005, with emphasis on results in the northern plains in 2005.

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RESULTS OF THE UNIFORM FUNGICIDE TRIAL ON BARLEY, NORTH DAKOTA, 2005

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ABSTRACT

As part of the national uniform scab fungicide trial, six fungicide treatments were compared for control of Fusarium head blight (FHB) in 'Robust' spring barley at the Fargo, ND Agriculture Experiment Station and in 'Tradition' spring barley at the Langdon Research Extension Center in northeast ND. The barley was planted on April 29th in Fargo and on May 9th in Langdon. Corn grain inoculated with *Fusarium graminearum* was spread evenly among plots in Fargo and naturally infected wheat seed was distributed among plots at Langdon. At Fargo, a misting system provided added water to the plots when the nighttime humidity dropped below 90%. Fungicides were applied on June 30th at Fargo and July 8th at Langdon, at early full head emergence (Feekes 10.5). Applications were with a backpack-type sprayer equipped with two XR8001 flat fan nozzles oriented toward the grain head at a 30 degree angle from the horizontal. The fungicides were applied at 18.5 gpa with 40 psi. Disease notes were taken at soft dough stage of development. The fungicide treatments included: Folicur (tebuconazole – A Bayer CropScience Section 18 compound) at 4 fl oz/A; Prosaro (19% prothioconazole + 19% tebuconazole - a Bayer CropScience experimental compound) at 6.5 fl oz/A; BAS555 (metconazole - a BASF experimental compound) at two rates, 13 fl oz/A and 10 fl oz/A; and Punch (flusilazole - a DuPont experimental compound) at two rates, 6 fl oz/A and 8 fl oz/A.

Fusarium head blight (FHB) field severity was very low at the Langdon location, only 0.6% in the untreated check, while at the Fargo location, it was 7.6%. Results indicated that all treatments significantly reduced FHB field severity at both locations. DON (deoxynivalenol) data was only available from Fargo at the time of this report. At Fargo, the Prosaro and BAS555 treatments significantly reduced DON over the untreated check. For yield, all treatments significantly increased yield at Fargo, but not at Langdon. The best yielding treatment at Fargo, Prosaro, increased yield by 29.5%.

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WHEAT UNIFORM FUNGICIDE TRIALS, ND, 2005

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OBJECTIVE

To evaluate experimental fungicides for control of Fusarium head blight (scab) in hard red spring and durum wheat in North Dakota.

INTRODUCTION

Uniform fungicide trials have been established across grain classes and environments as part of the U.S. Wheat and Barley Scab Initiative (McMullen and Milus 2002). Results of these fungicide trials across states are reported (Hershman and Draper, 2004). The purpose of these trials is to evaluate efficacy of fungicides in reducing Fusarium head blight severity (FHB), Fusarium damaged kernels (FDK), and deoxynivalenol (DON) levels and in increasing yield and test weight. North Dakota continues to participate in these trials at several locations across the state.

MATERIALS AND METHODS

The uniform fungicide trial was established at four locations: Fargo in the southeast; Langdon in the northeast; Carrington in the central part of the state; and at Minot in the north central region. Each site represents different environment, soil type, and cropping practices. Fungicides on hard red spring wheat were evaluated on FHB susceptible hard red spring wheat cultivars 'Reeder' at Carrington and Fargo and on 'Grandin' at Langdon, and on a moderately resistant cultivar 'Glenn' at Langdon. For durum wheat, evaluations were done on susceptible cultivars 'Lebsock' at Carrington and Langdon, and on 'Mountrail' at Minot.

A uniform set of six fungicide treatments were evaluated (Table 1). Fungicides tested included Folicur (tebuconazole), which had a Section 18 exemption for use on wheat in ND in 2005, Prosaro (equal parts prothioconazole and tebuconazole), an experimental fungicide from Bayer CropScience, BAS555 (metconazole), at two rates, an experimental product from BASF, and Punch (flusilazole) at two rates, an experimental product from DuPont. Artificial inoculum in the form of inoculated grain was dispersed in plots at Fargo and Langdon, wheat straw was distributed at Carrington, and natural inoculum was the source of infections at Minot. Natural rainfall was augmented by mist irrigation at Fargo and by some overhead irrigation at Carrington.

All treatments were applied at early flowering (Feekes 10.51) with a CO_2 backpack type sprayer, equipped with XR8001 nozzles mounted at a 60^0 angle forward and backward toward the grain heads. Water volume was 18-20 gpa applied at 40 psi. Disease ratings were taken at soft dough kernel stage. Plots were harvested with small plot combines. DON levels were determined by the NDSU Veterinary Toxicology Lab using gas chromatography and electron capture. Plots were in a Randomized Complete Block design and data were statistically analyzed across locations using ANOVA with locations as replicates.

RESULTS AND DISCUSSION

FHB field severities varied across sites and wheat class. FHB field severity on untreated hard red spring wheat averaged as high as 36% at Carrington and as low as 5.6 to 7.3% at Langdon. Durum FHB field severities in untreated plots ranged from 3% at Minot to 27.7% at Carrington. Overall, hard red spring wheat trials were planted earlier than the durum trials and had much more severe FHB than the durum trials, because record high rainfalls occurred in June in North Dakota in 2005, favoring FHB in wheat crops that flowered in June, including the spring wheat trials. Analysis of treat-

ment differences in durum often were numerically different than the untreated, but not significantly so, when analyzed across sites. For durum, only the percent Fusarium damaged kernels (FDK) were significantly affected by fungicide treatments, with all fungicide treatments significantly lower than the untreated, and the Prosaro treatment having significantly lower FDK than the Punch fungicide treatments. Table 1 contains data only from the ND hard red spring wheat uniform fungicide trials.

For hard red spring wheat, all fungicide treatments significantly reduced the percentage of FHB incidence, head severity, field severity and FDK over the untreated check (Table 1). The experimental treatments of Prosaro and BAS555 at the high use rate had the lowest FHB field severity, but not significantly lower than other treatments. For the one site reporting DON levels, treatments with Prosaro and BAS555 resulted in DON levels significantly lower than other fungicide treatments. All treatments significantly improved yield and test weight.

ACKNOWLEDGEMENT

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McMullen, M. and Milus, E. 2002. History and accomplishments of the USWBSI uniform fungicide and biological control trials, 1998-2002. Page 96 in: Proc. 2002 national Fusarium Head Blight Forum, Erlanger, KY, Dec. 7-9, 2002. U.S. Wheat and Barley Scab Initiative, Michigan State Univ., East Lansing, MI.

Table 1. Effect of fungicides on Fusarium head blight (FHB) incidence, head severity, field severity, DON, Fusarium damaged kernels (FDK), yield and test wt., averaged across four hard red spring wheat trials at Carrington, Fargo and Langdon, ND, 2005

Treatment and rate/acre[1]		FHB I[2] %	FHB HS[2] %	FHB FS[2] %	DON[3] ppm	FKD[4] %	Yield Bu/A	Test wt Lbs/bu
Untreated check		59.2	28.7	17.5	7.8	13.8	40.3	58.3
Folicur 3.6 EC	4.0 fl oz	41.1	19.6	9.0	5.8	7.7	49.7	59.1
Prosaro 421 SC	6.5 fl oz	35.0	17.6	7.4	4.0	5.9	53.7	59.9
BAS555 01 F	13.5 fl oz	38.4	17.5	7.8	4.1	7.0	51.4	59.5
BAS555 01 F	10.0 fl oz	42.4	21.2	9.2	4.6	5.6	50.4	59.5
Punch	6.0 fl oz	48.1	22.8	11.4	6.6	8.0	47.6	59.0
Punch	8.0 fl oz	44.0	19.3	10.1	6.0	8.1	47.3	59.1
LSD 0.05		11.0	5.9	4.6	1.1	2.8	2.3	0.7

[1] Folicur, Prosaro, and BAS555 treatments had 0.125% Induce added; Prosaro (19% prothioconazole + 19% tebuconazole) is an experimental fungicide from Bayer; BAS555 (metconazole) is an experimental fungicide from BASF; Punch (flusilazole) is an experimental product from DuPont

[2] FHB I = incidence; FHB HS = head severity; FS = Fusarium head blight field severity; field severity = incidence x head severity;

[3] DON (deoxynivalenol = vomitoxin) levels were only available from the Fargo location at the time of this report;

[4] FDK = Fusarium damaged kernels

CONTROL OF FHB IN WHEAT BY IMPROVED TECHNOLOGY AND FUNGICIDE CHOICE

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ABSTRACT

The European Union set binding limit values for important *Fusarium* toxins in all cereals including maize and Sorghum. For DON the limit value will be 1,250 mg/kg or 1,25 ppm for wheat. The commodities over this value cannot be processed for food and feed above 1.75 mg/kg. For this reason the control of Fusarium toxins will be the most important task for the growers. As resistant cultivars are not yet on the market in the extent we would need them, the chemical control will have a highly important role to secure food and feed safety. The consequence is that the efficacy of the chemical control should be described by the most important parameter will be the toxin contamination and not FHB or FDK severity. As most cultivars are susceptible, and their change for resistant ones takes longer time, for short run the only possibility is to use better technologies and better fungicides. Tests were made with three cultivars with differing resistance and artificial inoculation was made 24-48 hrs after fungicide treatment at full head coverage with four Fusarium isolates with differing aggressiveness. By this way 12 epidemic situations can be modeled.

Efficacy of the fungicides at full coverage is 2-3 times higher and for the best fungicides reaches 90 % or more. With the same fungicides under field conditions the efficacies vary between 0 and 50 %, exceptionally higher. Therefore we need a technology that enables much better coverage. The first field tests with the new technology were made 2005 by the Turbo Flood Jet alternating nozzles suggested by Hooker and Schaafsma on 1 acre plots. The tests were made with 9 fungicides, but symptomless fields were registered only after Prosaro 1 L/ha and Falcon 0.8 L/ha. In the tests with full coverage shows very high efficacies, only

Prosaro was able to secure lower DON than 1.25 ppm for three isolates, in one case at very high control DON value (51.96 ppm) even Prosaro was not enough. The efficacy above 90 % may also be sometimes not enough. It is worth to mention that Folicur with 250 g tebuconazole/l could not control FHB in any situations effectively. We can say that Prosaro is the best fungicide now, but in extreme epidemic situations the DON contamination may exceed limit values. For the other is granted.

ACKNOWLEDGEMENT

Projects: GAK ALAP-00073/2004, Bayer AG, Germany

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Mesterhazy A., Bartok, T., Lamper, Cs. 2003. Influence of cultivar resistance, epidemic severity, and *Fusarium* species on the efficacy of fungicide control of Fusarium head blight in wheat and deoxynivalenol (DON) contamination of grain. Plant Disease, 87:1107-1115.

Table 1. Fungicides against FHB in wheat, DON in ppm, Szeged, ,means for three cultivars, 2004.

Treatment	**Isolates**				**Mean**
L/ha	12377Fg	44Fg	12375Fc	12551Fc	
Prosaro 1.0	4.80	0.83	1.20	0.41	1.81
Input 1.0	4.15	1.09	2.42	1.06	2.18
F. solo 1.0	10.67	2.73	2.79	1.38	4.39
Falcon 0.8	13.62	1.84	2.13	1.87	4.87
Kolfugo 1.5	21.98	4.34	5.31	3.58	8.80
Fusarium contr.	51.96	35.59	22.72	18.02	32.07
Mean	17.86	7.73	6.10	4.39	9.02
LSD 5 %	6.74	6.74	6.74	6.74	3.37

NOVEL RESULTS ON FUNGICIDE APPLICATION AND CHOICE ON FHB IN WHEAT

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ABSTRACT

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Efficacy of the fungicides at full coverage is 2-3 times higher and for the best fungicides reaches 90 % or more. With the same fungicides under field conditions the efficacies vary between 0 and 50 %, exceptionally higher. Therefore we need a technology that enables much better coverage. The first field tests with the new technology were made 2005 by the Turbo Flood Jet alternating nozzles suggested by Hooker and Schaafsma on 1 acre plots. The tests were made with 9 fungicides, but symptomless fields were registered only after Prosaro 1 L/ha and Falcon 0.8 L/ha. In the tests with full coverage shows very high efficacies, only

Prosaro was able to secure lower DON than 1.25 ppm for three isolates, in one case at very high control DON value (51.96 ppm) even Prosaro was not enough. The efficacy above 90 % may also be sometimes not enough. It is worth to mention that Folicur with 250 g tebuconazole/l could not control FHB in any situations effectively. We can say that Prosaro is the best fungicide now, but in extreme epidemic situations the DON contamination may exceed limit values. For the other is granted.

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Input 1.0	4.15	1.09	2.42	1.06	2.18
F. solo 1.0	10.67	2.73	2.79	1.38	4.39
Falcon 0.8	13.62	1.84	2.13	1.87	4.87
Kolfugo 1.5	21.98	4.34	5.31	3.58	8.80
Fusarium contr.	51.96	35.59	22.72	18.02	32.07
Mean	17.86	7.73	6.10	4.39	9.02
LSD 5 %	6.74	6.74	6.74	6.74	3.37

EFFECT OF FUNGICIDES ON FHB AND DON IN WHEAT - 2005 UNIFORM FUNGICIDE TRIALS

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OBJECTIVES

Evaluate foliar fungicides for effectiveness in managing Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation in wheat across multiple trials representing different wheat classes and locations.

INTRODUCTION

FHB, caused predominantly by *Fusarium graminearum*, has had a great impact on every sector of the wheat and barley industries in North America. Wheat growers, millers, bakers, and consumers of wheat products all have been affected by this disease. This is largely due to the fact that in addition to yield losses associated with reduced kernel size and weight, reduced seed germination, and seedling blight, *F. graminearum* also produces a mycotoxin called deoxynivalenol (DON) (among other toxins) which may accumulate to unacceptable levels in harvested grain. DON levels above 2 ppm may render grain and their by-products unfit for commercialization and consumption. Efforts to minimize the impact of FHB and DON have been centered on the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application. Through collaborative research involving scientists from multiples states, representing various wheat-growing regions, Uniform Fungicide Trials (UFT) have been used to evaluate fungicide effectiveness against FHB and DON. These trials follow standard protocols and have been conducted annually since 1998. The results of the 2005 UFT trials form 25 trials across 10 states are presented herein.

MATERIALS AND METHODS

Each trial consisted of six fungicide treatments and an untreated control in a randomized complete block design, with four replicate blocks (one trial had five replicate block). The treatments were:

1 - Non-treated control;
2 - Folicur 432SC 4.0 fl oz + 0.125% Induce;
3 - Prosaro 6.5 fl oz/a + 0.125% Induce;
4 - BAS555 01/F 13.5 fl oz/a + 0.125% Induce;
5 - BAS555 01/F 10 fl oz/a + 0.125% Induce;
6 - Punch 6 fl oz/a (no surfactant); and
7 - Punch 8 fl oz/a (no surfactant).

Treatments were applied at early flowering (Feeke's 10.51) using CO_2-pressurized sprayers, equipped with Twinjet XR8002 nozzles or paired XR8001 nozzles mounted at a 60^o angle forward and backward.

Planting and crop production practices varied somewhat from trial to trial. See individual trial reports for details. Most plots were planted with a susceptible cultivar. To enhance disease development, plots were either planted into corn or wheat residue and/or artificially inoculated with *F. graminearum*-infested kernels. Many plots were mist-irrigated as a means of enhancing production of, and infection by fungal inocula. In each plot of each trial, percent FHB incidence (INC), diseased-head severity (SEV), index (IND; also known as plot severity), and *Fusarium*-damaged kernels (FDK) were measured as previously described (McMullen, et al., 1999). DON accumula-

tion was measured at one of the two USWBSI-funded DON Testing Laboratories.

For the purpose of data analysis, trials conducted at the same location, but using different cultivars, and trials conducted at different locations in the same state were considered separate studies. In the first part of the analysis, the data from all the trials with disease were pooled together. Trial was treated as a fixed effect, and a mixed effect model was fitted using PROC MIXED of SAS to determine the overall effects of trial, treatment, and the interaction between trial and treatment on the FHB and DON. The decision to treat trial as a fixed effect was based on the assumption that each location (or cultivar) was specifically chosen for the trial because of know characteristics, and researchers are interested in how these location- and cultivar-specific factors influence FHB and DON. In the second part of the analysis, each trial was analyzed separately to determine the most effective treatment within each trial. Linear contrasts were used to make pairwise comparisons between treatment means and means across groups of treatments. Studies with zero disease were not analyzed. A separate set of analyses was performed for each response variable IND, INC, SEV, and DON.

RESULTS AND DISCUSSION

FHB intensity varied from one trial to another. This was especially true when winter wheat trials were compared with spring wheat trials. In 2005, weather conditions in winter wheat areas were generally unfavorable for FHB development. Consequently, non-irrigated trials frequently had nominal disease development. Mean and maximum FHB index, across all replicates and treatments, ranged from 0 to 6.37 and 0 to 25.90%, respectively for winter wheat trials. For spring wheat trials, the corresponding values were 3.88 to 42.23% for mean index and 7.20 to 60.44% for maximum index (Table 1). In four of the 12 winter wheat trials, 0% index was observed in all treatments.

Based on the analysis of the pooled data, the treatment, trial, and the interaction between treatment and trial were significantly different from zero ($P < 0.05$). When averaged across trials, all treatments significant reduced FHB index relative to the check. When the data were grouped and analyzed according to wheat class, the effects of treatment and trial were significant for both winter and spring wheat trials; however, the interaction between treatment and trial was not significant ($P > 0.05$) among spring wheat trials. Within both groups of trials, all treatments significantly reduced FHB index relative to the check. In general, treatment 3 (Prosaro 6.5 fl oz/a + 0.125% Induce) was the most effective treatment, resulting in a greatest reduction in IND relative to the control. However, since there was a significant interaction between treatment and trial, the analysis was extended to determine the most effective treatment within each trial.

The mean level of disease and DON between each treatment and the untreated control was used to determine the most effective treatment within each trial. Similar results were observed for DON and for all measures of FHB intensity (IND, SEV, INC, and FDK), with the most effective treatment varying from trial to trial. Since IND is a direct function of INC and SEV (see Paul et al., 2005a,b), only the results for IND are summarized herein. The results for DON and FDK are presented in Tables 2 and 3. Treatment 3, application of Prosaro at a rate of 6.5 fl. oz per acre, resulted in the greatest reduction in IND relative to the untreated check in eight of the 21 trials analyzed (Table 1). In six of those trials, the difference in IND between Prosaro and the check was significantly different from zero ($P < 0.005$). A direct comparison between mean IND in Prosaro-treated plots and Folicur-treated plots showed that, although the absolute level of disease was generally lower in Prosaro-treated plots (in 15 of the 21 trials with disease), the difference between the two treatments was only significantly different from zero in two trials (Beltsville, MD and Fardo, ND). Treatments 4 (BAS555 01/F 13.5 fl oz/a + 0.125% Induce) and 5 (BAS555 01/F 10 fl oz/a + 0.125% Induce) were the most effective treatment in four trials each.

The percent control (Hershman and Milus, 2003) resulting from the most effective treatment was generally higher in trials with low levels of disease than in trials

with high levels of disease (overall mean and maximum disease IND in Table 1). This should be interpreted with caution since the ultimate effectiveness of a fungicide treatment should be based on results under high disease pressure. This can be done by jointly observing the percent control and the mean level of disease in the treated plots. In four of the seven trials with the highest levels of disease (mean IND across all treatments ranging from 10.95 and 42.23%), fungicide application significantly reduced IND when compared to the untreated check, with percent control between 21.42 and 59.36%. This is consistent with previously reported results (Hershman and Milus, 2003, Hershman and Draper, 2004). In the four trials with the highest levels of disease (Carrington 1 and 2 and Brookings 1 and 2), the mean index in plots treated with the most effective fungicides ranged from 16.25 to 27.72%.

The results for DON (Table 2) and FDK (Table 2) were very similar to those described for IND, with the treatment most effective at reducing DON and FDK varying among trials. The Prosaro treatment, treatment 3 was again the most effective. Based on the available data, fungicide treatment did result in a significant reduction in DON relative to the untreated check, with percent reduction being between 26.82 and 84.42%. Despite this reduction, however, DON levels in treated plots still exceeded critical thresholds in some trials. As was the case with IND, DON levels in Prosaro-treated plots was only significantly lower than DON levels in Folicur-treated plots in trials conducted at Beltsville, MD and Fardo, ND.

In summary, fungicide treatments did reduce FHB intensity and DON levels in harvested grain. The product and/or rate of application most effective at reducing FHB and DON varied from one trial to another; however, the application of Prosaro at a rate of 6.5 fl. oz per acre was the most effective treatment overall. The magnitude of FHB and DON reduction due to fungicide treatment depended on the level of disease. Under heavy disease pressure, relatively high levels of FHB and DON levels above threshold values may still occur in treated plots.

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Table 1. Fungicide effect on Fusarium head blight index.

Trial		Wheat	Most effective Treatment[a]				Disease index (%)	
State/PI	Location	Type	Treat	IND (%)	% Control	*P value*	Mean	Max
IL/Adee	Monmouth	W	2,3,4,6	0.02	91.81	0.002	0.07	0.67
IL/Fakhoury	Carbondale	W	7	0.11	88.5	0.089	0.81	3.24
IN/Shaner	Lafayette	W	…	…	…	…	0.00	0.00
	Butlerville	W	…	…	…	…	0.00	0.00
LA/Padgett	Macon Ridge	W	4	0.37	82.22	0.029	1.56	5.26
MD/Grybauskas	Beltsville	W	3	1.62	89.64	<0.001	6.37	25.90
MI/Hart	East Lansing 1	W	…	…	…	…	0.00	0.00
	East Lansing 2	W	…	…	…	…	0.00	0.00
MN/Hollingsworth	Crookston 1	S	3	3.36	45.4	<0.001	4.55	7.20
	Crookston 2	W	4	0.66	47.6	0.089	1.17	2.62
MO/Sweets	Columbia 1	W	6	0.00	100	0.151	0.55	3.15
	Columbia 2	W	6	0.00	100	0.170	0.59	2.80
ND/McMullen	Fargo	S	3	2.97	85.92	<0.001	6.91	23.00
	Carrington 1	S	3	19.50	45.45	0.004	25.68	55.00
	Carrington 2	S/D	3	16.25	41.44	0.069	21.54	37.00
	Langdon 1	S/D	4	1.60	71.93	0.067	4.36	16.00
	Langdon 2	S	3	2.25	59.82	0.015	3.88	8.00
	Langdon 3	S	7	3.15	56.55	<0.001	4.81	7.90
SD/Draper	Brookings 1	S	3	35.83	21.42	0.122	42.23	60.44
	Brookings 2	S	2	27.72	45.26	<0.001	39.21	54.62
	Watertown 1	S	5	10.70	49.17	0.057	14.16	37.14
	Watertown 2	S	5	6.91	59.26	0.008	10.95	26.44
	Groton 1	S	5	7.80	59	0.042	12.01	39.94
	Groton 2	S	5	6.09	28.57	0.299	7.23	14.00
VA/Stromberg	Warsaw	W	2	2.68	64.89	0.001	4.54	8.40

[a] Treat = the most effective treatment (s) within each trial based on the pair-wise difference between mean IND for each treatment and the check; IND (%) = mean index across plots receiving the most effective treatment; % control = percent control; *P* value = level of significance from *F* test of the difference between mean IND across plots receiving the most effective treatment and the untreated check. All tests of significance were done using arcsine-transformed IND.
… = Trials with no disease.

Table 2. Fungicide effect on DON.

Trial[a]		Wheat	Most effective Treatment[b]				DON (ppm)	
State/PI	Location	Type	Treat	DON	% Reduction	*P value*	Mean	Max
MD/Grybauskas	Beltsville	W	3	2.40	84.42	<0.001	7.90	24.50
MN/Hollingsworth	Crookston 1	S	3	2.23	53.40	<0.001	3.50	5.70
	Crookston 2	W	4	0.73	26.82	0.329	1.01	1.80
ND/McMullen	Fargo	S	3	4.00	48.37	<0.001	5.55	8.50
	Carrington 1	S	7	4.85	51.74	0.041	6.33	19.10

[a]DON data were not available for some trials or available but equally low (below 1 ppm) for all treatments.
[b]Treat = the most effective treatment within each trial based on the pair-wise difference between mean DON for each treatment and the check; DON (ppm = mean DON across plots receiving the most effective treatment; % reduction = percent reduction in DON; *P* value = level of significance from *F* test of the difference between mean DON across plots receiving the most effective treatment and the untreated check. All tests of significance were done using log-transformed.

Table 3. Fungicide effect on FDK.

Trial[a]		Wheat	Most effective Treatment[b]				FDK (%)	
State/PI	Location	Type	Treat	FDK (%)	% Reduction	*P value*	Mean	Max
IL/Fakhoury	Carbondale	W	6	6.75	32.50	0.440	10.43	28.00
LA/Padgett	Macon Ridge	W	4	9.75	22.00	0.0269	12.78	19.00
MD/Grybauskas	Beltsville	W	3	13.20	55.10	0.004	22.91	52.00
MN/Hollingsworth	Crookston 1	S	3	5.50	38.89	0.051	7.32	15.00
ND/McMullen	Fargo	S	3	7.50	61.54	<0.001	12.61	20.00
	Carrington 1	S	3, 4	10.75	52.22	<0.001	12.86	25.00
	Carrington 2	S/D	3	4.00	77.14	<0.001	9.04	20.00
	Langdon 2	S	7	3.25	63.89	<0.001	5.30	10.50
	Langdon 3	S	3	0.75	31.25	<0.001	2.25	7.90
SD/Draper	Brookings 1	S	4	32.5	13.33	0.025	36.79	45.00
	Brookings 2	S	3	32.5	13.33	0.059	36.07	40.00
	Watertown 1	S	5	2.50	50.00	<0.001	3.78	6.00
	Watertown 2	S	2, 3	2.25	43.75	0.006	3.26	6.00
	Groton 1	S	4	1.75	41.67	0.092	2.67	4.00
	Groton 2	S	5	2.00	33.33	0.218	2.44	4.00

[a] FDK data were not available for some trials or available but equally low for all treatments.

[b]Treat = the most effective treatment (s) within each trial based on the pair-wise difference between mean FDK for each treatment and the check; FDK (%) = mean FDK across plots receiving the most effective treatment; % reduction = percent reduction in FDK; *P* value = level of significance from *F* test of the difference between mean FDK across plots receiving the most effective treatment and the untreated check. All tests of significance were done using arcsine-transformed FDK.

SPRAYER NOZZLE CONFIGURATIONS AND EFFECTS ON FUNGICIDE SPRAY DEPOSITION ON WHEAT HEADS

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ABSTRACT

Fusarium head blight (FHB) continues to cause significant yield and quality losses of wheat and barley in the US, and was very significant in portions of the Northern Plains during 2005. Fungicide application remains an accepted method for FHB control. Previous studies concerning fungicide application and control of FHB did not address detailed parameters of spray deposition on varying wheat head structures and the relationship of this coverage to FHB control. The objective of this trial was continue previous work to take steps in quantifying the parameters surrounding spray deposition on wheat heads and to identify methods whereby optimized fungicide application for efficacy can occur. Spring wheat (cvs Oxen and Ingot) were planted at the South Dakota State University Agronomy Farm and treated at anthesis (Feekes growth stage 10.51) by spraying in a single direction in nearly calm wind conditions with a tank mixture of Folicur (tebuconazole) at a rate of 4 fl oz/a (292.30 ml/ha) and Induce adjuvant (0.125% v/v) supplemented with a fluorescent orange water soluble dye (3% v/v). The mixture was applied using a wheeled cart to control sprayer height above the crop canopy, with a pressurized sprayer set at 40 psi (275.79 kPa) and an application rate of 18.6 gpa (173.97 l/ha) . Nozzle configurations (treatments) included: 1) one flat fan nozzle pointing straight down (XR TeeJet 11002), 2) one flat fan nozzle angled 45 degrees forward (XR TeeJet 10002), 3) a twin-orifice flat fan nozzle (60 degrees between fans) (Twinjet TJ11002) and 4) a twin nozzle configuration (90 degrees between fans) (paired XR TeeJet 11001). Varieties and treatments were randomized in a 2 X 4 factorial design with four replications with varieties and nozzle types as factors. Plots were inoculated by spreading Fusarium graminearum (Fg4) inoculated corn (*Zea mays*) grain throughout the field and providing overhead mist irrigation on a 16 hr/ 8 hr on/off schedule (overnight mist) throughout anthesis. Wheat heads were evaluated for spray coverage, deposition pattern, FHB incidence, head severity, total FHB damage and location of diseased spikelets relative to direction of sprayer travel. Plot yield, test weight, and Fusarium damaged kernels (FDK) were also measured. Digital pictures of the incoming and outgoing side of the head were taken under UV light and spray coverage was analyzed digitally from the images. Spray coverage on the incoming side of heads was acceptable with all nozzles, while the side away from the application generally received much less product, regardless of nozzle configuration. No nozzle tested in this trial provided equal deposition on the incoming and outgoing sides of the head, although initial analysis shows that twin orifice configurations tend toward more uniform coverage on both sides of the head. Varietal and treatment differences were significant for yield, test weight and FDK. Initial data on FHB infection appears to show that there may be an effect of applied fungicide penetrating into the inner portions of the head, including the rachis, and thus limiting FHB infection to single spikelets and reducing FHB spread within the head. These data suggest that deep penetration of the spray droplets into the head may be as important into enhancing product efficacy and FHB control as total coverage of the head.

FERMENTATION AND FORMULATION: CRUCIAL FOCUS AREAS FOR EXPEDITING THE DEVELOPMENT OF BIOCONTROL PRODUCTS

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ABSTRACT

Developing an effective, commercially successful biological control product is a complex, labor intensive undertaking. The process must begin with a carefully crafted microbial selection procedure, proceed by employing biomass production protocols that optimize product quantity and quality, and end with devising a product formulation that preserves shelf-life, aids product delivery and enhances bioactivity. Selection procedures that require prospective microbial biocontrol agents to possess both efficacy and amenability to production in liquid culture enhance the likelihood of selecting agents with improved commercial development potential. Scale-up of biomass production procedures must optimize product yield without compromise of product efficacy and amenability to stabilization and formulation. Considerations critical to designing successful formulations of microbial biomass are many fold and include designing production processes that enhance biomass amenability to formulation; an awareness of the mode of action of the microbial agent; durability of the life-stage to be formulated; the physical, chemical, and biological characteristics present on the application target; and the equipment used for field application. Solutions to these formulation considerations will not necessarily be compatible. Data from several systems for biologically controlling plant pests, with emphasis on the biological control of Fusarium head blight of wheat, will be used to demonstrate many of these concepts.

USDA-ARS AND THE OHIO STATE UNIVERSITY COOPERATIVE RESEARCH: GREENHOUSE AND FIELD TESTS OF COMBINATIONS OF CHOLINE METABOLIZING STRAINS AND ANTAGONIST *CRYPTOCOCCUS NODAENSIS* OH 182.9 FOR REDUCING FHB OF WHEAT

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INTRODUCTION

The primary causal agent of Fusarium head blight (FHB) in North America is *Fusarium graminearum* Schwabe Group 2 (Aoki and O'Donnell, 1999) (perfect state = *Gibberella zeae* (Schwein.) Petch). Considerable experimental evidence suggests that wheat anthers and specifically the compounds choline and betaine in anthers can enhance FHB disease development (Strange and Smith, 1978). Previously, we isolated 738 strains from wheat anthers, determined 16.5% were able to metabolize choline as a sole carbon source, and found that several strains reduced FHB disease parameters in greenhouse and field tests (Schisler et al, 2004). We have demonstrated the potential of several antagonists including *Cryptococcus nodaensis* nomen nudum OH 182.9 (NRRL Y-30216) to significantly reduce the severity of FHB in field environments when biomass was produced in laboratory and pilot-scale quantities in liquid culture (Schisler et al., 2002; Khan et al., 2004). Combinations of biological control strains potentially increase the efficacy and consistency of biocontrol. The objective of this study was to determine if combinations of strain OH 182.9 and choline metabolizing strains (CMS) could be identified to enhance biocontrol compared to that obtained with individual strains.

MATERIALS AND METHODS

Antagonist combination tests (greenhouse)

Hard red spring wheat (cultivar Norm) was grown in plant growth chambers prior to conducting plant bioassays on greenhouse benches. Biomass of OH 182.9 and 3 CMS was produced by incubating flasks containing inoculated liquid medium (SDCL, Slininger et al. 1994) at 250 rpm and 25 C for 48 h. Conidial inoculum of *G. zeae* isolate Z-3639 was produced on clarified V8 juice agar under 12 h/day fluorescent light for 7 days at 24 C. At wheat anthesis, antagonist suspensions were individually misted onto approximately 14 wheat heads per treatment followed immediately by a mist application of a conidial suspension (5×10^5 conidia/ml). For treatments using single antagonist strains, fully colonized broths were diluted by one-quarter prior to use at log10 (CFU/ml) of 7.73, 7.11, 9.63, and 9.56 for OH 182.9, AS 55.2, AS 64.4 and OH 221.3, respectively (Fig. 1). Treatments that combined antagonists were produced by mixing equivalent volumes of treatment suspensions of each individual strain. Heads treated with water followed by the conidial suspension of *G. zeae* isolate Z-3639 served as a "pathogen only" control. Plants were placed in humidity tents for 3 days, scored for disease severity after 16 days, and data analyzed using one-way ANOVA. The reported means are results from pooled replicate experiments.

Field testing of CMS

Field trials were conducted in Peoria, IL (insufficient disease development, data not shown) and in Wooster, OH in 2005 (Table 1). Biomass of antagonists was produced in Fernbach flasks using SDCL medium.

Soft red winter wheat cultivars Elkhart (susceptible) and Freedom (moderately resistant) were grown. Biomass of antagonists was applied at the beginning of wheat flowering at concentrations of $\log_{10}$ (CFU/ml) of 7.18, 7.20, 9.36, and 9.35 for OH 182.9, AS 55.2, AS 64.4 and OH 221.3, respectively, and a rate of 80 gal/acre. The fungicide Folicur 3.6F was applied at the recommended AI rate as a chemical control and untreated plants served as an additional control. Corn kernels colonized by *G. zeae* were scattered through plots (~25-40 kernels/m^2) two weeks prior to wheat flowering and mist irrigation was provided periodically for approximately two weeks after treatment application. Heads were scored for disease incidence and severity 26 days after treatment using a 0-100% scale. Randomized complete block designs were used in all field trials. Analysis of variance and Fisher's protected LSD ($P<0.05$, Statistix 7 statistical software) were used to identify treatment means that were significantly different.

RESULTS AND DISCUSSION

All treatments that combined CMS with OH 182.9 reduced FHB disease severity with the exception of "OH 182.9 + OH 221.3" (Fig. 1) in greenhouse tests. Successful antagonist combination treatments did not reduce disease severity to a greater extent than treatment with OH 182.9 alone (Fig. 1).

Results varied in field testing depending on the wheat cultivar considered. The best disease control was obtained by combining the use of the resistant cultivar Freedom with antagonist combination treatments such as "OH 182.9 + AS 64.4" or OH 182.9 combined with all three CMS (Table 1). Folicur 3.6F and microbial treatments rarely reduced (and sometimes increased) FHB on the susceptible cultivar Elkhart (Table 1).

While enhanced efficacy of disease control was not demonstrated when OH 182.9 was combined with CMS, trends of enhanced consistency of biocontrol can only be determined with further data from field tests. Contrary to 2005 field results, 2003 field tests of individual CMS in Peoria, IL and Wooster, OH demonstrated significant reduction in FHB disease parameters on cultivar Freedom (Schisler et al., 2004). Optimization of liquid culture growth conditions enhanced the biocontrol efficacy of OH 182.9 biomass (Zhang et al., 2005). Medium optimization may be similarly effective in improving the efficacy of CMS individually or in combination with OH 182.9.

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DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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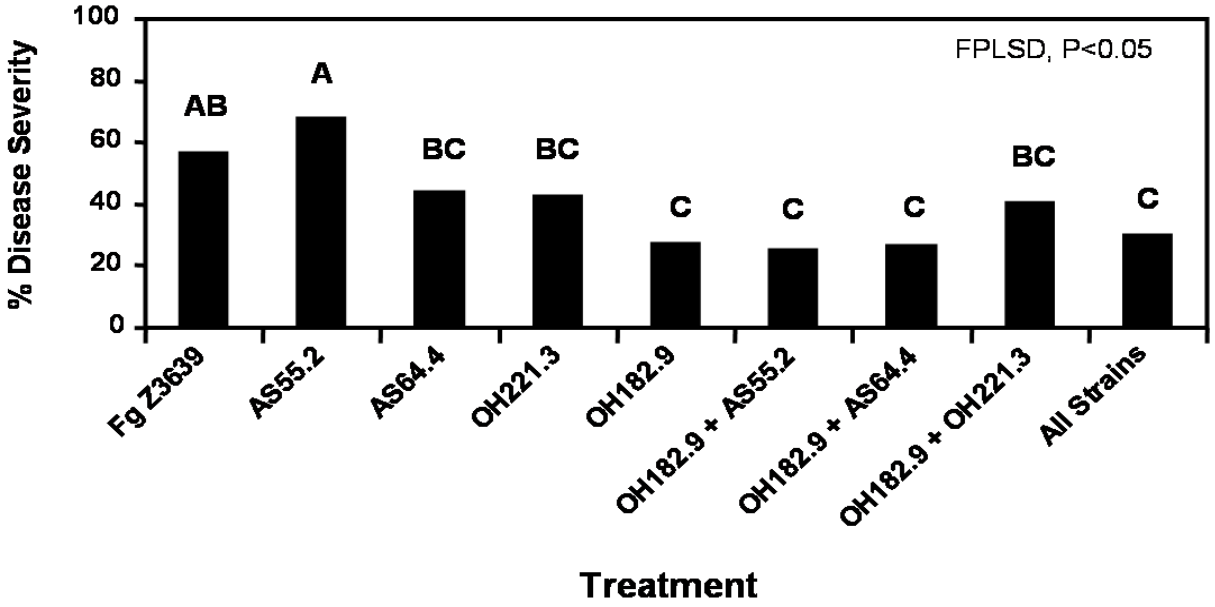

Figure 1. Influence of choline metabolizing strains AS 55.2, AS 64.4 and OH 221.3 alone or in combination with *C. nodaensis* OH 182.9 on FHB incited by *Fusarium graminearum* isolate Z-3639 in greenhouse tests.

Table 1. 2005 field trial results at Wooster, Ohio: influence of *Cryptococcus nodaensis* OH 182.9, choline metabolizing strains, Folicur 3.6F and combinations thereof on FHB disease parameters on two cultivars of winter wheat[1]

Treatment	Wheat Cultivar			
	Freedom		Elkhart	
	% Disease Severity	% Incidence	% Disease Severity	% Incidence
Untreated control	4.8	29.3	20.8	57.0
Folicur 3.6F	2.8*	19.7*	20.3	57.0
OH182.9	3.3*	22.3*	22.1	57.0
AS55.2	3.7	26.7	29.1*	66.7*
AS64.4	4.6	28.0	25.8*	52.0
OH221.3	4.2	25.3	17.4	55.0
OH182.9 + AS 55.2	4.1	21.0*	22.3	55.7
OH182.9 + AS 64.4	2.9*	21.7*	16.6	45.3*
OH182.9 +OH 221.3	3.3*	21.7*	17.3	54.0
182.9 + 55.2 + 64.4 + 221.3	2.9*	18.3*	18.0	50.0
55.2 + 64.4 + 221.3	5.6	32.3	20.3	52.7
$LSD_{(0.05)}$	1.5	6.9	5.0	7.9

[1]Within a column, values followed by an "*" are significantly different from the untreated control ($P \leq 0.05$).

EFFECT OF GLYPHOSATE ON THE *IN VITRO* GROWTH OF FUNGAL ORGANISMS

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ABSTRACT

Glyphosate [N-(phosphonomethyl)-glycine] is a broad-spectrum, non-selective, post-emergence herbicide used to control weeds in agricultural production systems. The application of glyphosate prior to wheat planting has been reported to be associated with increased Fusarium head blight incidence in the wheat crop. Wheat frequently follows soybeans in crop rotations; therefore, it is important to determine the effect of glyphosate on fungal communities. The objectives of this study were to: 1) determine the effect of glyphosate on mycelial growth of *Fusarium graminearum* (causal organism for Fusarium head blight, or wheat scab) and six other common soil microorganisms, and 2) determine the effect of glyphosate on macroconidia production by *F. graminearum*. Three isolates of *F. graminearum* were tested in addition to six randomly selected isolates of common soil fungal organisms. Mycelial growth was measured daily on the isolates grown on potato dextrose agar (PDA) amended with different concentrations of glyphosate. Macroconidia production was evaluated by growing *F. graminearum* in carboxymethyl-cellulose (CMC) liquid media for five days and counting the number of macroconidia produced. Macroconidia production was greatly reduced at the recommended field rate of glyphosate. The myclelial growth of all seven species was reduced at all rates of glyphosate. At very low glyphosate concentrations the growth of the *Fusarium* spp. was inhibited less than the other four species. The application of glyphosate may alter the soil microflora and allow the less affected *Fusarium* spp. to fill vacant niches left in the soil community by other negatively affected microorganisms. An increased abundance of *Fusarium* spp. in soils could potentially result in a higher incidence and severity of diseases such as *Fusarium* head blight and sudden death syndrome of soybeans.

STANDARDIZED EVALUATION OF BIOLOGICAL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT: 2005 RESULTS

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OBJECTIVE

To evaluate, using standardized methodology, a set of treatments involving biological control agents alone and in combination with a fungicide for effectiveness in managing Fusarium head blight (FHB) in wheat across a range of environmental conditions.

INTRODUCTION

Biological control has been investigated for the control of FHB by laboratories in the United States, resulting in a number of agents with the potential for controlling FHB being identified. These agents, including *Cryptococcus nodaensis* OH 182.9 (Khan et al., 2004), *Bacillus* spp. strains Trigocor 1448 (Stockwell et al., 2001) and 1BA (Draper et al., 2001), and *Lysobacter enzymogenes* C3 (Yuen and Jochum, 2002), were effective when evaluated separately in field tests. Evidence also was obtained suggesting that biocontrol agent-fungicide combinations could provide higher levels of FHB control than biological or chemical methods alone (DaLuz et al., 2003; Yuen and Jochum, 2004). In 2005, standardized tests supported by the USWBSI were conducted over a wide range of environmental conditions and crop genotypes to compare two agents *Bacillus* sp. 1BA and *L. enzymogenes* C3 applied alone and in combination with the fungicide tebuconazole. The results of these efforts are reported here.

MATERIALS AND METHODS

Five trials were conducted across three states on a range of wheat market classes (Table 1). The trials conducted on two cultivars in Missouri were separate experiments. In each trial, strain 1BA was provided by B. Bleakley and M. Draper, South Dakota State University and strain C3 was provided by G. Yuen, University of Nebraska-Lincoln as broth cultures. The pre-application population of each agent in the broth culture was determined by the local researcher using dilution plating. Each biocontrol agent was tested alone and in a tank mixture with tebuconazole (Folicur 3.6F; 4.0 fl oz/A; provided by Bayer Cropscience, Kansas City, MO). In addition, there was a non-treated control and a treatment with tebuconazole alone. All treatments were amended with the commercial surfactant Induce (0.125%) and applied at 20 gal. per acre. One application of each treatment was made at early flowering (Feekes 10.5.1) using a CO_2-pressurized sprayer (approximately 40 psi) equipped with flat-fan nozzles oriented forward and backward. The size and number of replicate plots varied among trials. Some of the trials were inoculated with *Fusarium graminearum* and utilized mist irrigation systems to stimulate infection. In all trials, FHB incidence (% heads infected per plot), severity (% spikelets infected per diseased head), and index (plot severity) were determined from at least 40 heads per plot around 3 weeks after anthesis. The incidence of Fusarium-damaged kernels (%FDK) was determined after harvest. Samples from each plot were sent to USWBSI-designated laboratories for analysis of deoxynivalenol (DON) content. Results from all trials first were analyzed separately using analysis of variance and then analyzed together, with each experiment being treated as a 'block'. Fisher's LSD test was used for means separation.

RESULTS

FHB pressure varied considerably among the trials, with incidence ranging from 8 to 94% and severity ranging from 5 to 91% in the controls (Table 2). Disease levels in MO experiments were too low to provide separation of treatments for any disease parameter. In the NE and SD experiments, significant treatment effects were found for some but not all disease parameters. Neither of the biological control agents or tebuconazole alone significantly reduced any disease parameter in more than one experiment. When data from all experiments were pooled, strain C3 and tebuconazloe alone reduced scab incidence and index compared to control, but the level of reduction was low. Biocontrol agent-tebuconazole combinations were no better than tebuconazole alone, in individual experiments and across all experiments. No significant treatment effect was found for incidence of Fusarium-infected kernels or for DON content in any experiment (data not shown).

DISCUSSION

The identification of a biological control agent that can be effective across a range of environments and wheat genotypes remains a challenge. The finding in this study that tebuconazole, currently the most commonly applied fungicide for FHB in the US, did not provide consistent disease control or affect DON levels, is further confirmation as to how difficult it is to manage this disease. Combining biological control agents with fungicides theoretically should confer an advantage in the management of FHB by bringing diverse modes of action to play (Da Luz et al., 2003); this advantage, however, was not realized in this study.

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Table 1. 2005 biological control trial locations, wheat cultivars, and researchers.

State and location	Wheat market class and cultivar	PI and Institution
MO	Soft red winter wheat 'Elkhart'	L. Sweets, University of Missouri
MO	Soft red winter wheat 'Roane'	L. Sweets, University of Missouri
NE - Havelock	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
NE - Mead	Hard red winter wheat '2137'	G. Yuen, University of Nebraska
SD	Hard red spring wheat 'Ingot'	B. Bleakley and M. Draper, South Dakota State University.

Table 2. Results across five uniform biocontrol trials on wheat, 2005

Treatment	MO 'Elkhart'	MO 'Roane'	NE Havelock	NE Mead	SD	Mean
SEVERITY (% spikelets infected)						
Control	6	5	20	31	91	31
Folicur	2	4	14	23	89	26
1BA	5	7	21	31	88	30
1BA + Folicur	3	2	19	21	85	26
C3	2	0	21	27	90	28
C3 + Folicur	4	5	16	17	95	27
P	NS	NS	0.006	NS	NS	NS
$LSD_{0.05}$	-	-	8	-	-	-
INCIDENCE (% heads infected)						
Control	8	8	94	71	57	47
Folicur	3	8	69	59	51	39
1BA	5	8	90	70	53	45
1BA + Folicur	5	3	90	58	48	41
C3	8	0	81	64	47	40
C3 + Folicur	10	8	88	55	50	42
P	NS	NS	NS	0.015	NS	0.05
$LSD_{0.05}$	-	-	-	13	-	6
INDEX (plot severity)						
Control	0.9	0.7	19	23	52	19
Folicur	0.2	0..7	10	14	46	14
1BA	0.5	0.7	19	22	47	18
1BA + Folicur	0.3	0.2	17	13	42	14
C3	0.7	0	17	17	42	15
C3 + Folicur	1	0.9	14	10	47	15
P	NS	NS	NS	0.006	NS	0.05
$LSD_{0.05}$	-	-	-	8	-	4

OTHER PAPERS

FUSARIUM HEAD BLIGHT: A SUMMARY OF THE SOUTH AFRICAN SITUATION

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ABSTRACT

In South Africa the main causal organisms of Fusarium head blight (FHB) are *Gibberella zeae* (=*Fusarium graminearum*), *F. culmorum* and *F. crookwellense*. *Fusarium graminearum* and *F. culmorum* are associated with warmer regions, and *F. crookwellense* with cooler regions. Sporadic FHB outbreaks occur principally in the irrigation areas of the country. In favourable years significant damage is caused. Effective control has not yet been achieved through breeding for resistance or chemical control. Unsustainable crop management systems are currently aggravating the problem and extensive FHB research is required.

INTRODUCTION

Head blight of wheat was first noted in South Africa in 1980. Since then, the use of center pivot irrigation, increasing no-till practices and continuous wheat / maize (corn) cropping systems have resulted in epidemic outbreaks of FHB in 1985, 1986 (Scott, De Jager and Van Wyk, 1988), 1994 (Scott and Smith, 1995) and 2000 (unpublished data). FHB can cause yield reduction of up to 70% under high inoculum pressure and favourable environmental conditions. The aim is to give an overview on the status of research on FHB in South Africa and the challenges it present.

SUMMARY OF RESEARCH

The predominant organism associated with FHB in South Africa is *G. zeae*, comprising between 48.4% (Boshoff, Pretorius and Swart, 1998) and 83.9% of total isolates studied (Minnaar-Ontong and Kriel, 2005, unpublished data). According to O'Donnell (personal communication) there are four clades of *F. graminearum* in South Africa, of which two, *F. graminearum* and *F. boothii*, are associated with FHB. Molecular screening using Amplified Fragment Length Polymorphisms (AFLPs) to distinguish between a representative collection of isolates is currently in progress. The AFLPs have already been standardised for reference isolates of *F. graminearum*, *F. crookwellense*, *F. avenaceum*, and *F. culmorum*, with outgroups including *F. sambucinum*, *F. pseudograminearum* and *F. equiseti* (Philippou, Herselman and Kriel, 2005, unpublished data). The results have shown that it is possible to use AFLPs to distinguish between these species which are difficult to differentiate morphologically. Current morphological data show *F. culmorum* to be the 2nd most important species in warmer regions (Minnaar-Ontong and Kriel, 2005, unpublished data), and *F. crookwellense* in the cooler regions (Boshoff et al., 1999a). Fusaria associated with maize stalk and cob rots include *F. verticillioides*, *F. subglutinans* and *F.graminearum* (Rheeder, Marasas, and Van Schalkwyk, 1993). Since *F. graminearum* is pathogenic on maize and wheat, disease control under current crop production systems in the irrigated areas are difficult.

Chemical control has proven to be ineffective in the reduction of FHB in the irrigation areas of South Africa. This could be due to varying flowering periods of wheat under large centre pivot irrigation systems (some exceeding 64 ha), thus complicating the timing of fungicidal spays. The efficacy of chemical control is also influenced by insufficient coverage obtained with aerial application and low efficacy of fungicides under field conditions. Boshoff et al. (1999b) found prochloraz (EC_{50}=0.027-0.337 µg/ml), bromuconazole (EC_{50}=0.415-1.126 µg/ml) and tebuconazole (EC_{50} = 0.45 µg/ml) to be the most effective against *F. graminearum* and *F. crookwellense*

in vitro, but differences were noted in the sensitivity of isolates.

It has been claimed that infected wheat seed is responsible for the introduction of *Fusarium* spp. in new wheat irrigation areas. The control of seedborne *Fusarium* spp. was tested with six seed treatment fungicides at three different dosages. The chemicals included two concentrations of tebuconazole (15 g/L and 60 g/L), two different formulations of carboxin/thiram (200/200 g/L), difenoconazole (30 g/L) and guazatine/tebuconazole (300/15 g/L). Results indicated a reduction of *Fusarium* colonization of the seed from 77.5% in the control to an average of 12.3% in the treated seed. Identification of the remaining isolates revealed the treatments to be ineffective against the Discolor section of *Fusarium*, including *F. graminearum* and *F. culmorum*. *Fusarium* spp. in the Liseola section, including *F. proliferatum* and *F. verticillioides* were controlled by the seed treatments (Kriel and Minnaar-Ontong, 2005, unpublished data).

Scanning electron microscopy studies revealed no differences in the infection processes employed by *F. graminearum* and *F. crookwellense* (Boshoff et al., 1999a). The fungus colonizes the anthers extensively, establishing itself in the floret. This is followed by penetration of the lemma and palea through stomata or wounds. The glumes, rachis, grain and peduncles are only colonized later. Bleached, necrotic symptoms can be seen on infected florets 4 days after inoculation. *Fusarium graminearum* is more pathogenic than *F. crookwellense*, with more extensive colonization of the head shown in artificial inoculation studies on the wheat cultivar Palmiet (Boshoff et al., 1999a).

Mycotoxin research in South Africa is performed by the Medical Research Council. Isolates of *G. zeae* (Group 1 and 2) and *F. crookwellense* were tested for mycotoxin production (Sydenham et al., 1991). Two chemotypes were identified within *G. zeae* Groups 1 and 2. Most isolates from wheat (crowns and scabby kernels) produced deoxynivalenol (DON), but none produced nivalenol (NIV) or fusarenon-X (FUS-X). Isolates from maize did not produce DON, but most produced NIV and / or FUS-X. All but one isolate of *G. zeae* produced zearalenone (ZEA). In the *F. crookwellense* isolates tested, none produced DON, all produced NIV and ZEA, and the majority produced NIV.

Suitable crop rotation systems for irrigation areas of the country are limited due to socio-economic factors. A possible rotation crop suggested by many agriculturalists is barley. Data from the US and Canada stated that FHB is more severe on barley than on wheat. FHB of barley has not been noted in SA before. Greenhouse trials were conducted to determine the relative susceptibility of SA barley cultivars, along with a susceptible wheat control, to *F. graminearum* cultures isolated from wheat (De Villiers, Kriel and Pretorius, 2004, unpublished data). Results indicated SA barley cultivars to be susceptible to FHB after artificial inoculation under controlled conditions, although the disease did not spread as fast as in wheat heads. The lack of symptoms in production fields could be attributed to morphological differences between the heads of barley and wheat.

Current SA cultivars do not have genetic resistance to FHB, but differences in tolerance have been noted (Scott, De Jager and Van Wyk, 1988). Some cultivars escape disease due to flowering windows not corresponding with favourable environmental conditions. Results of greenhouse trials of cultivar resistance were met with scepticism by many role players in the industry. Long term field data along with the introduction of resistant germplasm are necessary to clarify the status of commercial cultivars, but the sporadic nature of the disease and unfavourable conditions for disease development during the last three seasons have hampered progress in this regard. Some seed companies are breeding for resistance to FHB and the first cultivars with resistance incorporated from Sumai 3 sources should be available for commercial production within the next year or two (Koekemoer, Monsanto, personal communication).

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U.S. Wheat & Barley

Scab Initiative